Molecular Cloning of Pancreatic Group I Phospholipase A2 Receptor*

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We have recently reported that mammalian pancreatic group I phospholipase A2 (PLA2-I) has its specific receptor (PLA2 receptor) on a variety of mammalian cells and that various biological responses are elicited by PLA2-I via this receptor. In this study, we cloned cDNAs encoding a protein corresponding to the bovine PLA2 receptor purified from the corpora lutea on the basis of its partial amino acid sequences. The identity of a protein encoded by the cloned cDNA with the bovine PLA2 receptor was verified by a transient expression experiment using COS-7 cells. Interestingly, the deduced primary structure of the PLA2 receptor (1,463 amino acid residues) exhibits a close relatedness throughout the molecule to that of the macrophage mannose receptor, a unique member of C-type animal lectin family, in spite of their functional diversity. Based on this sequence similarity between these two receptors, the domain organization of the PLA2 receptor could be tentatively assigned as follows; 10 extracellular domains including 8 tandem repeats homologous to C-type carbohydrate-recognition domains (CRDs) and a single transmembrane region followed by a short cytoplasmic tail. The results of transient expression experiments for mutant PLA2 receptor supported this assignment and furthermore suggested the region responsible for PLA2-I binding corresponds to CRDs in the mannose receptor.

Phospholipases A2 (PLA2s, EC 3.1.1.4) catalyze the hydrolysis of the sn-2 bond of glycerophospholipid to release fatty acid and lysophospholipid. Mammalian PLA2s thus far identified are categorized into two types, secretory and cytoplasmic PLA2s, in terms of their localization, and the secretory PLA2s are further classified into two subgroups, pancreatic group I and arthritic group II, according to their characteristics in primary structure (1). The group I PLA2 (PLA2-I) has been thought to be a digestive enzyme for lipid-containing nutrients because of its abundance in digestive organs, whereas the group II PLA2s have been described in connection with inflammatory responses of the bovine corpus lutea, which clarified that the bovine PLA2 receptor, which allows us to deduce the complete primary structure of the receptor. We also describe some intriguing structural characteristics of the PLA2 receptor and the properties of the recombinant wild-type PLA2 receptor and its mutants.

EXPERIMENTAL PROCEDURES

Determination of Partial Amino Acid Sequences of the Bovine PLA2 Receptor—Bovine PLA2 receptor was purified to homogeneity from corpora lutea as described previously (9). The purified receptor protein was dissolved in 10 mM HCl containing 20 mM octyl thioglucoside and subjected to NH2-terminal analysis with a protein Sequencer (Applied Biosystems). The isolated fragment was analyzed with the protein Sequencer, and thus led us to search for its NH2-terminal sequence (ETAAWAVTPERLREWQDKFI, respectively. Total DNA synthesized with a modified moloney leukemia virus reverse transcriptase (SuperScript I) using cDNA fragments encoding the bovine corpus lutea, which clarified that the bovine PLA2 receptor is composed of a single glycosylated polypeptide chain with an apparent molecular mass of 190 kDa (9).

Here, we report the molecular cloning of a cDNA encoding the bovine PLA2 receptor, which allows us to deduce the complete primary structure of the receptor. We also describe some intriguing structural characteristics of the PLA2 receptor and the properties of the recombinant wild-type PLA2 receptor and its mutants.
Receptor—Using the 62-bp cDNA fragment encoding the NH₂-terminal one positive clone (pGE-1) was digested with HindIII, and the insert of the sequenced clones were found to code for the NH₂-terminal sequence (12). Some clones carrying the 62-bp insert region of the PLA₂ receptor labeled with 32P by PCR (13), were first probed for the subsequent cDNA library screening is indicated by the line shown above the nucleotide sequence. The splicing donor site (GT) is sequenced with a Sequenase Version 2.0 sequencing kit.

The genomic DNA fragment was subcloned for sequencing. The predicted genomic DNA fragment was randomly picked up and subjected to DNA sequencing using a sequencing kit (U.S. Biochemicals). One-third of the sequenced clones were found to code for the NH₂-terminal sequence of the PLA₂ receptor.

Screening and Isolation of Clones Encoding the Bovine PLA₂ Receptor—Using the 62-bp cDNA fragment encoding the NH₂-terminal region of the PLA₂ receptor labeled with 32P by PCR (13), we first screened λ phage bovine genomic library (Clontech). A λ phage DNA of one positive clone (pGE-1) was digested with HindIII, and the insert genomic DNA fragment was subcloned for sequencing. The predicted exon region in this genomic clone (residues 257–364 in Fig. 1) was amplified by PCR and used as a probe for screening of bovine cDNA libraries. Some positive clones carrying partial PLA₂ receptor cDNA were obtained from bovine placenta cDNA library ( oligo(dT) + random-primed, Clontech). Since the 3′-portion of the PLA₂ cDNA was not found in all the isolated clones, some cDNA libraries were rescreened with a cDNA probe generated from the most downstream region of the isolated clones. We eventually found some clones of the cDNA in a homemade plasmid cDNA library (cDNAs > 2.5 kilobase pairs) constructed from MDBK cells (bovine kidney-derived cell line) with a plasmid cloning kit (Life Technologies, Inc.). Two clones (pPL-1 and pMD-10, obtained from the bovine placenta and the homemade MDBK plasmid cDNA libraries, respectively) were completely sequenced with a Sequenase Version 2.0 sequencing kit (U.S. Biochemicals) or by the chemical method of Maxam and Gilbert (14).

RNA and DNA Blotting Analyses—Total cellular RNAs were routinely prepared by the method of Chomczynski and Sacchi (11) and further purified with a PolyATrac™ kit (Promega). Bovine brain poly(A)+ RNAs were purchased from Clontech. RNAs to be examined for hybridization, were first saturated with 125I-labeled porcine PLA₂-I at 4 °C for 2 h. After washing of the cells with phosphate-buffered saline twice, the internalization was induced by raising the incubation temperature to 37 °C. Forty min after the temperature shift, radioactivities internalized into the cells were measured, and a percentage of internalized PLA₂-I to the initially bound one was calculated.

RESULTS

Isolation and Characterization of cDNAs Encoding Bovine PLA₂ Receptor—In order to make library screening straightforward, we first prepared a cDNA fragment (62 bp) encoding the NH₂-terminal part of the receptor by PCR. Using this short cDNA probe, we screened a bovine genomic library (5 × 10⁸ clones) and isolated one positive clone (pGE-1). The sequence analysis of pGE-1 revealed that the 62-bp cDNA sequence was interrupted by an intron (Fig. 1), and a neighboring down-

Fig. 1. Nucleotide sequence around the exon included in the genomic clone pGE-1. The nucleotide sequence around the region hybridized with the 62-bp probe is shown. The nucleotide sequence corresponding to an NH₂-terminal translated region is given in bold letters with the deduced amino acid residues (the NH₂-terminal residue of the native receptor is numbered +1). The most 5′-end residue in the isolated cDNA clones is numbered +1, and the residues located upstream to this 5′-end residue are indicated by lowercase letters. The region (128 bp) used as a probe for the subsequent cDNA library screening is indicated by the line shown above the nucleotide sequence. The splicing donor site (GT) is underlined.
stream exon was not included in this clone. Hence, we next screened bovine cDNA libraries (about 10^6 clones per library) with a new cDNA probe generated from the predicted exon region (128 bp, indicated by the line above the sequence in Fig. 1) or the most downstream region of the isolated partial cDNA clones as described under "Experimental Procedures." We eventually isolated some cDNA clones (i.e., pPL-1 and pMD-10 as described under "Experimental Procedures") which collectively provided the complete structural information of the PLAZ receptor cDNA (Fig. 2).

It is interesting to note that pMD-10 contained a 114-bp insert at the nucleotide residue number of 2114 (Fig. 2A), whereas other isolated clones did not carry such an insert. This short insert, probably generated through alternative splicing, does not interrupt the open reading frame and thus results in an insertion of 38 amino acid residues at the middle of the receptor protein (Fig. 2B). A PCR-assisted analysis showed that the cDNA bearing this insert was minor in the MDBK cDNA library (data not shown).

Another alternative form of the PLAZ receptor cDNA was found in the MDBK cDNA library; a cDNA clone (pMD-11) carried a 3'-noncoding region shorter than that of pMD-10 by 1 kb (Fig. 2A). Poly(A) tails of pMD-10 and -11 are preceded by possible polyadenylation signals (ATTAAA and AATGAA, respectively), and thus these clones were probably derived from differentially processed mRNAs using alternative polyadenylation sites.

The predicted primary structure of the bovine PLAZ receptor is composed of 1,463 amino acid residues and includes all partial amino acid sequences obtained from lysylendopeptidase-digested fragments of the purified receptor (Fig. 2A). The calculated molecular mass of the mature receptor is 186.7 kDa, which is consistent with the highly glycosylated nature of the native protein (about 150 kDa) (9). There are several potential N-linked glycosylation sites (Asn-X-(Ser or Thr)-X, Pro is inhibited at the position of X (19)), which also agrees well with the highly glycosylated nature of the native receptor (9).

**Structural Relatedness of the PLAZ Receptor with the Mannose Receptor**—We subjected the nucleotide sequence encoding the PLAZ receptor to a computer-assisted homology search against DNA data bank (GenBank release No. 73.0) with FASTA program (20). The highest homology scores were given for macrophage mannose receptors, unique members of the Cya-dependent (C-type) animal lectin family (21, 22). The sequence similarity was not restricted in a specific region but observed throughout the molecule, and the overall identity between the amino acid sequences of the bovine PLAZ receptor and human mannose receptor is 29%. Although the sequence identity may not be remarkably high, the structural relatedness between these two receptors is confirmed by the conserved distribution of Cys and Trp; 51 out of a total 55 Cys and 47 out of total of 62 Trp in PLAZ receptor are found at corresponding positions in mannose receptor. Thus, these two receptor sequences can be aligned co-linearly according to the conserved positions of Cys and Trp. This structural similarity between the PLAZ and mannose receptors thus identified allowed us to tentatively assign the domain organization of the PLAZ receptor by analogy with that of the mannose receptor (21). The mannose receptor is a unique member of C-type animal lectins; it has eight tandem carbohydrate-recognition domains (CRDs), whereas all the other C-type lectins identified so far carry a single CRD per polypeptide (21). The possible domain organization of the PLAZ receptor is schematically shown in Fig. 3 together with a hydropathy plot of this receptor. The hydropathy plot pointed out the presence of two major hydrophobic regions in the PLAZ receptor, which is consistent with the tentative assignment of the domain organization of the PLAZ receptor; these two regions correspond to a secretory signal sequence and a membrane-spanning region as shown in Fig. 3. In summary, the PLAZ receptor can be described to include 13 domains as follows: a signal sequence (20 amino acid residues); a Cys-rich head domain (146 amino acid residues); a fibronection type II repeat-like domain (54 amino acid residues); eight tandem CRD-like domains (about 145 amino acid residues per domain); a membrane-spanning domain (29 amino acid residues); and a cytoplasmic domain (42 amino acid residues). Among these domain designations, the terms "Cys-rich" and "fibronectin type II repeat-like" are only descriptions of their structural characteristics, and their functional implications are not known even in the case of the mannose receptor (21).

CRD was originally defined as a minimal functional unit responsible for carbohydrate binding in C-type lectins and known to contain 14 invariant residues (24). Although all of these invariant residues are not conserved in the CRD-like domains of the PLAZ receptor, some of them are located at the conserved positions. Most importantly, 4 Cys, which are known to form two disulfide bonds in the CRD, are completely conserved in CRD-like domains of the PLAZ receptor (4), implying the tertiary structural similarity between the CRD and CRD-like domain. In contrast, some invariant residues, such as those directly involved in Ca2+ binding in the CRD (25), are completely replaced in the CRD-like domain (4), suggesting functional diversity between these domains.

It is interesting to note that a canonical internalization signal sequence (NPXY (26); Fig. 2A) is present in the cytoplasmic domain as expected from an endocytotic behavior of the PLAZ receptor (4). This is also coincident with the case of the mannose receptor (21).

**Genomic DNA and RNA Blotting Analyses for the PLAZ Receptor**—Genomic DNA blotting analysis showed a single band in each lane of digested genomic DNAs with different restriction enzymes, suggesting that this receptor gene is a single copy (Fig. 5A). No related genes were detected by this genomic DNA blotting analysis under the hybridization conditions employed here. Although we should use the full-length cDNA probe to search for genes encoding proteins related to the PLAZ receptor, the use of a long cDNA probe gave multiple bands on the blot because of the presence of many long introns in the PLAZ receptor gene and consequently made it difficult to discriminate the signals of the related gene(s) from those of the PLAZ receptor gene since we did not know the complete genomic structure of this gene.

RNA blotting analysis revealed that multiple mRNA species different in size were detected on the blot of the poly(A)^+ RNAs isolated from MDBK cells (Fig. 5B): the two bands (4.8 and 2.9 kb) were stronger than the remaining three bands (6.5, 6.2, and 5.4 kb). Although the larger transcripts could be detected by any probes derived from the cDNA, the 2.9-kb mRNA was not visualized by the 3'-end cDNA probe on the RNA blot (Fig. 5B), suggesting that this short mRNA was devoid of a region corresponding to the 3'-portion of the PLAZ receptor cDNA. These observations supported the notion that the long transcripts were derived by alternative polyadenylation and suggested that the short transcript encoded a PLAZ receptor-related protein lacking, at least, the CRD-like domains 7 and 8, and the membrane-spanning domain. In fact, we obtained evidence of the occurrence of alternative polyadenylation during the cDNA cloning (pMD-10 and pMD-11), and these clones were likely copies of 5.4- and 5.4-kb mRNAs, respectively. Furthermore, it is interesting to note that the poly(A)^+ RNAs isolated from the bovine brain contained a significant amount of the PLAZ receptor transcripts larger than 4.8 kb but lacked the 2.9-kb transcript (Fig. 5B).
FIG. 2. Nucleotide sequence of the bovine PLA₂ receptor cDNA. The nucleotide sequence corresponding to a full-length bovine PLA₂ receptor cDNA reconstituted from pPL-1, pMD-10, and pMD-11 is displayed together with the deduced primary structure (panel A). The nucleotide and amino acid residues are numbered as described in Fig. 1. The position of the 114-bp insert found only in pMD-10 is marked by a filled triangle.
I, the membrane-spanning domain. Tentatively predicted on the basis of that of the mannose receptor already clarified terminus and near the COOH terminus of the receptor, indicating the consistency of the domain assignment at least for the signal sequence and vertical and the horizontal axes represent hydrophobicity index and residue number, respectively. Two hydrophobic stretches are found at the NH, S, schematic drawing. The hydropathy plot highlighted in polyadenylation sites, through which two distinct PLAz receptor transcripts are generated, are indicated by open a stippled infected with the vehicle vector did not show any specific PLA,-I PLA, receptor expression plasmid whereas tants. In order to examine the identity of the encoded protein CRD-like domains 1-8 so as to demonstrate the conservation of the distribution of Cys and Trp in these domains. Fourteen invariant residues by the cDNA with the native receptor protein, we tried to check the CRD consensus CRD-like domains #1 (201) #2 (337) #3 (484) #4 (626) #5 (779) #6 (921) #7 (1079) #8 (1215) #1 RKHLGSEAEVF WGLNQLDGEDAQW WSDKTPLNYLN WKPWLNPFFPEVE YHCGTFNAFMP WAKESRDCESTLPFYV CKK #2 VTLLIDGENSKT WGLSHSSH1PVSF E WSGNSVSTFTN WHTLEPHIFRNS QLCVSADQGE GVKKWVCEETLFPY CKK #3 TSLISSVVKTDYF WIALQDNQNYGET WTAAQQOLPLEPWYTH7NRTQPRYS GCQVVRGRSHP GRELVRDRCRSKAMSLQKK #4 NELLHSDKFRTTEQROWPGNKNPRCNAGSWEDGTPVSYSLDNSYGEA RKKVVRKN TLPPSYGSDKREW CKK #5 HSKRALSRYGWNV WGLREESASDEFR WROGPSVYIYQ WKRKERSMGNL ES QMCOPISSIT GLLASEKESLNPST CKK #6 TMNLIGHTNV WGLQGOODYEK WLNQPVYSSN WSDPDPRTN1HPNHVVEKRK PELCOSNLFPNFPTOWYKD EROCVFY CKK #7 TVILRRVCYAN WIGLTDENGSLFD WEGQTFKPPSF WKGQDSSFL GOCQYFADTS GRSSTGASKSYLQCQWOVK #8 LEEALPRESVQMI WNLAPQDGQNETIK WPQGTPPOQSN WIGRPEVYHFPP HLCVALRIPE GVWQSSLDQDKKGT CKK

Expressions of the Recombinant PLAz Receptor and Its Mutants—In order to examine the identity of the encoded protein by the cDNA with the native receptor protein, we tried to check the functional properties of the encoded protein. Transient expression experiments revealed that a substantial PLAz-I binding activity was detected in COS-7 cells transfected with the PLAz receptor expression plasmid whereas COS-7 cells transfected with the vehicle vector did not show any specific PLAz-I binding under the same assay conditions (Fig. 6). The quantitative analysis of the PLAz-I binding to COS-7 cells expressing the PLAz receptor revealed that the $K_d$ between the recombinant PLAz receptor and PLAz-I was 1.8 nM, indicating that the recombinant PLAz receptor had almost the same affinity to PLAz-I as the native receptor (Fig. 6). Therefore, we concluded that the cDNA isolated in this study actually encoded the functional PLAz receptor protein which is probably indistinguishable from the native one at least in terms of the PLAz-I binding.

We further intended to verify our tentative assignment of the domain organization of the PLAz receptor by transient expression experiments using various mutant PLAz receptor expression plasmids. The first issue to be examined was the discrimination of extracellular domains from intracellular and and the actual nucleotide sequence of the insert and its deduced amino acid sequence are shown in panel B. The amino acid sequences elucidated by the protein chemical analyses are underlined. Some characteristic amino acid sequence motifs are highlighted in the deduced primary structure: a stippled box, Asn in a possible N-linked glycosylation site; a wavy underline, a consensus endocytosis signal. As described in the text, two possible polyadenylation sites, through which two distinct PLAz receptor transcripts are generated, are indicated by open boxes.
membrane-spanning ones. In order to test whether our tentative assignment of the membrane-spanning and cytoplasmic domains is correct, we constructed two types of expression plasmids directing the synthesis of deletion mutant PLA2 receptors; the mutant-MI lost both the membrane-spanning region and the cytoplasmic domain shown in Fig. 3, and the mutant-I was devoid of only the cytoplasmic domain. According to our tentative assignment, the recombinant mutant-MI was expected to be secreted into culture medium whereas the mutant-I was not. Therefore, PLA2-I binding activities in both transfected cells and the culture media were assayed in these experiments. Fig. 7 summarizes the results of PLA2-I binding assays of these mutants, indicating that the recombinant mutant-MI was secreted without lowering the binding affinity to PLA2-I. In contrast to the case of the mutant-MI, the PLA2-I binding activity of the mutant-I was detected mainly on the transfected cells with a slight decrease in the binding affinity whereas a small but significant amount of the PLA2-I binding activity was also found in the culture medium (Fig. 7). These results were coincident with the tentative assignment of the domain organization of the PLA2 receptor. Furthermore, these results suggested that some leakage of the mutant-I from cell surface might occur during culture because of destabilization of the membrane anchoring caused by the complete loss of the cytoplasmic domain. Thus, the decline in the PLA2-I binding affinity of the mutant-I might be ascribed to an artifact caused by leakage of the receptor-PLA2-I complex from cell surface during the binding assay.

The second issue investigated was the determination of the region responsible for the PLA2-I binding in the extracellular domains. We constructed an expression plasmid for a deletion mutant PLA2 receptor (the mutant-CT) lacking most of the Cys-rich domain, the complete fibronectin type II repeat-like domain, and a part of the CRD-like domain 1, introduced this expression plasmid to COS-7 cells and then assayed the PLA2-I binding ability of the transfected cells. As also shown in

**FIG. 5. DNA and RNA blotting analyses of the PLA2 receptor.** Panel A, bovine genomic DNAs were digested with various restriction enzymes and analyzed by hybridization. DNA sizes were estimated from λ phage DNA digested with HindIII, and the positions of the size marker DNA fragments are indicated at the left side of the autoradiogram. The probe (the nucleotide region, -363 to 320) was derived from pGE-1 by digestion with HindIII and PstI. Panel B, poly(A)+ RNAs isolated from MDBK cells (lanes 1 and 2) and from the bovine whole brain (lane 3) were analyzed by hybridization. RNA size markers (Life Technologies, Inc.) were co-electrophoresed with these RNA samples, and the positions of the size markers are indicated at the left side of the autoradiogram. The probe used for visualization of the PLA2 receptor transcript in lanes 1 and 3 was the full-length cDNA, whereas the RNAs in lane 2 were hybridized with the probe derived from the 3'-end portion of the coding region in the cDNA (the nucleotide residues 3540–4520).

**FIG. 6. PLA2-I binding assay of COS-7 cells transfected with the PLA2 receptor expression plasmid.** Actual data of the PLA2-I binding assays are shown. A specific PLA2-I binding activity, which was obtained from the difference between the amounts of bound 125I-labeled PLA2-I in the presence (nonspecific binding, an open bar) and absence (total binding, a shaded bar) of a large molar excess of cold PLA2-I. The inset shows the Scatchard plot of the binding data obtained by varying the amount of the added labeled PLA2-I, which indicates that $K_d$ was about 1.8 nM.

**FIG. 7. Properties of mutant PLA2-I receptors transiently expressed by COS-7 cells.** The structures of the mutant PLA2 receptors examined in this study are schematically diagrammed using the same domain abbreviations as in Fig. 3. The dominant locations of these recombinant receptors produced by transfected COS-7 cells and the estimated $K_d$ values are also indicated.
Fig. 7, the deletion of these NH2-terminal domains did not affect either the binding affinity or subcellular localization of the receptor protein. Therefore, it could be concluded that a region carrying the CRD-like domains was responsible for PLAz-I binding.

The third issue examined was the identification of a domain responsible for the receptor internalization on PLAz-I binding. According to our tentative assignment, an endocytosis signal sequence is located in the cytoplasmic domain and thus the mutant-I was expected to be incapable of undergoing the internalization. The transient expression experiments revealed that the mutant-I receptor on COS-7 cells internalized only 0.8% of the cell surface-bound PLAz-I during 40 min at 37 °C whereas about 60% of the ligand was internalized by the wild-type receptor under the same conditions, indicating that the mutant-I carried virtually no endocytotic ability.

DISCUSSION

An interesting finding in this study is the structural relatedness of the PLAz receptor to the macrophage mannose receptor, which greatly helped us to predict the domain organization of the PLAz receptor from the primary structure since the structure and function of the mannose receptor have been well studied to date (27, 28). Since all results of the transient expression experiments for the mutant receptor proteins were consistent with known properties of the PLAz receptor and the tentatively assigned domain organization based on the structure of the mannose receptor, the domain organization schematically shown in Fig. 3 seemed to be reasonable. According to this assignment, the PLAz receptor is structurally grouped into class I transmembrane protein categorized by von Heijne (29); the PLAz receptor consists of a large extracellular NH2-terminal portion (about 95% of the molecule) and a small membrane-spanning/cytoplasmic COOH-terminal portion. The short cytoplasmic tail must be responsible for transducing an extracellular signal (i.e. the binding of PLAz-I) to some intracellular machinery. Although an obvious function of this cytoplasmic tail was to direct endocytosis of the ligand as demonstrated in this study, we could not find any other characteristic sequence motifs in this region.

The PLAz receptor specifically binds only a mature form of mammalian PLAz-I, which does not carry any carbohydrate chains, in a Ca2+-independent manner (30). Since these functional properties are distinctively different from those of the mannose receptor (21), the structural relatedness of these two receptors was quite surprising. Furthermore, the results of the expression experiments of the mutant PLAz receptor devoid of the NH2-terminal domains indicated that the region including the CRD-like domains was responsible for binding of the ligand, i.e. PLAz-I, implying that CRD has been functionally converted into the PLAz-I recognition domain during evolution. These observations raised the question as to how the PLAz receptor achieves highly specific ligand recognition with the structural motifs diverged from carbohydrate binding proteins. This question from an evolutionary point of view is very intriguing and will be discussed in detail elsewhere.

The molecular cloning of the PLAz receptor made it possible to examine its expression at the mRNA level. Using the isolated cDNA probe, we found that a single PLAz receptor gene generated multiple transcripts distinguishable on the RNA blot probably through alternative processing after transcription as in the cases of many other receptor genes such as insulin receptor gene (31). A particularly interesting issue to be mentioned was the presence of the 2.9-kb mRNA which is too short to encode the PLAz receptor isolated from the bovine corpora lutea. Since we could detect this small transcript also in the poly(A)+ RNAs isolated from the corpora lutea (data not shown), the bovine corpora lutea might contain a certain protein structurally related to the PLAz receptor although we could isolate only a single type of PLAz-I binding protein from the membrane fraction of this tissue. Interestingly, this short transcript was not observed in the brain whereas other longer transcripts of the PLAz receptor were present in the bovine brain. These observations raised the question as to the function of the PLAz receptor in the brain and the physiological implication of the 2.9-kb transcript. Although the 2.9-kb transcript was abundant than the 5.4- or 6.2-kb PLAz receptor transcript in MDBK cells, we unfortunately failed to isolate a cDNA corresponding to this short transcript in the MDBK plasmid cDNA library where nearly full-length PLAz receptor cDNA clones were obtained. There is the possibility that the protein encoded by this short transcript is functionally related to the PLAz receptor as in the cases of some cytokine receptor genes which generate distinctive mRNAs encoding a membrane-bound and a soluble receptor as a consequence of alternative splicing (32, 33). Although we did not enter into detail on the characterization of this short transcript in this study, the elucidation of the structure of the protein encoded by this 2.9-kb mRNA would give an insight into its function and the relationship to the PLAz receptor.

The isolation of the PLAz receptor cDNA provided a powerful tool for further investigation on the physiological function of this receptor on a molecular basis. For example, we can produce a soluble PLAz-I binding protein with the same binding affinity to PLAz-I as the native PLAz receptor by deletion of a COOH-terminal part of the molecule as shown in the case of the mutant-1 in this study. The soluble PLAz-I binding protein is more suitable for further analyses on the PLAz-I recognition mechanism than the native receptor due to its high solubility even in the absence of detergents and might be used as a competitive inhibitor of the PLAz-I binding to the receptor. Furthermore, any kinds of structural alterations can now be introduced into the PLAz receptor by DNA recombinant technology, which would greatly facilitate investigation of the structure-function relationship of the PLAz receptor. On the other hand, an artificial expression of the PLAz receptor followed by close examination of changes in cellular response against PLAz-I will open a way to study the signal transduction pathway through which the PLAz-I binding evokes the cellular responses on a molecular basis. These courses of studies will shed much light on the structure and function of the PLAz receptor in the future.

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