

Identification of human endogenous calcitonin receptor in 293H cell line: characterization by cyclic AMP and calcium mobilization

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Introduction

Calcitonin receptor (CTR) belongs to an emerging subfamily (type 2) of seven transmembrane GPCR with pleiotropic coupling (Gs/Gq) in several recombinant systems. CTR has been identified in tissues such as kidney, brain, lung, placenta, ovaries.

There is only one human CTR gene but alternative splicing has generated three different isoforms.

The magnitude of the maximal cAMP response in isoform 2 compared to isoform 1 (10-100 fold) appears to reflect the importance of the absence of a 16 amino acids insert in isoform 1 implicated in the Gs protein coupling (Gorn et al, J.Clin.Inves. (1995) 95: 2680-2691).

The present study used cAMP, inositol phosphate 1 (IP1) measurements and calcium mobilization to characterize calcitonin receptor in 293H cell line.

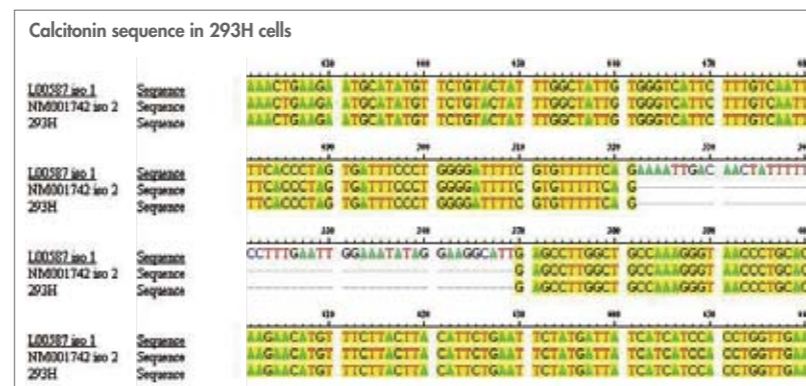
This presentation describes a new model of the endogenous isoform 2 calcitonin receptor. This cellular model is well adapted to serum-free medium and suspension culture for large scale production which can be used as a tool in primary functional screening.



Methods

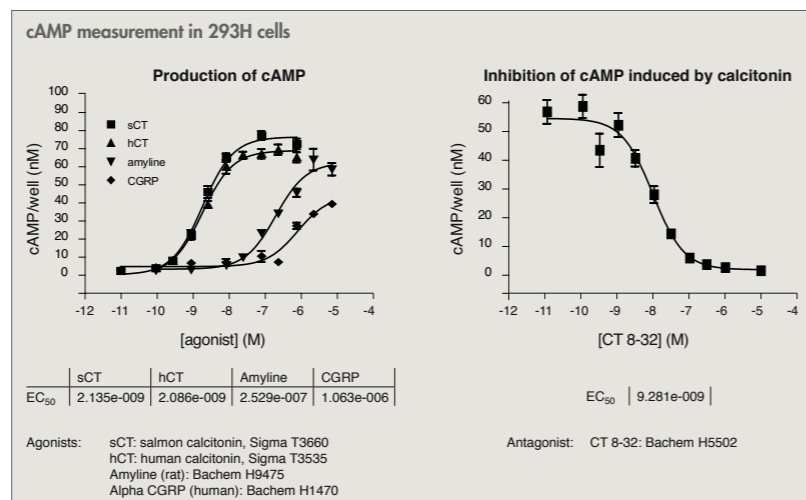
Cloning

- Cells were cultured in DMEM + 5% dialysed FCS at 37°C and 5% CO₂
- Total RNA was extracted from cultured cells using Tri-reagent (Sigma), cDNA produced by MMLV reverse transcriptase and random hexamers (Clontech).
- The cDNA was amplified by PCR and cloned into T/A cloning pCr2.1 vector (Invitrogen) then introduced into TOP10. Several clones were amplified and confirmed by sequencing and aligning analysis with human calcitonin gene (P30988), isoform 1 (L00587), isoform 2 (NM_001742).



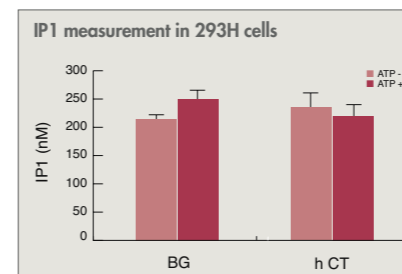
cAMP measurements

- cAMP measurements were performed via the HTRF® (homogeneous time-resolved fluorescence) technology (CisBio International).
- Cells were cultured under standard conditions. Cells were suspended in assay buffer (HBSS, glucose and IBMX) at a density of 20,000 cells/well. After incubation for 30 min at 37°C, an equal volume of assay buffer containing calcitonin agonist was added to the cells. After incubation for 30 min at room temperature, cAMP was measured.



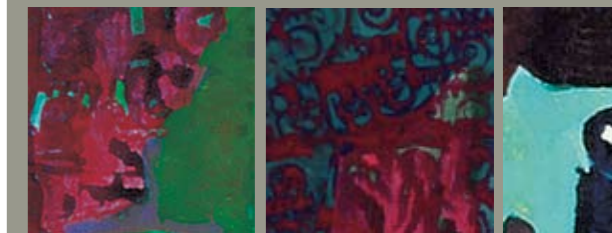
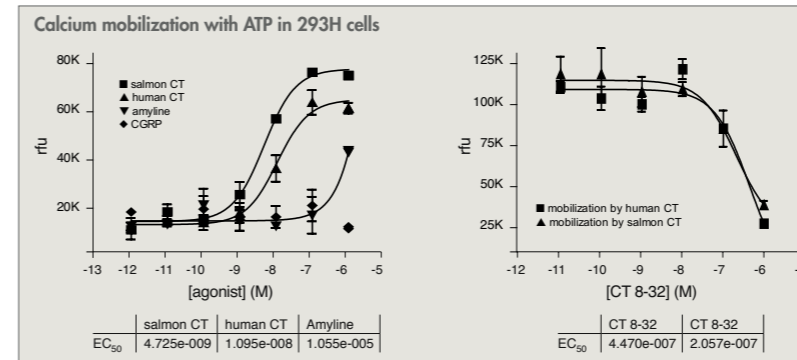
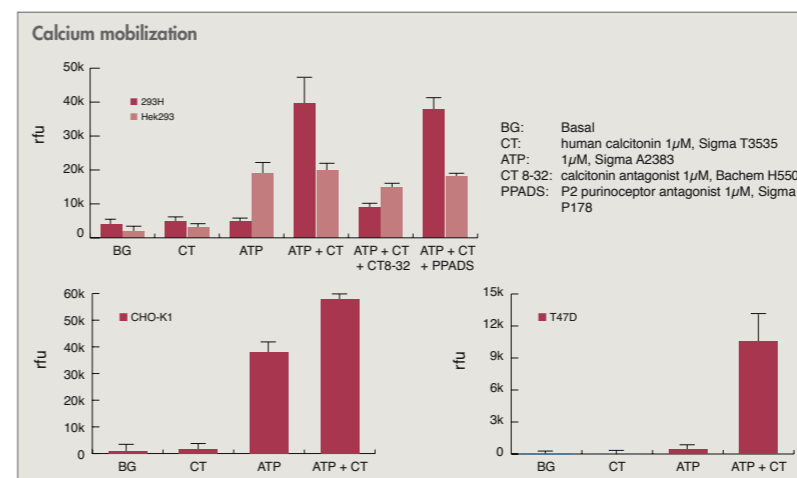
IP1 measurements

- IP1 measurements were performed via the HTRF® technology (CisBio).
- Cells were cultured under standard conditions. Cells were suspended in assay buffer at a density of 30,000 cells/well. After incubation for 5 min at 37°C, calcitonin agonist with or without ATP was added to the cells. After incubation for 30 min at 37°C, IP1 was measured.



Calcium mobilization

- Calcium mobilization was measured using a fluorescence calcium probe (Molecular Device).
- Cells were cultured under standard conditions. Cells were dispensed at a density 20,000 cells/well. After 24 to 48 hours of culture, an equal volume of dye loading buffer (containing HBSS, glucose, Probenicid, CaDye) was added to the cells. After 1 hour of incubation at 37°C, calcitonin agonist with or without ATP was added and calcium mobilization was then measured on a CellLux (Perkin Elmer).



Conclusion

- The presence of endogenous isoform 2 in the 293H cells was demonstrated where the parental cell line Hek293 does not express calcitonin receptor.
- We have confirmed the Gs coupling by cAMP enhancement induced by various calcitonin receptor agonists. This enhancement was further reversed by a selective antagonist validating the pharmacological properties on this receptor.
- The Gq coupling could not be demonstrated by the IP1 measurements in the endogenous model used in this study.
- ATP dependent calcium mobilization was demonstrated in 293H cell line. This phenomena was further observed in two other cell lines expressing endogenous calcitonin receptor. The pharmacological values found were similar to those obtained by cAMP measurements.
- The ATP dependent cytosolic Ca²⁺ increase is not mediated by purinergic receptor expressed in 293H suggesting an unknown mechanism.
- 293H cell line is a very interesting model for calcitonin receptor studies easily adapted for developing a HTS assay.

Acknowledgements

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