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Purification, molecular cloning, and functional expression of the cholecystokinin receptor from rat pancreas

(gastrointestinal peptide receptor/neuropeptide receptor/G-protein-coupled receptor/gastrin receptor)

STEPHEN A. WANK*†, RICHARD HARKINS‡, ROBERT T. JENSEN*, HAGIT SHAPIRA§, ANDREAS DE WEERTH*, AND TIMOTHY SLATTERY‡

*Digestive Diseases Branch, National Institute of Diabetes, Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD 20892; ‡Berlex Biosciences, 1401 Harbor Bay Parkway, Alameda, CA 94501; and §Laboratory of Neurochemistry, National Institute of Neurological Disorders and Stroke, National Institutes of Health, Bethesda, MD 20892

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ABSTRACT **The cholecystokinin (CCK) family of peptides and their receptors are widely distributed throughout the gastrointestinal and central nervous systems where they regulate secretion, motility, growth, anxiety, and satiety. The CCK receptors can be subdivided into at least two subtypes, CCK_A and CCK_B on the basis of pharmacological studies. We report here the purification of the CCK_A receptor to homogeneity from rat pancreas by using ion-exchange and multiple affinity chromatographic separations. This allowed partial peptide sequencing after chemical/enzymatic cleavage and synthesis of degenerate oligonucleotide primers. These primers were used for initial cloning of the cDNA from rat pancreas by PCR. The predicted protein sequence of the cDNA clone contained the five partial peptide sequences obtained from the purified protein. Seven putative transmembrane domains suggest its membership in the guanine nucleotide-binding regulatory protein-coupled receptor superfamily. *In vitro* transcripts of the cDNA clone were functionally expressed in *Xenopus* oocytes and displayed the expected agonist and antagonist specificity.**

The cholecystokinin (CCK) family of peptides was originally isolated from the mammalian gastrointestinal tract (1) and was one of the first gastrointestinal peptides to be discovered in the brain (2). Their receptors appear throughout the gastrointestinal and nervous systems and can be pharmacologically classified into two subtypes, CCK_A and CCK_B (3). CCK_A receptors mediate physiologic gallbladder contraction, pancreatic growth and enzyme secretion, delayed gastric emptying, relaxation of the sphincter of Oddi, and potentiation of insulin secretion (3). The CCK_A type receptor also appears in the anterior pituitary, the myenteric plexus, and areas of the central nervous system (midbrain) where CCK-containing dopaminergic neurons have been implicated in the pathogenesis of schizophrenia, Parkinson disease, drug addiction, and feeding disorders (4). Experimental rat pancreatic carcinogenesis is promoted by CCK through the CCK_A type receptor (5). CCK acting at peripheral CCK_A receptors and at central CCK_A and CCK_B gastrin receptors plays a significant role in the nervous system control of appetite (6).

Recently, antagonists highly selective and potent for each of the CCK receptor subtypes have been developed. The two most potent and selective antagonists are L-364,718 (7) and PD134308 (8) for CCK_A and CCK_B receptors, respectively.

In pancreatic acinar cells, CCK_A receptors are coupled to a guanine nucleotide-binding regulatory protein (G protein), which activates phospholipase C, breakdown of inositol phospholipids, mobilization of intracellular calcium, and activation of protein kinase C (3). Activation of this pathway in *Xenopus* oocytes by CCK receptors that have been functionally expressed in the plasma membrane after injection of either rat brain total RNA (9) or the rat pancreatic acinar carcinoma cell line, AR42J (10), mRNA (11) results in a depolarizing current due to Ca^{2+}-dependent chloride channels opening in the plasma membrane (12). Affinity labeling studies of CCK_A receptors from rat pancreas and partial purification demonstrate an 85- to 95-kDa, heavily glycosylated, binding subunit with a deglycosylated core protein of 42 kDa (13).

Further knowledge of the molecular structure of the CCK receptor would enhance the understanding of its subtype distribution, function, and regulation. Here we report the purification of the rat pancreatic CCK_A receptor to homogeneity, cloning of its cDNA‖, and functional expression in *Xenopus* oocytes.

METHODS

Purification of CCK_A Receptors from Rat Pancreas. Rat pancreatic membranes were prepared from 250 male Sprague–Dawley rat pancreases and solubilized in 2.5 liters of buffer [10 mM Hepes (pH 6.5), 1 mM EGTA, 5 mM $MgCl_2$, 1 μM dithiothreitol (DTT), 1 μM leupeptin, 1 μM pepstatin, 0.5 mM phenylmethylsulfonyl fluoride, bacitracin at 140 μg/ml, benzamidine at 200 μg/ml, and soybean trypsin inhibitor at 0.1 mg/ml at 4°C] with 1% (wt/vol) digitonin using methods similar to those described by others (14, 15). Soluble extract was applied to a S-Sepharose (Pharmacia) cationic exchange column (4 × 15 cm), the column was washed with 600 ml of buffer containing 100 mM NaCl and 0.2% digitonin, and the receptor was eluted with buffer containing 300 mM NaCl and 0.2% digitonin. Receptor purification was followed by a radiolabeled antagonist ([^{3}H]L-364,718) binding assay (7, 15) until final purification by SDS/PAGE. Eluted fractions containing [^{3}H]L-364,718 binding activity (15) were pooled, diluted with one-third volume of buffer, and applied to a wheat germ agglutinin-agarose (Vector Laboratories) affinity column (1.7-ml packed volume in a Bio-Rad Econo-Column). The column was washed overnight with 100 column volumes of buffer containing 150 mM NaCl and 0.1% digitonin and eluted stepwise with three 1.4-ml volumes of wash buffer plus 16 mM *N,N′,N″*-triacetylchitotriose. The pooled eluates

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Abbreviations: CCK, cholecystokinin; CCK-8, CCK octapeptide; G protein, guanine nucleotide-binding regulatory protein; DTT, dithiothreitol; RACE, rapid amplification of cDNA ends; MOPAC, mixed oligonucleotide-primed amplification of cDNA.

†To whom reprint requests should be addressed.

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‖The sequence reported in this paper has been deposited in the GenBank data base (accession no. M88096).

were then applied to a Cibacron Blue Sepharose (Pharmacia) column (0.5-ml packed volume in a Bio-Rad Econo-Column), the column was washed, and the receptor was eluted with five 0.5-ml volumes of buffer with 1 mM Cibacron F3GA in a method similar to that described above for wheat germ affinity chromatography. Purified receptor was then trace labeled with ^{125}I by the chloramine T method and subjected to preparative SDS/PAGE (11%) under reducing conditions (50 mM DTT). The major Coomassie brilliant blue-stained band corresponding to >90% of the radioactivity was cut, electroeluted, ethanol precipitated, and submitted for amino acid analysis and sequencing. Each step of the receptor purification was assayed for protein either by the method of Bradford (16) (corrected for the presence of digitonin) or amino acid compositional analysis (Beckman analyzer).

Automated Protein Sequence Analysis. Ten micrograms of intact purified rat pancreatic CCK_A receptor was subjected to automated sequence analysis on an Applied Biosystems model 475A gas-phase sequencer as described (17).

Chemical and Enzymatic Cleavage of the CCK Receptor. Cyanogen bromide (CNBr) cleavage of the CCK receptor was performed on the sequencer filter after five cycles of Edman degradation of the intact receptor using standard methods. The chemically cleaved receptor was then resequenced.

Endoproteinase Lys-C (Wako Pure Chemical, Osaka) digestion was performed on 10 μg of the purified CCK_A receptor in 150 μl of 0.1 M Tris·HCl (pH 9.0). Enzyme (1:20 relative to receptor weight) was added at 0 and 2 hr, and the reaction was continued for a total of 16 hr at 37°C. The digests were either directly used for sequence analysis of the peptide mixture or reduced with 25 mM dithiothreitol for 15 min at 37°C and then fractionated by HPLC.

Sequencing of the mixture of peptides was performed on chemical and enzymatic digests to determine the cycle at which proline appeared at the amino terminus. Primary amines of the mixture of peptides were blocked in subsequent sequencing runs where proline residues appeared by using *o*-phthalaldehyde (Pierce) at 0.2% (wt/vol) in *n*-butyl chloride containing 0.6% (vol/vol) 2-mercaptoethanol delivered through the S1 reservoir instead of R1 (phenylisothiocyanate) at predetermined cycles.

HPLC Separation of CCK Receptor Peptides. CCK receptor digestion products were fractionated on a 2.1-mm × 3-cm C_4 reverse-phase column (Aquapore BU-300; Brownlee Lab) as described (18).

Construction of a Rat Pancreatic cDNA Library and Isolation of cDNA Clones. mRNA was isolated from male, Sprague–Dawley rat pancreases as described (19). A library using oligo(dT)-primed cDNA [>2 kilobases (kb)] was constructed in λZap II (Stratagene) and *in vitro* packaged according to established methods (20). Approximately 7.5×10^5 plaques were screened with a ^{32}P-labeled, randomly primed probe using a 527-base-pair (bp) product (nucleotides 481–1007, Fig. 2) of the mixed oligonucleotide-primed amplification of cDNA (MOPAC) PCR described below. Samples on duplicate filters were hybridized at 42°C overnight, washed once at room temperature for 5 min in 2× standard saline citrate (SSC; 2× SSC = 300 mM NaCl/3 mM sodium citrate) and 0.1% SDS and twice at 45°C for 20 min in 0.1× SSC and 0.1% SDS, dried, and autoradiographed for 1–2 days by using methods previously described (20). Positive clones were plaque purified, and the phagemid pBluescript containing the insert was *in vivo* excised by using the helper phage R408 according to standard protocols (Stratagene).

DNA Sequencing. Sequencing of both DNA strands was done by the dideoxy chain-termination method of Sanger (21) with Sequenase version 2 (United States Biochemical).

cDNA Cloning by Using PCR. MOPAC (22) was performed with two groups of degenerate primers based on the amino acid sequence from peptides 1 and 3 (see Fig. 2). The sense group of primers, based on peptide 1, was 72-fold degenerate, included two inosines, and had the following sequence: 5′-ATGCCIATSAAYCTIATHCCNAA-3′ (Y = T or C; H = A, C, or T; N = A, C, G, or T; S = G or C). The antisense group of primers, based on peptide 3, was 80-fold degenerate and consisted of two groups of 32- and 48-fold degenerate primers with the following sequences, respectively: 5′-CCRTCRCTRTCYTCRTA-3′ and 5′-CCRTCDGARTCYTCRTA-3′ (R = A or G; D = A, G, or T).

The remaining 3′ coding and untranslated sequence was obtained by using the methods of rapid amplification of cDNA ends (RACE) (23) and anchored PCR. RACE/PCR was performed by using the gene-specific primer 5′-GCCAGCCAGAAGAAATCTGCC-3′ (nucleotides 928–948, Fig. 2) for the first round and the nested primer 5′-AGCCGAGCACTGGCAGCAGCA-3′ (nucleotides 959–979, Fig. 2) for the second round. Anchored PCR utilized the unamplified cDNA library constructed in λZap II described above as template DNA, the gene-specific primer containing an *Xba* I site and a 9-bp cap on the 5′ end (5′-ACTGACTAGTCTAGATCAGCTGCCAACCTGATAGCC-3′; nucleotides 1102–1122, Fig. 2), and the anchored primer from the vector also with an *Xba* I site and 9-bp cap (5′-ACTGACTAGTCTAGATAATACGACTCACTATA GGGCG-3′). The PCR product was digested with *Xba* I, subcloned into pGEM (Promega), and sequenced by using standard methods (20).

The CCK_A receptor open reading frame with 5′ and partial 3′ flanking sequence (nucleotides 5–1506, Fig. 2) was cloned by PCR using the following sense and antisense primers each with an *Xba* I site and 9-bp cap: 5′-ACTGACTAGTCTAGAAATGCTTGCCCAGATGCTCTG-3′ (nucleotides 5–25, Fig. 2) and 5′-ACTGACTAGTCTAGACAGTGGACCAGGTGGAGTTCA-3′ (complementary to nucleotides 1506–1486, Fig. 2). Single-stranded cDNA reverse transcribed from rat pancreatic mRNA (20) served as the DNA template. The PCR product was digested with *Xba* I, ligated, and cloned in the vector pCDNA-1 (Invitrogen). These clones were used for sequencing and *in vitro* transcription described below.

Northern Blot Analysis of mRNAs. Poly(A)$^+$ mRNA was isolated (19) from tissue or cell culture lines, electrophoretically separated on a 1.4% agarose/formaldehyde gel, blotted onto Nytran (Schleicher & Schuell), hybridized with a CCK_A receptor cDNA probe labeled with ^{32}P by random priming (24), washed, and autoradiographed for 4 days as described (20).

***In Vitro* Transcription of the CCK_A Receptor and Expression in *Xenopus* Oocytes.** DNA was *in vitro* transcribed by using T7 RNA polymerase and a CCK_A receptor PCR product (nucleotides 5–1506, Fig. 2) cloned in pCDNA-1 (5 μg linearized with *Apa* I) as a template in the presence of the cap analog $m^7G(5')ppp(5')G$ as recommended by the manufacturer (Promega). *Xenopus* oocytes were injected with 50 nl (≈25 ng) of transcribed RNA. At 1–2 days, oocytes were voltage clamped at −70 mV, ligands were applied rapidly and directly to the constantly perfused bath, and the ligand-dependent Cl^- current was measured as described (25).

RESULTS AND DISCUSSION

Two hundred and fifty rat pancreases were used to purify the CCK_A receptor to homogeneity. A crude membrane preparation derived from the whole organ was solubilized in 1% digitonin and sequencially purified over three chromatographic columns: cationic exchange resin, wheat germ agglutinin agarose, and Blue-Sepharose. By starting with 11.7 g of membrane protein, this resulted in an ≈14,600-fold increase in specific radiolabeled antagonist ([^3H]L-364,718)

binding activity in 260 μg of purified receptor protein (data not shown). Radiolabeling of the purified receptor with ^{125}I by the chloramine T method followed by SDS/PAGE under denaturing conditions resulted in a single, broad band (suggesting heavy glycosylation) with a molecular mass of 85–95 kDa (Fig. 1). These results are consistent with previous pharmacological (3) and biochemical affinity crosslinking studies (13) demonstrating that CCK receptors from rat pancreas are of the CCK_A subtype and are highly glycosylated, with a molecular mass of 85–95 kDa. Purification on preparative SDS/PAGE and electroelution yielded 200 μg of homogeneous receptor for amino acid sequencing.

Initial attempts to obtain sequence on 10 μg of purified intact CCK_A receptor were unsuccessful and suggested that the amino terminus of the receptor was blocked to Edman degradation. CNBr cleavage of the receptor on the sequencing filter followed by *o*-phthalaldehyde blocking of the peptide mixture on the first cycle allowed sequencing of only peptide 1 (Fig. 2). This same technique of mixture sequencing and *o*-phthalaldehyde blocking was also applied to a Lys-C digest of the intact CCK_A receptor where a proline was observed at cycle 2 of the mixture sequence. This resulted in the sequence of peptide 3 (Fig. 2). Further sequence analysis was performed on peptides obtained from Lys-C digestion of intact CCK_A receptor and HPLC separation on a C_4 column. This resulted in peptides 2, 4, and 5 (Fig. 2).

Based on the sequences of peptides 1 and 3 (Fig. 2), we synthesized two groups of mixed degenerate oligonucleotides and used MOPAC PCR with single-stranded cDNA reverse transcribed from rat pancreatic mRNA as template. This resulted in a 527-bp product (corresponding to nucleotides 481–1007, Fig. 2), which, after ^{32}P random primer labeling, was used for hybridization screening of an oligo(dT)-primed cDNA library constructed from rat pancreas in the vector λZap II. Twenty-six strongly hybridizing clones were identified on initial screening of $\approx 7.5 \times 10^5$ clones. However, after three rounds of plaque purification, only six clones remained. The six clones were *in vivo* excised with R408 helper phage into pBluescript and sequenced. All six of these clones lacked the 3′ end of the hybridizing sequence. Therefore, only a partial sequence corresponding to the 5′ untranslated and partial 5′ coding region (nucleotides 1–985, Fig. 2) was obtained.

The remainder of the CCK_A receptor sequence was obtained by using various PCR methods to circumvent the unusually high rate of mutation occurring during plaque purification. To correct for sequence errors introduced by multiple rounds of PCR amplification, PCR products were either sequenced directly or, when products were cloned, more than one clone was sequenced. With knowledge of the 5′ end of the CCK_A receptor cDNA sequence, gene-specific primers were synthesized corresponding to nucleotides 928–948 and 959–979 (Fig. 2) and used in the first and second rounds, respectively, of the RACE protocol. This resulted in only an additional 366 bp of sequence (nucleotides 986–1351, Fig. 2) because the PCR preferentially amplified truncated products (23). The remaining 3′ sequence was then obtained by the method of "anchored" PCR by using a gene-specific primer corresponding to nucleotides 1102–1122 (Fig. 2) and the λZap II vector-specific T7 primer/promoter. An additional 155 bp of sequence completed the 3′ coding and part of the 3′ untranslated sequence to give a total of 1506 bp. The first in-frame ATG consistent with a consensus translation initiation site (27) represents the start codon of a single long

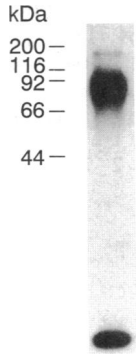

FIG. 1. Autoradiogram of the purified CCK_A receptor after radiolabeling and SDS/PAGE. CCK_A receptor purified from rat pancreas by cation exchange, wheat germ agglutinin, and Blue Sepharose chromatography was trace labeled with ^{125}I by the chloramine T method and subjected to SDS/PAGE (11%) under reducing conditions (50 mM DTT). Greater than 95% of the radioactivity (data not shown) appears in a single symmetric broad band with a molecular mass of 85–95 kDa. A faint band at $\approx$180 kDa represents aggregated receptor (data not shown), and the radioactivity at the bottom of the lane is coincident with the dye front.

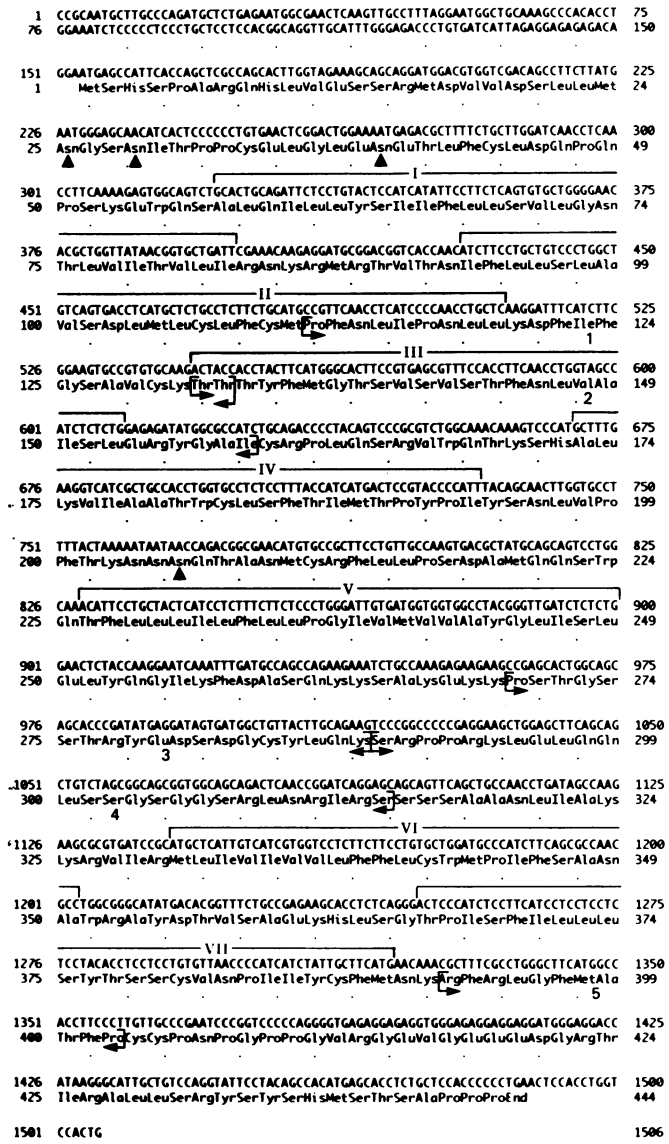

FIG. 2. Nucleotide and deduced amino acid sequences of the rat pancreatic CCK_A receptor cDNA clone. The solid lines labeled with Roman numerals delineate the putative seven transmembrane domains predicted by Kyte–Doolittle criteria (26) and homology with other G-protein receptor superfamily members. Amino acid sequences enclosed within brackets and labeled with arabic numbers correspond to the five internal peptide sequences obtained following CNBr cleavage or Lys-C digestion of the purified CCK_A receptor protein. The triangles indicate four potential sites of N-linked glycosylation.

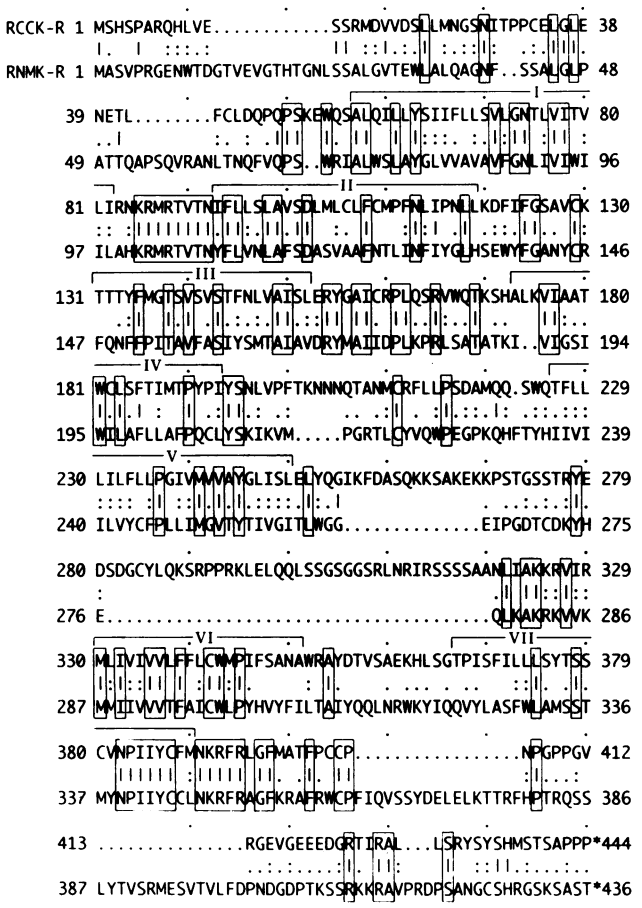

FIG. 3. Alignment of the rat CCK_A (RCCK-R) and neuromedin K (RNMK-R) receptor protein sequences. By using the GAP program sequence analysis package of the Genetics Computer Group (33), the CCK_A receptor deduced protein sequence was aligned for maximal homology to the five protein sequences (rat neuromedin K, bovine substance K, mouse gastrin-releasing peptide, rat substance P, and rat β_1-adrenergic receptors) (18, 32, 34–36) found to be most homologous upon searching all available protein data banks. Shown here, using the one-letter code for amino acids, is the alignment between the CCK_A receptor and the most homologous protein, the rat neuromedin K receptor. Solid lines denote amino acid identity, and dotted lines denote conservative substitutions. Amino acid identities between the CCK_A and neuromedin K receptors also found to be identical in at least one of the other four G-protein-coupled receptor protein sequences are enclosed in boxes. Solid lines labeled with Roman numerals indicate the seven putative transmembrane domains (Fig. 2).

open reading frame encoding a unique 444 amino acid protein with a calculated molecular mass of 49.6 kDa. The five independent peptide sequences obtained from the CNBr cleavage and Lys-C digestion of the purified CCK_A receptor protein are present within the predicted protein sequence (Fig. 2) and confirm that the combined DNA sequence derived from cDNA cloning by library hybridization and PCR codes for the purified protein having high affinity for the specific antagonist L-364,718. The sequence allows for four potential N-linked glycosylation sites, three in the amino terminus and one in the second extracellular loop (Fig. 2), which is consistent with the heavily glycosylated 85- to 95-kDa band seen on Coomassie staining and subsequent four-step reduction to a final molecular mass of ≈42 kDa following deglycosylation with endoglycosidase F (13) (Fig. 2). There are four potential sites for protein kinase C phosphorylation (28), three on serine in the large intracellular fifth loop (residues 260, 264, and 275) and one on threonine in the cytoplasmic tail (residue 424), which is consistent with previous data indicating predominately serine, minor threonine, and no tyrosine phosphorylation of the CCK_A receptor in rat pancreas following CCK and phorbol 12-myristate 13-acetate stimulation and inhibition of phosphorylation by staurosporine (29).

A hydropathy plot of the predicted amino acid sequence using the criteria of Kyte and Doolittle (26) and homology to other G-protein receptor superfamily members identifies seven regions of hydrophobic residues corresponding to putative transmembrane domains expected for members of the G-protein-coupled superfamily of receptors (30). This is consistent with evidence that G proteins couple CCK_A receptors to phospholipase C in exocrine pancreas (31). A comparison of the CCK_A receptor-deduced protein sequence with all protein sequences in available data banks (Protein Identification Resource, release no. 29; Swiss-Prot, release no. 19) found that the five most homologous proteins [rat neuromedin K (32) (Fig. 3), bovine substance K (34), mouse gastrin-releasing peptide (18), rat substance P (35) and rat β_1-adrenergic receptors (36)] having 27–30% amino acid identity and 50–54% similarity were all members of the G-protein receptor superfamily.

High-stringency Northern blot analysis of organ- and tissue-specific polyadenylylated RNA using a full-coding-region probe revealed a 2.7-kb hybridizing transcript in rat pancreas and a rat pancreatic acinar carcinoma cell line, AR42J (Fig. 4). No hybridization was observed in the rat brain, an organ known to possess CCK receptors, presumably because of either low-level expression, a small mount of expressing cell representation in this organ, a different receptor subtype that is unable to hybridize under the stringent conditions employed, or a combination of these factors. As expected, no signal was observed in liver, muscle, or kidney. The size of the hybridizing transcript is consistent with the cloned cDNA size and the 3-kb size estimated from sucrose gradient fractionation of AR42J mRNA functionally expressed in *Xenopus* oocytes (11).

To further demonstrate that the protein sequence encoded by the cDNA represents a functional CCK_A receptor, a capped *in vitro* transcript of a cDNA clone containing the entire coding region and 5′ untranslated sequence (nucleotides 5–1506, Fig. 2) was injected into *Xenopus* oocytes and assayed for specific, cell surface, functional expression 1 and 2 days later. The oocytes responded to CCK octapeptide (CCK-8) (Fig. 5*A*) but not to gastrin-releasing peptide (Fig. 5*A*), substance P (Fig. 5*A*), or acetylcholine (data not shown). Repeated challenges with CCK-8 caused only a moderate desensitization of the response (Fig. 5*A*) and allowed an internal positive control for the effect of the specific CCK_A receptor antagonist L-364,718. Application of the specific CCK_A receptor antagonist L-364,718 after an initial response to CCK-8 inhibited any further response to repeated applications of CCK-8 (Fig. 5*B*). Inhibition was specific for CCK_A receptors

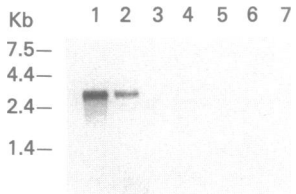

FIG. 4. Northern blot analysis of RNA from rat tissues and a rat cell line. A radiolabeled probe of the CCK_A receptor coding region was hybridized to 4 μg of poly(A)$^+$ mRNA from each tissue per lane and washed under conditions of high stringency. A 2.7-kb hybridizing mRNA can be identified in lanes 1 and 2, corresponding to rat pancreas and the rat pancreatic acinar carcinoma cell line AR42-J. No hybridizing mRNA could be identified in lanes 3–7, corresponding to rat brain stem, cerebral cortex, muscle, liver, and kidney, respectively.

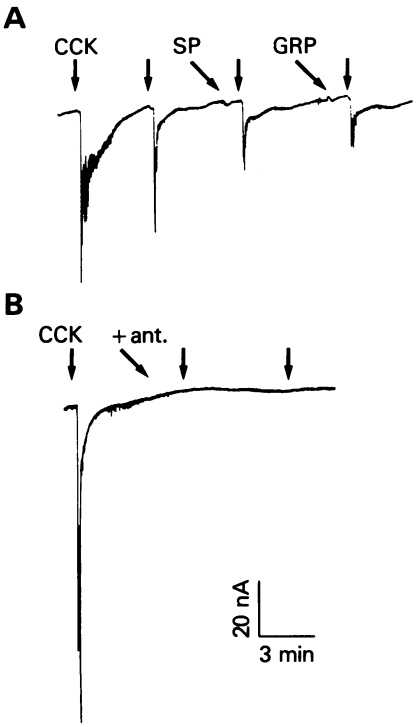

FIG. 5. Expression of the CCK_A receptor in *Xenopus* oocytes. Ligand-induced chloride currents were measured in the same oocytes 1 (*A*) and 2 (*B*) days after injection of mRNA (25 ng) *in vitro* transcribed from CCK_A receptor cDNA cloned from rat pancreas. (*A*) Response to agonists. Application of 1 μM CCK-8 (vertical arrows) elicited a response that desensitizes with repeated applications in the same oocyte. Application of 1 μM gastrin-releasing peptide (GRP) and 4 μM substance P (SP; diagonal arrows), interspersed between response-evoking applications of CCK-8, failed to elicit responses. (*B*) Inhibition by the specific CCK_A receptor antagonist (ant.) L-364,718. Application of 1 μM CCK-8 (vertical arrows) to the same oocyte shown in *A* 2 days after mRNA injection elicited a response that was completely inhibited by the coapplication of 5 μM L-364,718 (diagonal arrow). The response to 1 μM CCK-8 could not be restored following antagonist application despite prolonged washes with buffer. Experiments were repeated several times (n = 12 for *A* and n = 10 for *B*) in different oocytes, with similar results.

(i.e., there was no inhibition of substance P or acetylcholine response in oocytes injected with their respective receptor mRNAs; data not shown). Oocytes injected with total rat pancreatic mRNA showed a typical CCK-8-evoked response. Coinjection of the same mRNA with an antisense oligonucleotide (reverse complement of nucleotides 265–295) completely abolished the response to CCK-8 (data not shown).

The CCK/gastrin family of peptides interacts with at least two receptor subtypes widely distributed throughout the gastrointestinal and nervous systems, with some cells possessing both subtypes. The present results should allow better assignment of CCK receptor subtype distribution and function on the basis of such studies as *in situ* hybridization, cloning of CCK_B/gastrin and perhaps other subtypes using low-stringency hybridization methods, production of large quantities of pure receptor for immunization, and transfection in mammalian cells to facilitate screening of more potent and selective agonists and antagonists. This should ultimately allow better understanding of disease pathophysiology and targeting of therapy to diseased organs of the gastrointestinal and nervous systems while sparing uninvolved organs possessing different CCK/gastrin subtypes.

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