Identification of multiple human calcitonin receptor isoforms: heterologous expression and pharmacological characterization

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ABSTRACT

The human breast carcinoma cell line T47D is known to express high-affinity calcitonin receptors (CTRs). PCR amplification of the CTR cDNA from T47D mRNA resulted in the identification of two different cDNAs that encode distinct receptor isoforms, hαCTR and hβCTR. The two cDNAs are identical except that the hαCTR cDNA contains a 48 bp insert sequence that encodes a 16 amino acid domain in the first cytosolic loop of the receptor. Stable transfection of each receptor cDNA into murine erythroleukaemia (MEL) cells resulted in the expression of receptors with high affinity for radiolabelled salmon calcitonin (hαCTR $K_d$ 0.09 nm, hβCTR $K_d$ 0.12 nm). Ligand competition binding studies did not reveal any significant pharmacological difference between the receptor isoforms. In transfected MEL cells and COS-1 cells the hβCTR isoform was expressed at tenfold higher levels than the hαCTR. A reporter gene assay that monitored the coupling of CTR to adenylate cyclase by increases in β-galactosidase activity indicated that both receptors were able to stimulate cyclic AMP production in response to ligand binding. Journal of Molecular Endocrinology (1995) 14, 179–189

INTRODUCTION

Calcitonin (CT) is a 32 amino acid peptide hormone that is secreted from the thyroid gland in response to elevations in serum calcium levels (Copp et al. 1962). High-affinity receptors for CT have been demonstrated in a range of tissues including the central nervous system (CNS) (Fischer et al. 1981), bone (Warshawsky et al. 1980), kidney (Warshawsky et al. 1980) and lung (Fouchereau-Peron et al. 1981). In the CNS, CT is thought to induce analgesia, gastric acid secretion and inhibition of appetite (Sexton 1991), whereas, in the periphery, the hormone has a hypocalcaemic effect that is mediated by inhibition of bone resorption by osteoclasts (Nicholson et al. 1986) and an increase in calcium excretion from the kidney (Carney & Thompson 1981). cDNAs encoding CT receptor (CTR) subtypes have recently been cloned from a number of sources including a porcine kidney cell line (LLC-PK1) (Lin et al. 1991), a human ovarian carcinoma cell line (BIN67) (Gorn et al. 1992) and rat brain (Albrandt et al. 1993, Sexton et al. 1993). Each cDNA encodes a seven transmembrane G protein-coupled receptor which, by sequence homology, belongs to a receptor subfamily containing the receptors for secretin (Ishihara et al. 1991), parathyroid hormone/parathyroid hormone-related peptide (Juppner et al. 1991), glucagon (Jelinek et al. 1993), glucagon-like peptide (Thorens 1992), vasoactive intestinal peptide (Ishihara et al. 1992) and growth hormone-releasing hormone (Mayo 1992). In this report we describe the identification of two different CTR cDNAs from the human breast carcinoma cell line T47D which encode CTR isoforms. Both receptors exhibit high-affinity binding of salmon and human CT when expressed in murine erythroleukaemia (MEL) and COS-1 cells. A reporter gene assay which measures intracellular cyclic AMP (cAMP) levels has been used to demonstrate that both receptor isoforms can couple to adenylate cyclase and elevate intracellular cAMP.
MATERIALS AND METHODS

Peptides

Salmon CT was obtained from Cambridge Research Biochemicals (Northwich, Cheshire, UK). 125I-Labelled salmon CT was obtained from Amersham International plc (Aylesbury, Bucks, UK). Amylin and CT gene-related peptide (CGRP) were obtained from Peninsula Laboratories (St Helens, Merseyside, UK) and human CT, adrenocorticotrophic hormone (ACTH) and parathyroid hormone (PTH) were purchased from Sigma Chemical Co. (Poole, Dorset, UK).

Recombinant DNA technology

Unless stated otherwise recombinant DNA techniques were as described by Sambrook et al. (1989). Total RNA was prepared from T47D cells that had been cultured in the presence of 50 μg hydrocortisone/ml. First strand cDNA was synthesized from 2.5 μg total RNA using the BRL superscript kit (BRL, Bethesda, MD, USA) and the primer 5'-AAGGATCCGTCGACATCGATAATACGACTCATATAGTTAGA(T)17-3'. To amplify CTR cDNA specifically four primers were used: primer 1 (positions 248-283), 5'-ATGAGTTCTACATTTACAAAGCCGGTGCTTGGCCTG-3'; primer 2 (positions 967-931), 5'-AAAATGCAAATCTTGCGAGCTCACCCGCTCCCTCG-3'; primer 3 (positions 884-919), 5'-ATTATCATCACCACCTGTTGGAAGTAGTACCCCAAT-3'; primer 4 (positions 1720-1865), 5'-TCAAGCAGATGACTCCTTGGCTGTATGATTCAAACGG-3'. PCR was performed at 92 °C for 2 min, 65 °C for 2 min and 72 °C for 2 min for 40 cycles using Taq polymerase (AmpliTaq) under conditions described by the manufacturer (Perkin Elmer Cetus, Norwalk, CT, USA). Amplified DNA fragments of the expected size were cloned directly into pCR11 (Invitrogen, San Diego, CA, USA) and sequenced on both strands. Full length cDNAs were constructed by fusing the 5' and 3' fragments at the unique SacI site (Fig. 1). Both cDNAs were modified to include SalI and NotI sites at the 3' end by insertion of the oligonucleotide 5'-CTGTGTGTGACGCGCCGCCGCA-3' at a unique Spel site in the pCR11 polylinker. For expression in MEL cells the 5' end of both cDNAs were modified by PCR to introduce a consensus translational initiation sequence 5'-GCCCACC-3' (Kozak 1984) immediately upstream of the ATG codon. The modified human CTR (hCTR) cDNAs were then cloned into the MEL cell expression plasmid (Needham et al. 1992) as BglII–NotI fragments. For expression in COS-1 cells the hCTR cDNAs were cloned into pRC/CMV vector (Invitrogen).

MEL cell expression studies

MEL-C88 cells were cultured in Dulbecco's modified essential medium supplemented with 10% fetal calf serum (FCS) and 2 mM glutamine. Prior to transfection, 50 μg of the appropriate expression construct were linearized by cleavage at the unique PvuI site in the ampicillin gene of the plasmid backbone. Linearized plasmid was transfected into MEL-C88 cells by electroporation as described by Needham et al. (1992). Individual clones were picked 7-10 days after transfection. For expression studies, cells were maintained in exponential growth for a period of at least 4 days and then dimethylsulphoxide was added to a final concentration of 2% (v/v) to induce differentiation and hence expression (Antoniou 1991). Samples for ligand binding were taken at 4 days post-induction.

Ligand binding assays

Cells were harvested, washed twice and resuspended in binding assay buffer (BAB; RPMI 1640, 10% heat-inactivated FCS, 15 mM Heps, 1 mg bacitracin/ml, pH 7.4) at a density of 10^6 cells/ml. This suspension (100 μl; 10^5 cells) was added to siliconized microcentrifuge tubes; a further 50 μl BAB were added to total binding assays, or 50 μl 4 x 10^-6 M unlabelled salmon CT to determine non-specific binding. The tubes were incubated at room temperature for 15 min and then 50 μl 4 x 10^-10 M 125I-labelled salmon CT were added to each reaction. Binding was allowed to continue at room temperature for 2 h. Ice-cold PBS (1 ml) was added to each tube to terminate the reaction. Bound and free ligand were separated by centrifugation.

FIGURE 1. Cloning of two isoforms of the hCTR. (a) Four oligonucleotides (P1–P4) were used to amplify the hCTR cDNA in two fragments. DNA sequence analysis identified two different forms of fragment A which differed by the presence of a 48 bp insert sequence encoding a 16 amino acid domain in the first cytosolic loop of the receptor; this domain is present in the haCTR and absent from the hbCTR. (b) Amino acid sequence of the hCTR and predicted membrane topology. The 16 amino acid insert present in the haCTR is located in the first cytosolic loop of the receptor as shown. DNA sequence analysis of fragment B from a number of cell types identified a single amino acid difference (Pro48,Leu) from the hCTR sequence presented in Gorn et al. (1992).
(10,000 g/5 min), cell pellets were washed twice with 1 ml PBS and the tubes were then counted in gamma counter vials. Triplicate data for each experimental point were averaged and the percentage of total counts was determined. Total counts for each assay were determined by counting a 50 μl aliquot of 125I-labelled salmon CT. $K_d$ values were determined directly from theoretical curves fitted by non-linear regression using the programme EnzFitter (Biosoft, Cambridge, Cambs, UK). Cell membranes were prepared from MEL cells by homogenization in lysis buffer (20 mM Hepes, pH 7.4, 100 mM NaCl, 0.1 mM phenylmethylsulfonyl fluoride, 1 mM iodoacetamide, 5 mM EDTA, pH 7.4) followed by centrifugation at 100,000 g for 45 min. Membranes were resuspended in 20 mM Hepes (pH 7.4) using a Teflon homogenizer. Protein concentrations were determined using the Pierce BCA reagent (Pierce, Rockford, IL, USA). Membranes were incubated at 25°C with $10^{-10}$ M 125I-labelled salmon CT in buffer (20 mM Hepes, 120 mM NaCl, 0.25% BSA, 0.1% bacitracin, pH 7.4) in a final reaction volume of 90 μl. Membrane concentrations were adjusted to provide 10% specific binding in all studies; under these conditions non-specific binding was approximately 1% of total added counts. Membrane-bound radiolabel was collected on DEAE-impregnated glass fibre filters pretreated with 0.1% polyethyleneimine. Filters were washed with ice-cold 10 mM Tris and 150 mM NaCl, pH 7.4, dried and then bound label was determined by scintillation counting.

**cAMP responsive reporter gene system**

The oligonucleotide 5'-TGACGTCACTCTGCTGCCGCTCA-3', containing a cAMP response element designed from the native sequences in the vasoactive intestinal peptide gene (Fink et al. 1988), was cloned into a vector containing the the β-galactosidase coding sequence and a minimal β-globin promoter (M Needham, unpublished observations). T47D and COS-1 cells were transiently transfected with this plasmid by the calcium phosphate co-precipitation method (Wigler et al. 1979). Twenty-four hours after transfection cells were treated with salmon CT ($10^{-7}$ M) or forskolin ($10^{-5}$ M; Sigma), and incubated for a further 48 h. Cells were then scraped from plates, harvested and resuspended in assay buffer (Lumi-Gal 530; Lumigen, Detroit, MI, USA). Aliquots were

**Figure 2.** Binding of 125I-labelled salmon CT to whole cells. (a) Binding to T47D cells, calculated $K_d$ by Scatchard analysis was 0.13 nM (inset). (b) Binding to MEL cells stably transfected with hCTR, calculated $K_d$ by Scatchard analysis was 0.09 nM (inset). (c) Binding to MEL cells stably transfected with hCTR, calculated $K_d$ by Scatchard analysis was 0.12 nM (inset).
**FIGURE 3.** Competition of $^{125}$I-labelled salmon CT binding. Membranes from MEL cells expressing (a) haCTR or (b) hßCTR were incubated with $^{125}$I-labelled salmon CT in the presence of increasing concentrations of unlabelled competitor peptide. No significant competition of binding was seen at either receptor with the two structurally unrelated competitor peptides PTH and ACTH. Each point represents the mean value from quadruplicate data sets.

**TABLE 1.** Competition binding studies. $K_i$ (± 95% confidence interval) values for binding of $^{125}$I-labelled salmon CT by a number of CT-related peptides to MELaCTR and MELßCTR membranes. Data were derived from two independent experiments, in which each experimental point was performed in triplicate.

<table>
<thead>
<tr>
<th>Peptide</th>
<th>hCTR</th>
<th>hßCTR</th>
</tr>
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<tbody>
<tr>
<td>Salmon CT</td>
<td>$1.3 (1.17-1.85) \times 10^{-10}$ M</td>
<td>$1.03 (0.9-1.2) \times 10^{-10}$ M</td>
</tr>
<tr>
<td>Human CT</td>
<td>$9 (6.5-12.9) \times 10^{-8}$ M</td>
<td>$2 (1.5-2.8) \times 10^{-8}$ M</td>
</tr>
<tr>
<td>CGRP</td>
<td>$4.7 (3.9-5.7) \times 10^{-7}$ M</td>
<td>$5.3 (4.3-6.5) \times 10^{-7}$ M</td>
</tr>
<tr>
<td>Amylin</td>
<td>$8 (4.2-15.6) \times 10^{-7}$ M</td>
<td>$7.9 (5.1-12.2) \times 10^{-7}$ M</td>
</tr>
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dispensed into Microlite tissue culture-treated 96-well microtitre plates (Dynatech Labs, Chantilly, VA, USA) and β-galactosidase activity measured by a 4-h incubation at 37 °C with the chemiluminescent substrate LumiGal.

**Northern hybridization**

Approximately $4 \times 10^7$ cells were harvested 4 days after induction, washed twice with saline and resuspended in 3 ml ice-cold NTE (0·1 m NaCl, 10 mM Tris–HCl, pH 8·0, 1 mM EDTA, pH 8·0) containing 2% Nonidet P-40. An equal volume of phenol–chloroform (1:1, v/v) was added to the suspension and mixed thoroughly. Following centrifugation (10 000 g/10 min) the aqueous layer was removed into a fresh tube containing a second volume of phenol–chloroform. RNA was precipitated from the final aqueous layer by the addition of 0·1 vol. 3 M NaOAc, pH 5·5, 2 vols ethanol, and incubation at −70 °C for 60 min. Precipitated RNA was harvested by centrifugation, washed with ice-cold 70% ethanol, and dissolved in distilled water. RNA was separated by electrophoresis in agarose gels containing 2·2 M formaldehyde, and then blotted onto nylon membrane (Hybond-N) in 20 × SSC (Sambrook et al. 1989). Membranes were hybridized under stringent conditions (Church & Gilbert 1984) using a $^{32}$P-labelled DNA fragment corresponding to the entire CTR cDNA.

**RESULTS**

**Cloning of two human CTR isoforms**

The human breast carcinoma cell line T47D has been shown to possess specific high-affinity CTRs (Findlay et al. 1980). We have identified two distinct CTR isoforms in T47D cells by PCR.
amplification of the hCTR cDNA from T47D mRNA. Oligonucleotide primers were designed to amplify the cDNA in two portions: a 5' fragment (A) and a 3' fragment (B) (Fig. 1a). Amplified DNA fragments of the expected size were cloned directly into the TA cloning vector, pCR II (InVitrogen), and sequenced on both strands. Analysis of recombinant clones carrying the 5' fragment revealed that two distinct cDNAs had been isolated that were identical in sequence except for the presence of a 48 bp sequence at positions 523–571 (where position 1 is the A residue of the ATG codon). The amino acid sequence of the hCTR from BIN67 cells is 73% identical to the porcine receptor (Gorn et al. 1992), but the hCTR contains an insert of 16 amino acids between transmembrane domains I and II. We have isolated two human cDNA fragments from T47D cells, one of which encodes a receptor with the 16 amino acid sequence and a second that does not. We conclude, therefore, that there are at least two isoforms of the hCTR expressed in T47D cells. We have designated the receptor with the 16 amino acid insert hCTR, and the receptor lacking the insert hβCTR. DNA sequencing of a number of recombinants carrying fragment B confirmed that the 3' sequences of our clones were identical to the cDNA from BIN67 cells except for a single base change (C1387T) which resulted in a Leu463Pro codon change in the predicted receptor sequence (Fig. 1b).

Two lines of evidence indicate that this sequence is authentic: first, the nucleotide change was observed in fragments isolated from a number of independent PCRs, and secondly, the sequence of the hCTR isolated from human kidney mRNA carried the same codon change (data not shown).

**Heterologous expression and pharmacological characterization of hCTRs**

Binding of radiolabelled CT to T47D cells indicated the presence of a single class of CTR with an apparent $K_d$ of 0.13 nM (Fig. 2a). To characterize the ligand-binding properties of the hCTR and hβCTR isoforms, receptor cDNAs were expressed in the LCR/MEL system (Needham et al. 1992, Shelton et al. 1993). This expression system allows the rapid and simple identification of stable high-level expressing cell lines. Full length cDNAs were created by splicing the two different 5' fragments with a 3' fragment at a unique ScaI site. The 5' end of each receptor cDNA was modified to include a consensus translation initiation sequence (Kozak 1984) and then cloned into the MEL cell expression vector (Needham et al. 1992). Expression levels were determined by binding of $^{125}$I-labelled salmon CT to cells. Two cell lines expressing the hCTR (MELαCTR) or the hβCTR (MELβCTR) that displayed high-level specific binding of ligand were isolated. MEL cells
transfected with the expression vector alone did not exhibit any specific binding of 125I-labelled salmon CT. Scatchard analysis of 125I-labelled salmon CT binding revealed that both receptor isoforms have similar affinities for ligand: hCTR has an apparent $K_d$ of 0.09 (± 0.006) nM and is expressed at approximately 2.8 x 10^6 sites per cell (Fig. 2b); the hβCTR has an apparent $K_d$ of 0.12 (± 0.03) nM and is expressed at 1.04 x 10^6 sites per cell (Fig. 2c). In competition binding studies using membranes from MEL cells expressing either hCTR or hβCTR, both receptors demonstrated similar affinities for a range of CT-related ligands (Fig. 3). Salmon CT potently inhibited binding of 125I-labelled salmon CT to both receptors, human CT was approximately two orders of magnitude less potent than salmon CT, and two CT-related peptides, CGRP and amylin, were only weak inhibitors. Two peptides unrelated to CT, PTH and ACTH, did not compete for binding at either receptor (Table 1).

Both CTR isoforms couple to adenylate cyclase

T47D cells undergo a rapid activation of adenylate cyclase when treated with CT and it is sustained for long periods of time following removal of the hormone (Lamp et al. 1981). The hCTR and hβCTR isoforms contain a motif (R-X_11-K-A-V-K_359) in the third cytosolic domain that is postulated to be involved in coupling to $G_{q}$ (Okamoto et al. 1991). We have used a reporter gene assay to investigate the functional coupling of CTRs to adenylate cyclase. Increased levels of cAMP result in activation of protein kinase A (Edelman et al. 1987) and induction of gene transcription via cAMP response elements in the promoter region of target genes (Roesler et al. 1988). To monitor cAMP levels, the expression of the E. coli lacZ gene was placed under the control of a synthetic cAMP response element, based on the native response sequences found in the promoter of the vasoactive intestinal peptide gene (Fink et al. 1988); increases in intracellular cAMP levels therefore result in increased β-galactosidase activity. T47D cells expressing native CTRs were transiently transfected with the reporter gene plasmid (Fig. 4a), and were then treated with salmon CT and the intracellular β-galactosidase activity was measured. The reporter gene produced a low basal level of gene expression which was induced five- to sevenfold upon exposure to salmon CT (Fig. 4b); the accumulation of β-galactosidase activity peaked 24 h following addition of hormone and remained stable for up to 72 h. Direct measurement of cAMP in T47D cells by RIA indicated a five- to tenfold increase in cAMP levels following exposure to 10^{-7} M salmon CT.
(data not shown); we therefore believe that this reporter gene system accurately monitors coupling of receptors to adenylate cyclase. To determine if haCTR and hβCTR can couple to adenylate cyclase, receptor expression plasmids and reporter gene constructs were transiently co-transfected into COS-1 cells. Transfected cells were incubated with \(10^{-7}\)M salmon CT for 48 h and then \(\beta\)-galactosidase activity was determined. COS-1 cells transfected with either CTR exhibited a three- to fivefold increase in \(\beta\)-galactosidase activity (Fig. 5). Control COS-1 cells transfected with only the reporter gene construct or the receptor expression plasmid did not express \(\beta\)-galactosidase activity after salmon CT treatment; all cells exhibited equivalent cAMP production in response to \(10^{-5}\)M forskolin, which directly activates adenylate cyclase (Seamon & Daly 1986). We conclude, therefore, that in COS-1 cells both receptor isoforms are coupled to adenylate cyclase and that ligand binding stimulates intracellular cAMP levels.

**Differences in the levels of receptor expression**

Saturation binding of \(^{125}\)I-labelled salmon CT to MEL cells expressing human CTRs indicated that the hβCTR was expressed at higher levels than the haCTR. The results presented in Fig. 2 indicate a fourfold difference in the number of ligand binding sites. Analysis of other MEL cell clones expressing receptor cDNAs revealed that the β isoform was always expressed at four to ten times the levels of the haCTR. The two receptor expression plasmids used in these experiments were identical except for the presence of the 48 bp insert in the hCTR cDNA. To investigate further the effect of this sequence on receptor expression levels, receptor cDNAs were transiently expressed in COS-1 cells. In a typical experiment the haCTR was expressed at \(1.17 \times 10^5\) ligand binding sites per cell (195 fm/10\(^6\) cells) and the hβCTR at \(6.57 \times 10^5\) sites/cell (1092 fm/10\(^6\) cells). Similar results were obtained when the receptors were expressed in mouse fibroblast cells (data not shown). We conclude that the level of functional ligand binding sites is influenced by the 16 amino acid sequence and that this phenotype is not cell-type dependent.

Transcription of receptor cDNAs in MEL cells was examined in a Northern hybridization experiment in which the receptor cDNA was hybridized to total RNA (Fig. 6), the RNA was also probed with the mouse \(\beta\)-globin cDNA as a control for the induction of MEL cell differentiation (Needham et al. 1992). We failed to detect any significant difference in the levels of CTR mRNA in MELαCTR and MELβCTR cells, indicating that the different levels of receptor expression were not due to increased hβCTR mRNA levels.

**DISCUSSION**

The peptide hormone CT stimulates several physiological responses when administered to animals. The hormone is implicated in calcium homeostasis, modulation of nociception and food intake, and is a potent natriuretic agent. This is consistent with the action of CT at multiple organs and the existence of multiple hormone receptor subtypes. In this paper we report the identification of two high-affinity CTRs, haCTR and hβCTR, from the human breast cancer cell line T47D. To our knowledge this is the first documentation of multiple CTR isoforms from a single cell line. The predicted amino acid sequences of the two receptors are identical, except for the presence of a 16 amino acid sequence in the first cytosolic domain of the haCTR receptor. The two receptor mRNAs may be expressed from independent genes or they may arise from alternative splicing of a primary transcript. Alternative splicing to produce altered receptor isoforms has been previously documented in a number of G protein-coupled receptors; two dopamine receptor isoforms (D2A and D2B) that differ by a 29 amino acid insert in the third intracellular domain have been demonstrated in human, bovine and rat brain (Dal Torso et al. 1989). Recently, two isoforms of CTR that are identical in amino acid sequence except for the presence in one receptor of a 37 amino acid insert in the second extracellular domain have been demonstrated in rat brain. It has been suggested that the mRNAs encoding these receptors are generated by alternative splicing, although no consensus splice sequences were found in the nucleotide sequences surrounding the insert sequence (Albrandt et al. 1993, Sexton et al. 1993).

The haCTR and hβCTR exhibited similar affinities for radiolabelled salmon CT when heterologously expressed in the LCR/MEL system. The two receptors also displayed essentially equivalent responses to hCT and CT-related peptides in ligand competition binding experiments. We have therefore been unable to identify any pharmacological differences between the two receptor subtypes. This contrasts with the two CTR isoforms isolated from rat brain, where the presence of a 37 amino acid insert in the second extracellular loop results in significantly lower affinity for salmon CT and only negligible affinity for hCT (Albrandt et al. 1993, Sexton et al. 1993).
Heterologous expression of each receptor subtype has revealed a potentially important difference between the haCTR and hßCTR. We consistently observed a four- to tenfold increase in ligand binding sites in cell lines expressing hßCTR compared with cell lines expressing the haCTR. This difference was observed in a number of different cell types and was independent of whether the cell was stably or transiently transfected. We speculate that the 16 amino acid domain, present in haCTR, influences some post-transcriptional event in the biosynthesis of the receptor that is required for ligand binding activity, such as receptor folding in the membrane, or that the 16 amino acid domain interacts with a cytosolic component(s) that prevents ligand binding. Insertion of the 16 amino acid domain into haCTR generates the sequence Arg-Lys-Leu-Thr, where only the Arg residue is present in hßCTR. This sequence is recognized by cAMP-dependent kinase (Kennelly & Krebs 1991), and consequently haCTR and hßCTR could be differentially affected by cAMP levels. The presence of a putative phosphorylation site in the first intracellular loop could result in uncoupling of the receptor from G protein or a susceptibility to down-regulation and internalization.

Recent studies have revealed that the CTR has a dual function as a hormone receptor and as a calcium sensor (Stroop et al. 1993). Baby hamster kidney cells expressing a recombinant human receptor were observed to respond to extracellular levels of calcium chloride by a rapid and sustained increase in intracellular levels of free calcium, and in these experiments the increase in intracellular calcium was proportional to the number of receptors expressed. Further studies are required to establish if the haCTR and hßCTR isoforms can both function as calcium sensors, but the difference in expression levels between the two receptors may provide a mechanism for calcium homeostasis, by which cells expressing CTRs can control their response to extracellular levels of calcium.

There is increasing evidence of heterogeneity in the CTR family. To date, five receptor cDNAs have been characterized from which the porcine CTR, the hßCTR and the rat C1a receptor appear to define a receptor prototype; the rat C1b receptor has a 37 amino acid insert in an extracellular domain that confers altered ligand specificity, and the haCTR has a 16 amino acid sequence in an intracellular domain that influences the number of receptor binding sites. Further evidence of heterogeneity within the CTR family is the ability of the porcine CTR to regulate both cAMP production and intracellular calcium (Chabre et al. 1992), in a cell cycle-dependent manner (Chakraborty et al. 1991). The physiological significance of different receptor isoforms and the coupling to multiple signal transduction pathways is unclear, but is indicative of divergent pathways that are responsive to CT. Further characterization of the repertoire of CTRs in particular cell types and the response of these receptors to ligand binding is required, to understand better the multiple actions of CT; our current studies are addressing some of these questions.

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