«Research Note»

Structure and Tissue Distribution of Cholecystokinin-1 Receptor in Chicken

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Chicken cholecystokinin-1 receptor (chCCK1R) cDNA was isolated from a chicken brain and tissue distribution of the chCCK1R mRNA was determined by RT-PCR. The chCCK1R is composed of 429 amino acid residues and showed approximately 73% and 50% identity with those of mammalian CCK1R and CCK-2 receptor (CCK2R) at the amino acid level, respectively. In phylogenetical analysis, the chCCK1R belonged to a cluster of CCK1R and CCK-CHR, another CCK receptor cloned from chicken, was placed into a cluster of CCK2R. By RT-PCR analysis, whilst both chCCK1R and CCK-CHR mRNAs were widely expressed in the chicken tissues, the expression pattern for each receptor was slightly different. Abundant chCCK1R mRNA was observed in multiple tissues, while CCK-CHR mRNA expression was dominantly observed in the brain and proventriculus in the chicken. Taken together, chCCK1R is a potent peripheral CCKR and CCK-CHR cloned and characterized previously is classified as CCK2R in the chicken. Furthermore, mRNA expression for chicken CCKRs is possible to be regulated by tissue specific manner and that may be associated with the diverse roles of CCK and gastrin in the chicken.

Key words : cDNA cloning, chicken, cholecystokinin, receptor

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Introduction

Cholecystokinin (CCK) and gastrin share the same carboxyl-terminal tetrapeptide sequence that constitutes the minimal structure necessary for biological activity for both peptides (Johnsen, 1998). In mammals, the peptides modulate gallbladder contraction, exocrine pancreatic secretion and gastric acid secretion in the gastrointestinal tract, and also regulate feeding behavior and emotional responses in the central nervous system (Wank, 1995 ; Noble and Roques 1999). The roles of these two hormones have been extensively studied in birds as well as in mammals. For instance, CCK and gastrin were physiological regulators of feeding (Savory and Gentle, 1980 ; Furuse *et al.*, 1999). CCK modulated gastroduodenal and intestinal motility in chickens

(Rodríguez-Sinovas *et al.*, 1994 ; Martin *et al.*, 1995 ; Martinez *et al.*, 1995). In addition, bioactive forms of CCK and gastrin as well as cDNAs for prepro-CCK and prepro-gastrin were also identified in birds (Dimaline *et al.*, 1986 ; Bjornskov *et al.*, 1992 ; Wu *et al.*, 1995 ; Jonson *et al.*, 2000).

The physiological functions of CCK and gastrin are mediated by the specific receptor belonging to a member of the G protein-coupled receptor superfamily. By pharmacological characterization, two subtype receptors for CCK and gastrin were revealed. The CCK-1 receptor (CCK1R) was highly selective for sulfated CCK analogue, whereas CCK-2 receptor (CCK2R) is a non-selective receptor for CCK and gastrin (Jensen *et al.*, 1989). In chickens, the existence of two receptor subtypes for CCK was also suggested by a binding study for CCK and its

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analogues (Rodríguez-Sinovas *et al.*, 1995). Furthermore, Nilsson and his colleagues recently cloned cDNA encoding the CCK receptor from the chicken brain and termed it CCK-CHR (Nilsson *et al.*, 2003). The structure of CCK-CHR was similar to that of mammalian CCK2R and the binding property with CCK/gastrin agonists also resembled that of mammalian CCK2R, although it showed higher affinity for CCK1R antagonist than CCK2R antagonist. Thus CCK-CHR is likely to be a counter part of the central CCK receptor, CCK2R, but pharmacological profiling did not establish that the CCK-CHR is functionally identical to that of mammalian CCK2R.

On the other hand, cDNA for CCK1R has not been reported in birds. Therefore, this study aimed to clone the cDNA encoding CCK1R from chickens to extensively understand the avian CCK/gastrin system, and we compared the mRNA expression profile of the chicken CCKRs to characterize the target tissues for CCK and gastrin in chickens.

Materials and Methods

Animals

One-day-old White Leghorn male chicks were obtained from a local hatchery (Niinobe Farm, Kagawa, Japan) and kept in an electrically heated battery cage with free access to commercial feed and water. At four weeks of age, the birds ($n=3$) were killed by cervical dislocation and tissues were immediately removed and frozen in liquid nitrogen before RNA extraction. The brain tissues collected were the cerebrum, cerebellum, hypothalamus and anterior pituitary gland. Peripheral tissue fragments were collected from the proventriculus, gizzard, duo-

denum, small intestine, cecum, liver, pancreas, gallbladder, kidney, adrenal gland and testis. Animal procedures were performed according to the guideline of the Experimental Animal Committee of Kagawa University.

Primers for Polymerase Chain Reaction (PCR)

For PCR analysis, oligonucleotide primers were synthesized by Sigma Genosys Japan (Tokyo, Japan). Sequences of the primers used in this study are listed in Table 1. Primers termed CCKR-1 and CCKR-2 were designed from the conserved nucleotide sequence among mammalian CCK1R (human ; accession No. L13605, rat ; accession No. D50608 and mouse ; accession No. NM009827). Other primers in Table 1 were synthesized as specific primers to chicken CCK1R cDNA or CCK-CHR cDNA.

Reverse Transcription (RT)-PCR Based Cloning of Chicken CCK-1 Receptor (*chCCK1R*)

Total RNA was extracted from a chicken brain using a commercial kit (TRIZOL, Invitrogen, Tokyo, Japan) and followed by poly (A)⁺ RNA isolation using Oligotex-dT30 (TaKaRa, Tokyo, Japan). One microgram of poly (A)⁺ RNA isolated from the chicken brain was subjected to cDNA library construction using a Marathon cDNA amplification kit (Clontech Laboratories, Palo Alto, CA, USA). A cDNA fragment for *chCCK1R* was amplified by 30 cycles of PCR with CCKR-1 (forward) and CCKR-2 (reverse) using Marathon cDNA library as the template. The PCR parameters to amplify the cDNA fragment of *chCCK1R* were as follows : denaturation for 30 sec at 95°C, annealing for 30 sec at 58°C and extension for 1 min at 72°C. At the final PCR cycle, extension at 72°C was extended to 5 min

Table 1. List of oligonucleotide primers used for PCR

Name	Derection	Sequence (5'-3')
CCKR-1	forward	GTCTCTGGCCCTCAGTGACCTCATG
CCKR-2	reverse	GAGCATGCGGATCACGCGTTCTTGCC
CCK1R-1	reverse	GCTGTTGCTCATAGTAGATAGCTG
CCK1R-2	reverse	GATGGCAACAAGGTTGAATGTAGA
CCK1R-3	forward	CACTTACTTCATGGGTATCTCTGTG
CCK1R-4	forward	GGAAGTACCAGCATCGCCAAATAC
CCK2R-1	forward	AGAGGCCGTCTGCAAGTTGAT
CCK2R-2	reverse	GGTGGGCAACGGTTCTGTGCT
S17-1	forward	TGATCATCGAGAAGTACTAC
S17-2	reverse	TGATCTCTGATCGAGAGCA

to terminate PCR reaction. The 5'- and 3'-ends of the chCCK1R cDNA were amplified by the rapid amplification of cDNA ends (RACE) with the Marathon cDNA library using CCK1R-1 (initial primer ; reverse) and CCK1R-2 (nested primer ; reverse) for 5'-RACE and CCK1R-3 (initial primer ; forward) and CCK1R-4 (nested primer ; forward) for 3'-RACE under the manufacturer's instructions. All cDNA fragments were subcloned into pGEM-T easy vector (Promega, Madison, WI, USA) and sequenced by Applied Biosystems 373A automated sequencer or ABI PRISM 310 automated sequencer.

RT-PCR Analysis for the Tissue Distribution of chCCK1R and CCK-CHR

Total RNA extracted from tissues of 4-week-old male chicks was subjected to the RT-PCR analysis to determine tissue expression of chCCK1R and CCK-CHR. Each one microgram of total RNA was reverse-transcribed with oligo-dT₁₇ primer and ReverTra Ace (TOYOBO, Osaka, Japan). Each cDNA fragment for chCCK1R and CCK-CHR was amplified using GoTaq DNA polymerase (Promega, Tokyo, Japan) with specific primers for chCCK1R (CCK1R-1 and CCK1R-3) and CCK-CHR (CCK2R-1 ; forward and CCK2R-2 ; reverse), respectively by 35 cycles of PCR consisting of denaturation for 30 sec at 95°C, annealing for 30 sec at 60°C and extension for 30 sec at 72°C. Ribosomal protein S17 cDNA was amplified using a pair of primers (S17-1 ; forward and S17-2 ; reverse, Trüeb *et al.*, 1988) as the internal control with the same condition described above except the number of cycles was reduced to 25 cycles. The PCR product was separated by electrophoresis on a 1% agarose gel and followed by staining with ethidium bromide to visualize the amplified bands.

Results

Structure of chCCK1R cDNA

The chCCK1R cDNA encoded a protein comprised of 429 amino acids (Fig. 1), and that showed approximately 75% and 50% sequence identity at the amino acid level with mammalian CCK1R and CCK2R, respectively. The chCCK1R satisfactorily conserved the seven transmembrane domains that are common structural features of the G-protein coupled receptor superfamily (Fig. 2).

Expression Profile of chCCK1R and CCK-CHR mRNAs in Chicken Tissues

Whilst both chCCK1R and CCK-CHR mRNAs were widely expressed in the chicken tissues, the expression pattern for each receptor was slightly different. As for chCCK1R mRNA expression, abundant expression was observed in multiple tissues except cerebrum, proventriculus, gizzard, liver and kidney in the chicken. On the other hand, strong CCK-CHR expression was observed in the brain areas and proventriculus, and less CCK-CHR mRNA expression was found in the intestines, pancreas and gallbladder where chCCK1R mRNA was highly expressed (Fig. 3).

Discussion

By pharmacological characterization, two CCK receptor subtypes have also been suggested to exist in chickens (Rodríguez-Sinovas *et al.*, 1995). Recently, Nilsson and colleagues reported CCK-CHR cDNA, which is structurally similar to the mammalian CCK2R from chicken brains (Nilsson *et al.*, 2003). Here we cloned chicken CCK-1 receptor (chCCK1R) cDNA by PCR technology and expression of the receptor was analyzed. The chCCK1R showed higher similarity with mammalian CCK1R than CCK2R in mammals. In addition, phylogenetic analysis among CCK receptors in vertebrates indicates chCCK1R and CCK-CHR were placed in a cluster for CCK1R and CCK2R, respectively (Fig. 4). This is in good agreement with the structural similarity of both chicken CCKRs to those of mammals. Due to advances in genome research in poultry, a number of new genes were mapped on the chromosome and the chicken CCK1R gene is mapped on chromosome 4 (accession No. XM420751). However, hypothetical chicken CCK1R based on the Genome Project is 33-amino-acids longer than chCCK1R. This discrepancy might have occurred from estimation of the splicing donor site for the chicken CCK1R gene. While mammalian CCK1R gene is composed of a structure of 5 exons and 4 introns, the chicken CCK1R gene derived from the Genome Project possesses a structure of 6 exons and 5 introns. This is because a 99-bases extra exon is inserted after the third exon of the gene. Several variant forms of CCK2R are reported in mammals, but variant CCK1R produced by alternative splicing is not reported in any vertebrates. In addition, we

AAGCAACCTTGCAAGGGGTTAACACTGTAATCTTTTCTAGTCTGTTTATAATCCCAACAGTAGGCCAGTAACAGAAGAGGGTAGATCTA	90
AAAAAGTACAGGAGGGGATGTGTGCATGTGTGCATATGTCTGAATGTGTGTCTGCGTGCTTGCCCTGCTTTTCTAAACTGCCTCGTATGG	180
CATAAATCTTTTTCAGCCTGACCAGAGCGTTCTGATGGATGGTTAAAGAGCTTACTTGACACCTCTGCCTCTCTCGTATTCTCTTGGAG	270
ACTTCATTCAGAAGATATTGTGTTACTGAAAGAATGGGCAGATAGTTACAAACCAGCCTTCTTCTAGCAGCTAACCTGTGGATCCTGAA	360
GGATGGAAATAGTTGATGCTAGCTTCTTGAGAACAGTACCAACATCACTGCTTTGCTGTGTGATATCCTCTTGAAAAATGAGACTTTCT	450
M E I V D A S F L E N S T N I T A L L C D I L L E N E T F	29
ACTGTGTGGATGATCCACCTTATTCTTCTAAAGATTTCATCAGATAATCCGGATCCTGCTGTATTGTTGATATTTCTGCTCAGCGTTT	540
Y C V D D P P Y S S K D L H Q I I R I L L Y C L I F L L S V	59
TGGGAACATTCTGGTCATCACTGTGCTGATAAGAAACAAACAGATGAGAACGGTCACCAACACATTTCTGCTGTCCCTGGCAGTCAGTG	630
L G N I L V I T V L I R N K Q M R T V T N T F L L S L A V S	89
ACTTAATGCTCTGCCCTCTCTGTCATGCCATTACCCCTCATTCCTCAACCTGCTGAAAGATTTTATTTTGGGAAGCACTGTTTGCAAACTG	720
D L M L C L F C M P F T L I P N L L K D F I F G S T V C K T	119
CCACTTACTTCATGGGTATCTCTGTGAGTGTGTCTACATTCAACCTTGTGTCATCTCTTTGGAGCGGTACAGTGCCATTTGCAAACTC	810
A T Y F M G I S V S V S T F N L V A I S L E R Y S A I C K P	149
TTCAGTCCAGGGTCTGGCAGACAAAAATCCCATGCTTGAAGTGATTGCTGCTACCTGGTGTGTTTCTTTTACCATCATGTACCATATC	900
L Q S R V W Q T K S H A L K V I A A T W C V S F T I M S P Y	179
CAATTTACAGCAAGCTGGTCCCTTTCACCAAGTACAACAACAGCACAGCCAACATGTGTGCGCTCCTTTGGCCAAGTGATGTCTTCAGC	990
P I Y S K L V P F T K Y N N S T A N M C R L L W P S D V I Q	209
AGTCTTGGTACACCTTCTGCTGCTCATACTCTTTCTTATACCTGGGATAATAATGATGGTTGCGTATGGCCTCATTTCATTGGAACCTC	1080
Q S W Y T F L L L I L F L I P G I I M M V A Y G L I S L E L	239
ACAGAGGAATAAAATTTGATACCGCCAGAGAAGATCTTCAAGAGAAAGAAAGAGTACCAGCATCGCCAAATACGAGGATGGAGACG	1170
Y R G I K F D T S Q R R S S R E R K G S T S I A K Y E D G D	269
GGTGTACCTGCACAAAGCCAAAAGGAAAGGAAAGTGCCGTTGCAACAGCTATCTACTATGAGCAACAGCAAAATAGACAGGGTGAGAA	1260
G C Y L H K A K R K R K V P L Q Q L S T M S N S K I D R V R	299
GCAGCAGCTCTTCTGCCAACCTGATGGCCAAGAACTTGTCTATCCGATGCTGATGGTGATAGTGGTTTTGTTTTTCATTGTGCGACTC	1350
S S S S S A N L M A K K L V I R M L M V I V V L F F I C W T	329
CCATCTTCAGCGTGAACCGCTGGCGCGCATTTGACACCGCTCCGAGACCTGCATCTCTCGGAGCTCCCATCTCCTTTTATCCACCTGC	1440
P I F S V N A W R A F D T A S A D L H L S G A P I S F I H L	259
TGTCCTACACTTCTGCTGCGTGAACCTATCATATACTGCTTCATGAACAAGCGTTTCCGATGGGTTTTCTAGCCACTTTCACTGCT	1530
L S Y T S A C V N P I I Y C F M N K R F R M G F L A T F T C	389
GCGCTAAGCAGAAGCCGCTGTGATTAGAGGAGAGGTTGGTGATGAAGAGGAGGGGAAGACGACAAGGGCTTCGCTCTCCAAGTGCTCTT	1620
C A K Q K P P V I R G E V G D E E E G K T T R A S L S K C S	419
ACACACACATGAACATGTCTGACCCCTCTGAGCTGCATCAGAAGGGGTACAAGTGGCCACAGAAATCAAGTGACATCATCTGGTC	1710
Y T H M N M S A P P *	429
TTGGGAAGTGGCTGTGGCATTAGTTGATATGATTTAGTTCCAAGTAATGAAACACTTCAGCTGACAGTAAACGCTGTGAGAACTTCATG	1800
GGAAATGGATGCTGTCTCTGTCTGAGCTTTATATCATTTTCCCTTCAAAGTACAAAGGAGGAAGAGGGCTCTTTGAAGACAAGGACTT	1890
TATAAAAGCCTTAGAAGATGGTCTATTTGTCTATAAATCTCCTGTAAAAAATGGTGTTCTTTAACATCAGCAGTTTAGCTCAGTATT	1980
CAGCTGCACTTCTAAGCACCTCCGAAAACGTCCAAGATGCCAATAGATTCTCCTCAAACACAAATAAATGTATTTTAGTCTTCACTTCAG	2070
TATCTCTGCTTACAATCAAATCATTCAGTTACATTTGAAGTACTTTTTTACATTATTTTAGCTGAGCTAATACGTATCGAGATCATG	2160
CTGGTGCCATAGTCAAGTACAAGCATTTGGGGTGGGAGAATGGGCATGTCCATGTGAAAATAATGCTTTCAAGTGAAACTGTTTTTTT	2250
TTTTTTTTTTTTCTAAACAAAACAAAAATAAATACTGATGACTTCAAATGTGGACGGAAGCATTCTTCACCTGGCAATAACCTATGAT	2340
AAATTACCATCATTTAAGTGATCTTTGTTTGAATAAAAAACACAGAGCAAGCACAGAAAAA	2418

Fig. 1. Nucleotide and deduced amino acid sequence of chCCKR cDNA. The deduced amino acid residues are shown in the single-letter code, and asterisk indicates the termination codon. The numbering for each nucleotide and amino acid is given on the right margin. The putative polyadenylation signals are underlined. The nucleotide sequence has been submitted to the GenBank/EMBL/DBJ Data Bank with the accession number AB214534.

chCCK1R	1	M-----EIVDASELENSTNITALLCDILLENETFYCVDDPPYSSKDLHQIIRILLYCLIFLLSVLGNLIVITVLRNKKOM	75
human CCK1R	1	M-----DVVD-SLLVNGSNITP-PCELGLENETLFCLDQ-PRPSKEWQPAVQILLYSLIFLLSVLGNLIVITVLRNKKRM	72
rat CCK1R	1	MHSHPARQHLVESSRMDVVD-SLLMNGSNITP-PCELGLENETLFCLDQ-PQPSKEWQSAILOILLYSLIFLLSVLGNLIVITVLRNKKRM	87
CCK-CHR	1	MDPRLNES-LQ-ELLCRPGNGTGSANGTGTPTWNGS-ACDLLRRGLRGPQAPKELDTVRVLLYALIFVLVSGCNALVAVLLNRRLL	87
human CCK2R	1	MELLKLNR-SVQ-GTGPGGASLCRPGAPLLNSSSVGNLSC-EPPR-IRG-AGTRELELAIRITLYAVIFELMVGNNLITVVLGSRRL	85
rat CCK2R	1	MELLKLNR-SVQ-GPGGSGSSLCRPGVSLNSSSAGNLS-C-DPPR-IRG-TGRELEMAIRITLYAVIFELMVGNNLITVVLGSRRL	85
TM2			TM3
chCCK1R	76	RTVTNIFLLSLAVSDLMCLFCMPFNLIPNLKDFHFGSTVCKTATYFMGHSVSVSTFNLVAISLERYSAICRPLQSRVWOTKSHALKVI	165
human CCK1R	73	RTVTNIFLLSLAVSDLMCLFCMPFNLIPNLKDFHFGSAVCKITTYFMGHSVSVSTFNLVAISLERYGAICRPLQSRVWOTKSHALKVI	162
rat CCK1R	88	RTVTNIFLLSLAVSDLMCLFCMPFNLIPNLKDFHFGSAVCKITTYFMGHSVSVSTFNLVAISLERYGAICRPLQSRVWOTKSHALKVI	177
CCK-CHR	88	RTVTNIFLLSLAVSDLMCLFCMPFNLIPNLKDFHFGSAVCKITTYFMGHSVSVSTFNLVAISLERYGAICRPLQSRVWOTKSHALKVI	177
human CCK2R	86	RTVTNIFLLSLAVSDLMCLFCMPFNLIPNLKDFHFGSTVCKTATYFMGHSVSVSTFNLVAISLERYSAICRPLQSRVWOTKSHALKVI	175
rat CCK2R	86	RTVTNIFLLSLAVSDLMCLFCMPFNLIPNLKDFHFGSTVCKTATYFMGHSVSVSTFNLVAISLERYSAICRPLQSRVWOTKSHALKVI	175
TM4			TM5
chCCK1R	166	AATWCVSFTIMSPYPIYSKLVPTKYNNTA-NMCRLLWPSDVIQSWYTFLLLLFLIPGIIMMVAYGLISLELYRGIKF-DTSQRRSS	253
human CCK1R	163	AATWCVSFTIMSPYPIYSKLVPTKYNNTA-NMCRLLWPSDVIQSWYTFLLLLFLIPGIIMMVAYGLISLELYRGIKF-EASQKKSA	250
rat CCK1R	178	AATWCVSFTIMSPYPIYSKLVPTKYNNTA-NMCRLLWPSDVIQSWYTFLLLLFLIPGIIMMVAYGLISLELYRGIKF-DASQKKSA	265
CCK-CHR	178	ASTWLFSAALLMPYAVYSTTRLLPVPGARPPLSQCTHAWPSEHVROAMVYVLLVLLFFIPGVVMIVAYGLISRELYRGIRFELDIDKGEAA	267
human CCK2R	176	VATWLLSGLLVMPYVYT--VVQPV-GPR-VL-QCVRWPSARVQTMVSVLLVLLFFIPGVVMIVAYGLISRELYRGIRFELDIDKGEAA	260
rat CCK2R	176	LATWLLSGLLVMPYVYT--MVQPV-GPR-VL-QCVRWPSARVQTMVSVLLVLLFFIPGVVMIVAYGLISRELYRGIRFELDIDKGEAA	260
TM6			TM7
chCCK1R	254	RERK-GST-SIAKYED--GD-G-C--Y-LHKAKRR--KVPL--QQ--LS-TMSNSKIDRVRS--SSS--ANLMAKKRVIRMLVIVVLFLLFC	327
human CCK1R	251	KERK-PSTTSSGKYED--SD-G-C--Y-LQKTRPP--KLEL--RQ--LSTGS-SSRANRIRNS--SSA--ANLMAKKRVIRMLVIVVLFLLFC	325
rat CCK1R	266	KEKK-PSTGSTRYED--SD-G-C--Y-LQSRPP--KLEL--RQ--LSSGSGSRLNRIR--SSA--ANLMAKKRVIRMLVIVVLFLLFC	341
CCK-CHR	268	AQ-RSTEPLP-TCDEG----DG-C--Y-LQLS-RPGG-ALEL--RA--LGAAGAQQERARINS--S-EAKLVAKRRVIRMLVIVVLFLLFC	339
human CCK2R	261	SRVRNQGGLP-G-A---VHQNGRCRPETGAVGEDSDGCCVQLPRSRPALLETALTAPGPGSGSRPTQAKLLAKRRVIRMLVIVVLFLLFC	345
rat CCK2R	261	SRARNQGGLPGGAAGPVGHQNGCRPVTSGAVGEDSDGCCVQLPRSRLEMTLTTPGPGVPGPRPQAKLLAKRRVIRMLVIVVLFLLFC	350
TM7			
chCCK1R	328	WPIPIFSANAWRAEDTASADLHLSGAPISFIFLLSYISACVNPDIYCFMNRFRFMGLATFTCCAKQKPPVIRGEVGDDEEGKTTRASLSK	417
human CCK1R	326	WPIPIFSANAWRAYDTASAERRLSGTPISFIFLLSYISACVNPDIYCFMNRFRRLGEMATFPCCPNPGPPGAGEVGEDEEGTTGASLSR	415
rat CCK1R	342	WPIPIFSANAWRAYDTVSAEKHLSGTPISFIFLLSYISACVNPDIYCFMNRFRRLGEMATFPCCPNPGPPGAGEVGEDEGRTIRALLSR	431
CCK-CHR	340	WPIPIFAANTWRAFAAPRAAQRALSGTPISFIFLLSYISACVNPDIYCFMNRFRSAFLGTCA-GC-R--RACPRAPDEADVANAN-ASLSK	424
human CCK2R	346	WLPVYSANTWRAFDGPGAQRALSGAPISFIFLLSYISACVNPDIYCFMNRFRSAFLGTCA-GC-R--RACPRAPDEADVANAN-ASLSK	435
rat CCK2R	351	WLPVYSANTWRAFDGPGAQRALSGAPISFIFLLSYISACVNPDIYCFMNRFRSAFLGTCA-GC-R--RACPRAPDEADVANAN-ASLSK	440
chCCK1R	418	CSYTHMNSAPP-	429
human CCK1R	416	FSYSHMSASVPPQ	428
rat CCK1R	432	YSYSHMSTASAPP	444
CCK-CHR	425	FSYITVSSLGPP-	436
human CCK2R	436	LSYITITSLGG-	447
rat CCK2R	441	LSYITITSLGG-	452

Fig. 2. Multiple alignment of the amino acid sequences of CCK receptors among vertebrates. Conserved residues are shaded, and identical residues in the all species are black-boxed. Amino acid sequences of CCK receptors from chicken, human and rat aligned using Clustal W (Thompson *et al.*, 1994). The position of putative transmembrane domain is overlined. TM ; transmembrane domain.

Fig. 3. Expression profile of chCCK1R and CCK-CHR mRNAs in the chicken tissues. The data represents a RT-PCR result from three independent experiments. The same reverse-transcribed samples were used for the three PCR reactions.

could not identify such a long isoform CCK1R predicted by the Chicken Genome Project from the chicken tissues (data not shown). Therefore, the present result will be useful in revising the structure

of the chicken CCK1R gene.

Chicken CCK receptors showed higher affinity for a mammalian CCK1R antagonist, devazepide, than a mammalian CCK2R antagonist, L-365,260

Fig. 4. **Phylogenetic tree of CCK receptors in vertebrates.** The tree was constructed by the neighbor-joining method using a distance matrix based on amino acid sequences. Horizontal lines indicate genetic distance. The sequences were obtained from EMBL/GenBank Data Bank : CCKAR from human, mouse and rabbit (Accession No. L13605, NM_009827 and U86601, respectively), CCKBR from human, mouse and dog (L07746, NM_007627 and AD001537, respectively), CCK-CHR (AJ489483) and Xenopus CCKR (U49258).

(Vigna *et al.*, 1986 ; Rodríguez-Sinovas *et al.*, 1995). This was also confirmed by Nilsson *et al.* (2003) that CCK-CHR, structurally resembling CCK2R, showed high affinity for the CCK1R antagonist. It was reported that a valine in the sixth transmembrane of the human CCK2R is critical in determining the binding affinity for devazepide or L-365,260, and where substitution of the valine with an isoleucine increases the affinity for devazepide (Beinborn *et al.*, 1993). In chicken CCK receptors, the corresponding amino acid in the sixth transmembrane domain that would interact with the CCK antagonists is isoleucine (I331 of chCCK1R and I343 of CCK-CHR in Fig. 2).

The reason devazepide is a potent antagonist for chicken CCK receptors could, in part, be explained by the V/I polymorphism existing in the sixth transmembrane domain in both receptors.

In RT-PCR analysis, whilst mRNA for CCK1R and CCK-CHR was expressed widely in the chicken tissues, the expression profile for both receptors was slightly different (Fig. 3). CCK modulates intestinal motility (Martinez *et al.*, 1993 ; Rodríguez-Sinovas *et al.*, 1994) and biliary flow in the gallbladder (Duke *et al.*, 1987), where chCCK1R mRNA was highly expressed. We found that CCK-CHR was dominantly expressed in the paraventriculus which is suggested to be a target of gastrin in chickens

(Campbell *et al.*, 1994 ; Furuse *et al.*, 1999) and turkeys (Dimaline and Lee, 1990). We also demonstrated that the amplification signal for CCK1R mRNA was strong and that for CCK-CHR was faint. A binding study using CCK agonists revealed that CCK1R is predominant over CCK2R in chicken pancreas (Vigna *et al.*, 1986 ; Rodríguez-Sinovas *et al.*, 1995). Furthermore CCK stimulates amylase secretion from the pancreas in chickens and ducks (Sato *et al.*, 1994 ; Xiao and Cui, 2004). Consequently, tissue specific regulation of CCKR expression may suggest varying CCK and gastrin functions in chickens.

In conclusion, we cloned chicken CCK-1 receptor cDNA in this study. Based on the sequence similarity, phylogenetic analysis and tissue distribution, chCCK1R is a potent peripheral CCKR and CCK-CHR is classified as CCK2R in chickens. Furthermore, mRNA expression for both chCCK1R and CCK-CHR may be regulated by tissue specificity and that would be associated with the diverse roles of CCK and gastrin in the chicken.

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