《Original Article》

Distribution of CCK receptors in guinea-pig gastrointestinal tract

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[Abstract]

Cholecystokinin (CCK) is a regulatory peptide in the gastrointestinal tract. The cell/tissue distribution of CCK receptors is variable among species. Guinea-pig is a useful animal model to study the physiology of gastrointestinal tract. However, its structure and distribution are not known. In the present study, we have cloned guinea-pig CCK-BR and examined the distribution of CCK receptors in guinea-pig gastrointestinal tract and pancreas. A large amount of CCK-AR mRNA was detected in pancreatic acinar cells, gallbladder, and stomach mucosal layer. CCK-BR was expressed in pancreatic acinar and duct cells and mucosal and muscle layers of duodenum and stomach. However, only a truncated isoform of CCK-BR with a deletion of exon 1 (Δexon 1) was detected in pancreatic duct cells.

Key words; CCK-1 receptor, CCK-2 receptor, gastrin, truncated isoform

[Introduction]

Cholecystokinin (CCK) belongs to a family of gastrointestinal peptides that regulate various functions in the gastrointestinal tract including stimulation of gastric acid production, induction of gastric smooth muscle contraction, and promotion of growth and differentiation of the stomach mucosa[1]. CCK also mediates enzyme secretion from pancreatic acinar cells and functions as a neurotransmitter in the central nervous system[2, 3].

Receptors for CCK have been pharmacologically classified into two subtypes, CCK-1 receptor (CCK-A receptor; CCK-AR) and CCK-2 receptor (CCK-B receptor; CCK-BR)[4]. CCK receptors belong to the superfamily of G protein-coupled receptors which are characterized by seven transmembrane domains connected by intracellular and extracellular loops with an extracellular N-terminal and intracellular C-terminal.

CCK-AR has been identified in various tissues such as gallbladder, sphincter of oddi, and pancreatic acinar cells[5, 6, 7, 8]. CCK-BR is typically found in gastric parietal cells, gastrointestinal smooth muscle cells and in the central nervous system[2]. However, the cell/tissue distribution of CCK-AR and CCK-BR is variable among species. For example, human pancreatic acinar cells lack CCK-AR and only express CCK-BR, while mouse and rat acinar cells possess CCK-AR. CCK-AR and CCK-BR have several isoforms. Among them N-terminally truncated isoform shows low affinity with CCK[9, 10].

Guinea-pig is a useful animal model to study the physiology of gastrointestinal tract because of some similarities to humans. Guinea-pig has gallbladder and its pancreatic juice contains high concentrations

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of HCO_3^-. Guinea-pig CCK-AR cDNA was cloned[14] and the mRNA was expressed in gallbladder, pancreas, stomach[2]. However, guinea-pig CCK-BR has not been cloned and the distribution of CCK-BR in gastrointestinal tract are not known. In the present study, we have cloned guinea-pig CCK-BR and examined the distribution of CCK receptors including the truncated isoform of CCK-BR in guinea-pig gastrointestinal tract and pancreas.

[Material and Methods]

Animals and tissue isolation

Female guinea pigs were obtained from Japan SLC (Hamamatsu, Japan). All protocols were approved by the Animal Use Committees of Nagoya University and the National Institute for Physiological Sciences. Guinea-pigs were killed by cervical dislocation and the pancreas, duodenum, stomach, and gallbladder were removed. The pancreatic acini and ducts were isolated as described previously[15, 16]. The duodenum and stomach were separated to mucosal and muscle layers as described previously[17].

Total RNA extraction

Total RNA was extracted using RNeasy Protect Mini Kit (QIAGEN GmbH, Germany). Reverse transcription was performed using an oligo-dT primer and TaqMan Reverse Transcription Reagents (Roch, Branchburg NJ, U.S.A.).

Direct sequencing

Polymerase chain reactions (PCR) were carried out using the Ex Taq (TaKaRa, Japan). The PCR protocol was: 94°C, 1 min; 60°C, 1 min; 72°C, 2 min; 30 cycles for first PCR and 94°C, 30 s; 60°C, 60 s; 72°C, 30 s; 25 cycles for second PCR using GeneAmp PCR System 9700 (Applied Biosystems, Foster, CA).

The oligonucleotide primers used for first PCR were; sense 5'-GGGCGGAAACTAGGAGGGGC-3' in 5' untranslated reasion (AF264177), antisense 5'-TGTGCTGATGGTGGTATAGCT-3' in exon5 (NM007627); for second PCR were; sense 5'-ATGTGGTGGGCGGGAGCCTGAGC-3' in 5' untranslated reasion (AF264177), antisense 5'-GATTGGGCAGGAGGGTGAA-3' in exon 2 (NM176875), sense 5'- CGCAGTGATCTTCCTGATGAGCG-3' in exon 2 (NM176875), antisense 5'-TGCAGTGGTCGGCAGATGGCG-3' in exon 3 (NM176875), sense 5'-GTGTCTGTGAGCGTGTCCACG-3' in exon 3 (this paper), antisense 5'-CTGCTCTGGCTCTCACTGTCG-3' in exon 4 (this paper), sense 5'-TATGGACTCATCTCCCGCGAA-3' in exon 4 (NM007627), antisense 5'-TGTGCTGATGGTGGTATAGCT-3' in exon5 NM013165). The primer selection was based on the region found in each GenBank accession no. that has high homology in human, mouse, and rat sequences.

PCR products were purified using the High Pure PCR Product Purification Kit (Roche Diagnostics GmbH Mannheim, Germany) and sequenced directly using automated sequencer (BECKMAN COULTER, Inc Fullerton, CA) after terminator reaction using DNA Sequencing Kit CEQ™ DTCS-Quick Start Kit (BECKMAN COULTER, Inc Fullerton, CA).

RT-PCR

Polymerase chain reactions (PCR) were carried out in a 50 μL reaction mixture containing 50 ng cDNA, 5 μL of 10×Ex Taq buffer, 4 μL of 2.5 mM dNTP mix, 50 pmol of each primer and 1.5 U Ex Taq DNA polymerase (TaKaRa, Shiga, Otsu Japan). The conditions were; denaturation for 3 min at 94°C, then 35 cycles of denaturation for 30 sec at 94°C, annealing for 30 sec at 62°C, extension for 30 sec at 72°C, followed by 7 min at 72°C using GeneAmp PCR System 9700 (Applied Biosystems, Foster CA). The oligonucleotide primers used for CCK-AR were; sense 5'- ATGGACGTGGTAGACAGCCTT-3' in exon 1, antisense 5'-ACATCGTTTGGCAGTAGGAAG -3' in exon 3 (S68242, 576 bp), for CCK-BR were; sense 5'- CAGGCAACGTCAGCTGCGAAA-3' in exon 1 (this paper), antisense 5'-GATTGGGCAGGAG-

GGTGAA-3' in exon 2 (NM176875, 246 bp). sense 5'- TTCACCCTCCTGCCCAATC -3' in exon 2, antisense 5'-AGTGGCTAAAATCACGCGAGC-3' in exon 3 (this paper, 207 bp). The primers for GAPDH were; sense 5'- ACCACAGTCCATGCCATCAC-3', antisense 5'-TCCACCACCCTGTTGCTGTA-3'.

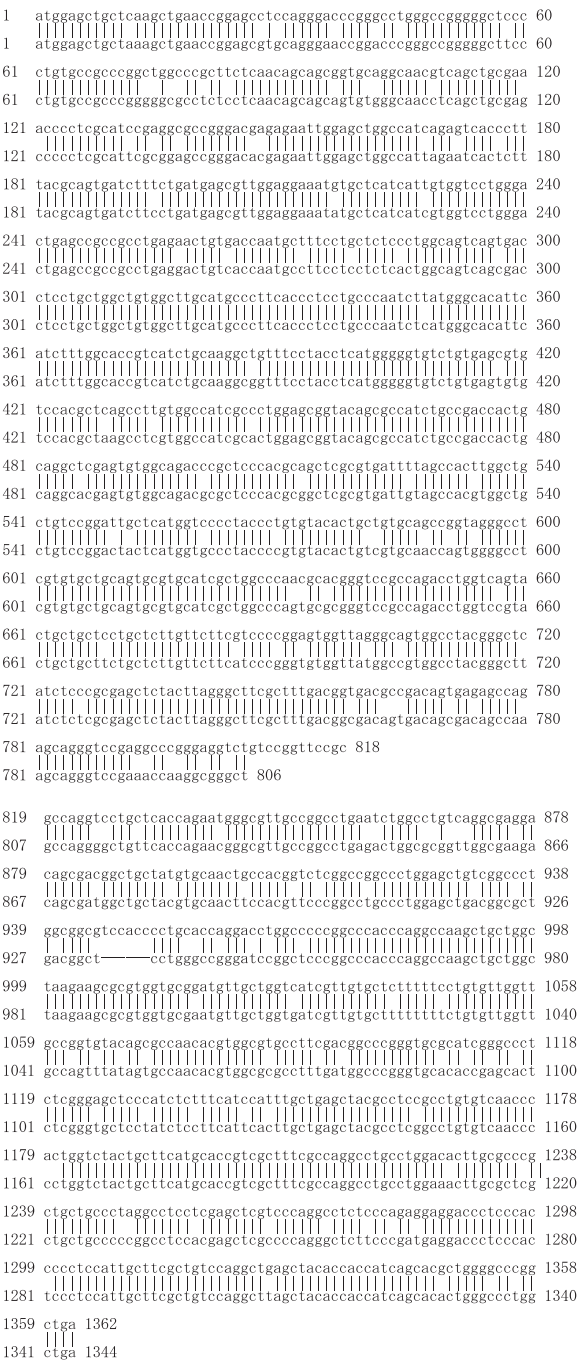

Fig. 1 Alignment of nucleotide sequences for guinea pig CCK-BR (upper) and human CCK-BR (lower).

[Result]

Alignment of guinea pig CCK-B receptor and human CCK-B receptor

Alignment of guinea pig CCK-B receptor and human CCK-B receptor are shown in Fig. 1 for nucleotide sequences and in Fig. 2 for deduced protein sequences. They have 88% and 91% homology respectively. The homology to mouse and rat CCK-BR is 83% for nucleotide sequence and 87% for deduced protein sequence.

Expression of CCK-AR and CCK-BR mRNA in guinea-pig gastrointestinal tract and pancreas

RT-PCR analysis of the tissue distribution of CCK-AR and CCK-BR is shown in Fig. 3. The fragments amplified with primers for exon 1 (sense) and exon 2 (antisense) of CCK-BR indicate mRNA expression of a complete isoform (wild type) of CCK-BR. The fragments amplified with primers for exon 2 (sense) and exon 3 (antisense) of CCK-BR indicate mRNA expression of both complete isoform (wild type) and truncated isoform (Δexon 1) of CCK-BR. A large amount of CCK-AR mRNA was detected in pancreatic acinar cells, gallbladder, and stomach mucosal layer while its amounts in pancreatic duct cells, duodenum mucosal and muscle layers, and stomach muscle layer were small. CCK-BR was

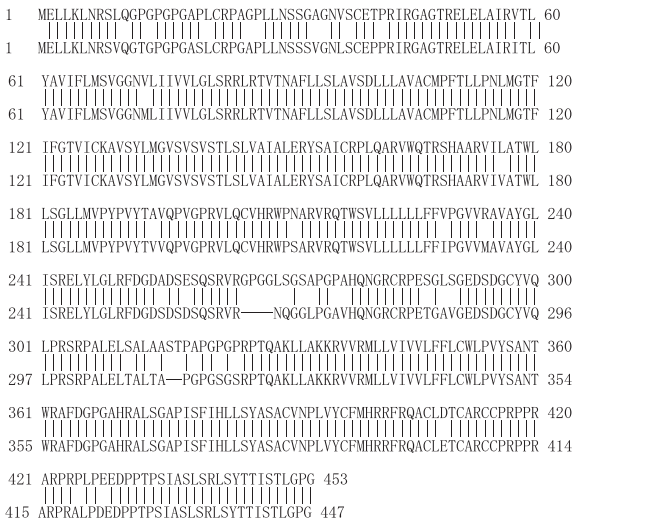

Fig. 2 Alignment of deduced protein sequences for guinea pig CCK-BR (upper) and human CCK-BR (lower).

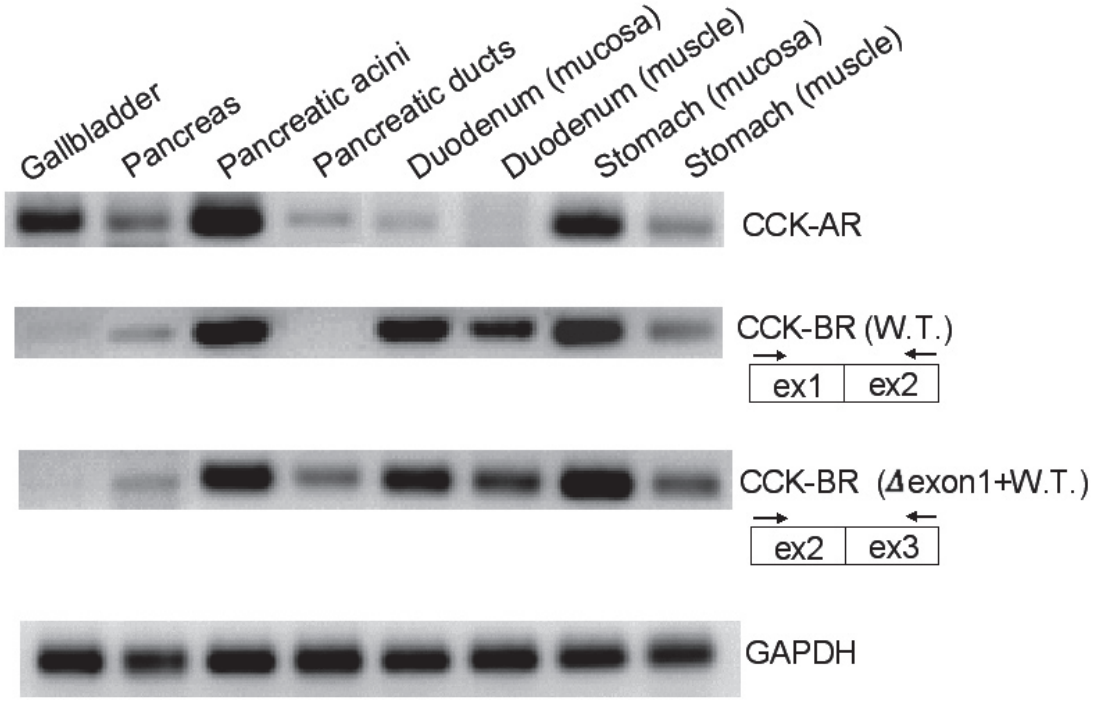

Fig. 3 Distribution of CCK-AR and CCK-BR mRNA in guinea-pig gastrointestinal tract and pancreas.

expressed in pancreatic acinar and duct cells and mucosal and muscle layers of duodenum and stomach. However, only a truncated isoform of CCK-BR with a deletion of exon 1 (Δexon 1) was detected in pancreatic duct cells [Fig. 3].

[Discussion]

In the present study we have successfully obtained cDNA sequence of guinea-pig CCK-BR. The deduced amino acid sequence of CCK-B receptor is 50% identical to the CCK-A receptor. Wank et al. reported that CCK-A receptors have 1000 fold higher affinity for CCK than for gastrin. CCK-B receptor have similar affinity for CCK and gastrin[18].

In guinea-pig the expression of CCK-A receptors were detected in pancreatic acini, but not in pancreatic ducts. Mice pancreatic ducts express CCK-A receptors but not secretin receptors[19]. Only a truncated isoform of CCK-BR with a deletion of exon1 was detected in guinea-pig pancreatic ducts. The exon1 codes initial 50 amino acids which is the N-terminal extracellular domain of CCK-BR. The N-terminally truncated form of the human CCK-BR shows about 10 fold lower affinity for CCK-8 and 100 fold lower affinity for gastrin than the complete form of CCK-BR[9]. The CCK-AR which lacked the first 42 amino acids did not directly bind CCK-9[10]. Truncated isoforms of CCK-BR are identified in the tumor cells[11] and so their function may be related to proliferation and growth of the cells.

Rat duodenum mucosa expresses a small amount of CCK-AR and does not express CCK-B receptor[12]. Human duodenum mucosa and muscle layer express CCK-AR and does not express CCK-BR[13]. The present study demonstrated that guinea-pig duodenum predominantly expresses CCK-BR while stomach mucosa expresses both CCK-AR and CCK-BR. CCK- BR in the duodenum may play an important role in the intestinal and neural phase of the regulation of pancreatic exocrine secretion.

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和文抄録

モルモット消化管における CCK 受容体の分布

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［要旨］

CCK（Cholecystokinin）は消化管ホルモンの一つで、その受容体の分布は動物種によって異なる。モルモットは消化管研究における実験モデルとして有用だが、CCK 受容体の構造や分布については明らかではない。

本研究において、我々は CCK-B 受容体の cDNA をクローニングすることに成功した。CCK-A 受容体と共にそれらの消化管における分布を mRNA レベルで調べた。その結果、CCK-A 受容体は膵腺房細胞、胆嚢、胃粘膜層に多く発現しており、CCK-B 受容体は膵腺房細胞、膵導管細胞、十二指腸および胃の粘膜層と筋層に発現していた。しかし、膵導管細胞における CCK-B 受容体はエクソン 1 が欠損したアイソフォームであった。

キーワード；CCK-1レセプター，CCK-2 レセプター，ガストリン，選択的スプライシング

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