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MOLECULAR CLONING AND STRUCTURAL ANALYSIS OF THE RABBIT GASTRIN/CCK$_B$ RECEPTOR GENE

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SUMMARY: Gastrin and cholecystokinin exert a variety of physiological actions in the central nervous system and digestive tract that are mediated through one or more receptors exhibiting similar affinity for the two peptides. We isolated genomic clones encoding the rabbit gastrin/CCK$_B$ receptor by screening a rabbit EMBL phage library with a cDNA probe based on the nucleotide sequence of the human gastrin/CCK$_B$ receptor. The gene contained a putative 1356-bp open reading frame consisting of five exons interrupted by 4 introns and encoded a protein of 452 amino acids. The putative protein-coding region of the gene exhibits 93 to 97% amino acid similarity with corresponding cDNAs previously identified in human, canine and rodent brain or gastric tissues.

Gastrin and cholecystokinin (CCK), two members of a family of peptide hormones characterized by an identical carboxyl-terminal pentapeptide sequence, are widely distributed throughout the central nervous system and the digestive tract [1]. The physiological actions of gastrin include stimulation of gastric acid secretion [2], modulation of smooth muscle contraction [3], and regulation of mucosal cell growth and differentiation [4]. CCK mimics the actions of gastrin in the stomach and exerts other physiological effects in the central nervous system and digestive tract [1].

On the basis of both ligand-binding and functional studies, the target receptors for the gastrin/CCK family can be distinguished into two main classes, CCK$_A$ and CCK$_B$ [5]. The CCK$_A$ receptors possess much higher affinity for CCK than for gastrin, are mainly localized to the digestive tract and certain areas of the brain, and are inhibited by the selective antagonist L-364,718 [5, 6]. Recently, cDNAs encoding the CCK$_A$ receptor have been cloned from rat pancreas [7] as well as human [8, 9] and guinea pig gallbladder [10]. CCK$_B$ receptors display similar affinity for both gastrin and CCK, are abundantly expressed throughout the central nervous system, and are

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inhibited by the selective antagonists L-365,260 and PD 134,308 [5, 11, 12]. However, these antagonists are unable to discriminate between CCK_B and gastrin receptors located on gastric parietal cells [5]. The cDNAs encoding the gastrin/CCK_B receptor have been cloned from human [13-15], canine [16], rat [17], and *Mastomys* [18] cDNA libraries. In addition, we have cloned the human gastrin/CCK_B receptor gene and determined that it is alternatively spliced to yield two different receptor isoforms [19].

The localization and the relative importance of gastrin receptors mediating the acid secretory response in different species is a subject of considerable debate. Canine parietal cells can be stimulated directly by gastrin [20] via receptors that induce membrane inositol phospholipid turnover and increases in intracellular Ca^{2+} [21, 22]. The effects of gastrin on rabbit parietal cells were more difficult to demonstrate, thus the stimulation of acid secretion by gastrin in this species initially was proposed to be indirect in nature [23]. However, subsequent studies demonstrated that gastrin is able to stimulate rabbit parietal cells directly through the activation of CCK_B-like receptors [22, 24, 25]. It is noteworthy that specific CCK_B receptor antagonists can inhibit the effects of gastrin with high potency in rabbit parietal cells [25], whereas L-365,260 is a weak antagonist of both gastrin action and binding to canine parietal cells [26]. This latter phenomenon has been attributed to a single amino acid in the structure of the canine receptor [27]. In view of the apparent differences in both expression and function of parietal cell gastrin/CCK_B receptors we undertook the present study to clone and analyze the structure of the rabbit gastrin/CCK_B receptor gene.

METHODS

MATERIALS. A λEMBL-3 rabbit genomic library was purchased from Clontech Laboratories, Inc. (Palo Alto, CA). The human gastrin/CCK_B receptor cDNA was generated by polymerase chain reaction (PCR) as previously reported [19], and used as a probe in screening for the rabbit gastrin/CCK_B receptor gene.

LIBRARY SCREENING. The rabbit genomic library was plated and the DNA in phage plaques was transferred to nitrocellulose filters. After baking for 2 h at 80°C, filters were prehybridized for 3 h at 55°C in 0.1 M HEPES (pH 7.5), 5x saline-sodium citrate (SSC), 5x Denhardt's solution and 100 μg/ml salmon sperm DNA [28]. The probe was labeled by random priming with [^{32}P]-dCTP [29]. Hybridization was performed for 12-16 h at 55°C in the same solution as used for prehybridization. Filters were washed for 60 min at 55°C in 2x SSC, for 60 min at 60°C in 1x SSC, and for 20 min at 65°C in 0.2x SSC. Positive plaques were detected by autoradiography using Kodak XAR-5 films with intensifying screens. The DNA from positive phage plaques was isolated and digested with *Bam* HI or *Sst* I.

DNA SEQUENCING. Restriction fragments from positive clones were subcloned into the M13mp18 sequencing vector and then sequenced in both directions by the dideoxynucleotide method [30]. Oligonucleotides used as sequencing primers were synthesized with an Applied Biosystems 380B oligonucleotide synthesizer. Computer analyses of nucleotide sequences were performed using the Genetics Computer Group Program (University of Wisconsin Biotechnology Center, Madison, WI).

RESULTS AND DISCUSSION

The random-primed [^{32}P]-labeled human gastrin receptor cDNA probe gave positive hybridization signals with two clones after screening $3x10^5$ plaques from the rabbit genomic

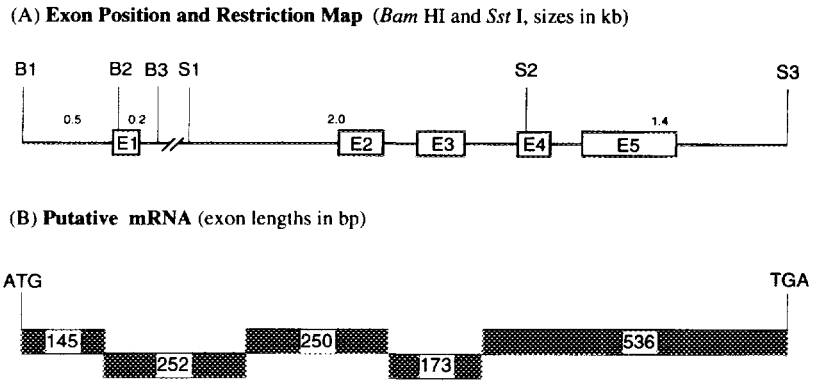

FIGURE 1. Structure of the rabbit gastrin/CCK_B receptor gene. (A) *Bam* HI (B1-B3) and *Sst* I (S1-S3) restriction sites are shown with the sizes of fragments subcloned into M13 sequencing vector. E1 to E5 indicate exon position; the fragment size in intron 1 between the B3 and S1 sites was not determined. (B) Lengths of exons are depicted by solid boxes and the locations of the initiation ATG and the termination TGA codons are shown.

library. The two positive clones (R5 and R22) were digested with *Bam* HI or *Sst* I and nucleotide sequences were determined from resulting restriction fragments of 0.2 to 2.0 kb in length (Fig. 1A). One clone (R5) lacked nucleotide sequences of the 5' flanking region including exon 1. The available sequence of the rabbit receptor gene exceeded 4 kb in length and contained a putative 1356-bp open reading frame which was interrupted by four introns (Fig. 1). The putative protein-coding region of the gene is encompassed by five exons, ranging from 145 to 536 bp in length (Fig. 1B). The four introns of the gene range in size from 176 bp to more than 900 bp (the fragment size of intron 1 in the restriction sites between *Bam* HI and *Sst* I was not determined). Examination of the exon-intron splice junctions of the gene revealed that the splice donor and acceptor sites conform to standard convention, beginning with the nucleotides GT and terminating with AG [31] (Fig. 2). The 5' region immediately upstream from the ATG initiation codon to nucleotide -308 manifests a high G+C content (77%). A polyadenylation site (AATAAA) occurs 508 bp downstream from the stop (TGA) codon (Fig. 2). The deduced amino acid sequence of the open reading frame delineates the rabbit gastrin/CCK_B receptor as a member of the G protein-coupled heptahelical receptor superfamily. Exon 1 encodes the putative extracellular amino terminus of the receptor, exons 2 and 3 encode transmembrane regions I-IV, exon 4 encodes the fifth transmembrane region and an initial portion of the third intracellular loop, and exon 5 encodes the remainder of this intracellular loop, transmembrane regions VI and VII, and the intracellular carboxyl terminus (Fig. 3).

The rabbit gastrin/CCK_B receptor exhibits a high degree of amino acid similarity with human, canine, rat and *Mastomys* gastrin/CCK_B receptors [13-18]. When the comparison is restricted only to the portion encoding the putative third intracellular loop, which is an important site for effector coupling and regulatory phosphorylation in most G protein-coupled receptors, the rabbit receptor has greater similarity to the human receptor than to the canine receptor (Table. I). Some

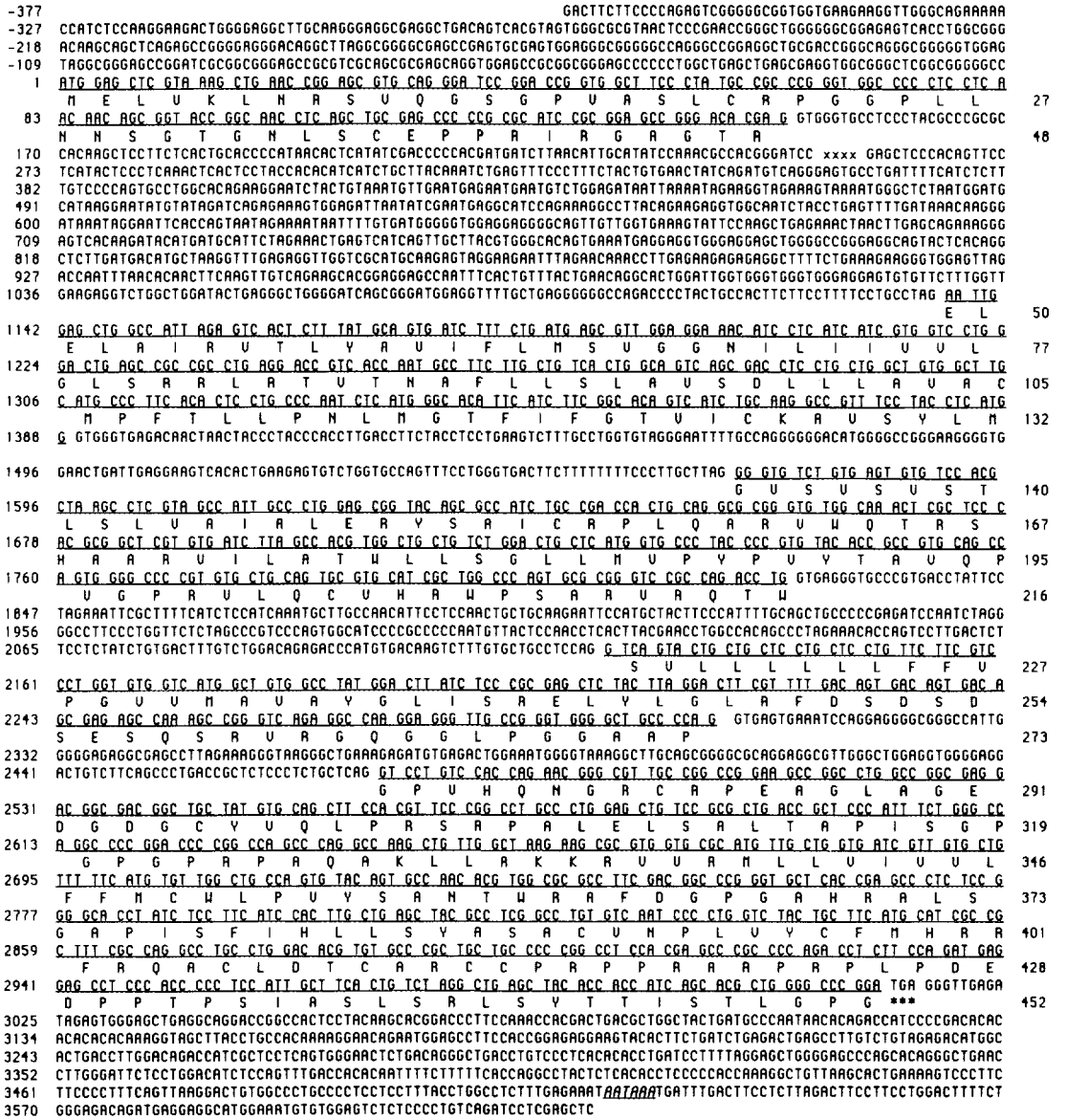

FIGURE 2. Nucleotide and deduced amino acid sequence of the rabbit gastrin/CCK$_B$ receptor gene. The five exons are underlined. The nucleotide sequence is numbered relative to the first nucleotide of the translation initiation codon (on the left); amino acids are numbered on the right and are depicted in the single-letter symbols below each triplet codon. The fragment size in intron 1 at bp 253-256 was not determined. The putative stop codon (TGA) at bp 3013-3015 is underscored by asterisks, and a polyadenylation consensus motif (AATAAA) is underlined at bp 3524-3529.

notable differences between the rabbit and other species are present. In particular, the rabbit gastrin/CCK$_B$ receptor lacks two amino acids (Gly-Ser or Gly-Pro) in the extracellular amino terminus, but it has the longest third intracellular loop, the other species lacking at least two amino acid residues at different positions (Fig. 3). As we have reported previously, the human

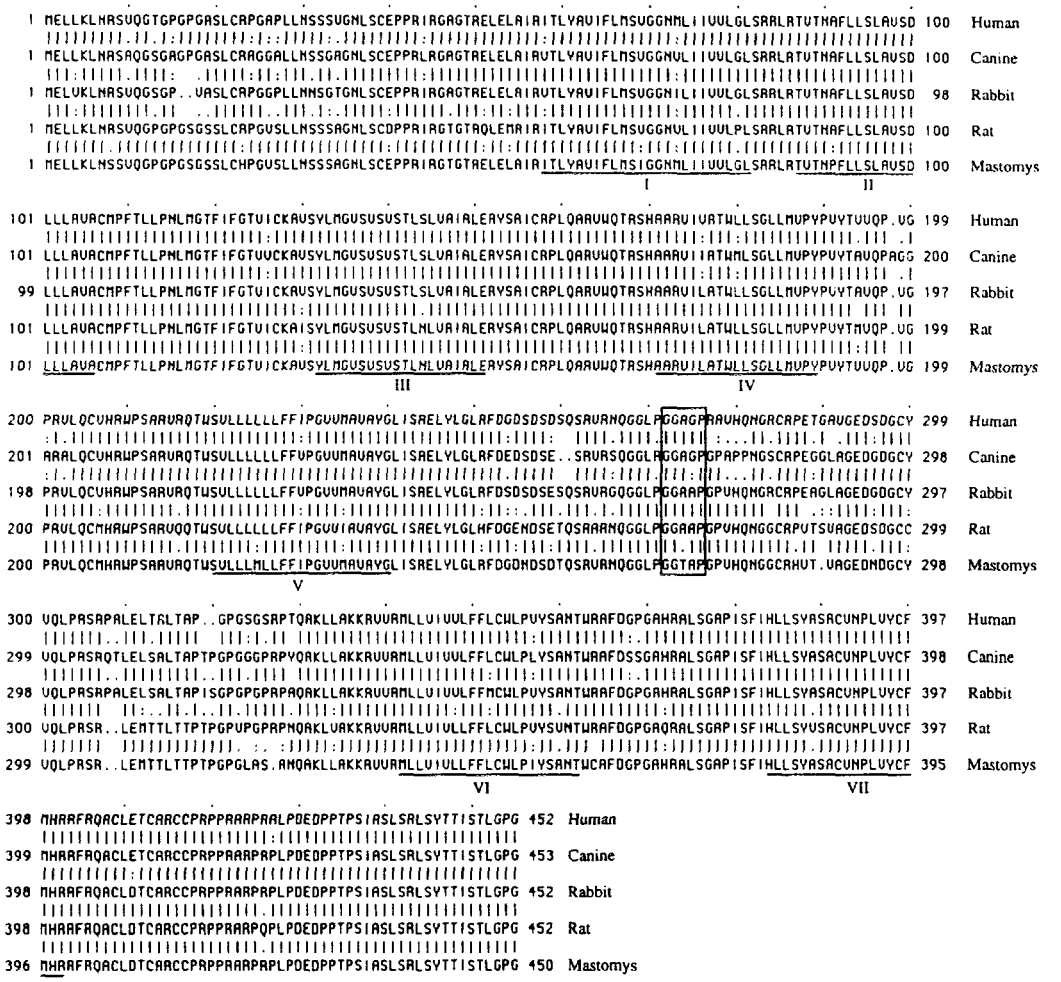

FIGURE 3. Alignment of the deduced amino acid sequences for gastrin/CCK$_B$ receptors of various species. Putative membrane-spanning regions I-VII are underlined. A region of exon 4 of this gene in human appears to be alternatively spliced to yield two different mRNAs, one lacking and the other containing the boxed pentapeptide cassette (GGAGP) at position 271-275 in the third intracellular loop [19].

gastrin/CCK$_B$ receptor gene is alternatively spliced in exon 4 [19] to produce two mRNA variants: the long isoform, containing a block of 15 bp in exon 4 that encodes the pentapeptide cassette (GGAGP), and the short isoform, lacking this sequence [19]. This pentapeptide cassette (GGAAP) is also present in the putative cDNA of the rabbit gastrin/CCK$_B$ receptor as shown in the boxed area of Fig. 3. It is also noteworthy that both rabbit and human gastrin/CCK$_B$ receptors share complete amino acid identity in their sixth transmembrane domain wherein a single amino acid variation in the canine gastrin/CCK$_B$ receptor alters affinity for the non-peptide antagonists L-364718 and L-365260. This observation may account for the pharmacological profile exhibited by gastrin receptors of isolated rabbit parietal cells in functional experiments [25].

TABLE I. The deduced amino acid sequence similarity of the gastrin/ CCK_B receptor between species

	% Similarity in Coding Region (3rd intracellular loop)			
	Canine	Rabbit	Rat	Mastomys
Human*	95.1 (84.5)	96.9 (90.9)	94.0 (85.6)	94.2 (83.3)
Canine		95.8 (87.8)	92.9 (84.5)	93.1 (82.1)
Rabbit			93.6 (85.9)	93.5 (82.5)
Rat				96.9 (92.8)

*The long form is compared with the other species.

Our studies indicate that the rabbit gastrin/CCK_B receptor differs in subtle but definable fashion from the canine gastrin/CCKB receptor. It is possible that these variations might represent a structural basis for the differences reported in the physiology and pharmacology of the gastrin/CCK_B receptors from the two species [26]. Successful cloning and expression of the rabbit gastrin/CCK_B receptor cDNA should permit more definitive studies to examine further this question.

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