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


Introduction
Calcitonin receptor (CTR) belongs to a 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.

Methods

Cloning
- Cells were cultured in DMEM + 5% dialysed FCS at 37°C and 5% CO2
- Total RNA was extracted from cultured cells using Trizolreagent (Sigma), cDNA produced by MMVI 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 (P30998), isoform 1 (100587), isoform 2 (NM_007172).

CAMP measurements
- cAMP measurements were performed via the HTTR® (homogeneous time-resolved fluorescence) technology (Caltia International).
- Cells were cultured under standard conditions. Cells were dispensed at a density 20,000 cells/well. After 24 to 48 hours of culture, a equal volume of dye loading buffer (containing HBSS, glucose and IBMX) 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).

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 30,000 cells/well. After 24 to 48 hours of culture, a equal volume of dye loading buffer (containing HBSS, glucose, Probenicid, CaDye) was added to the cells. After 30 min at 37°C, cAMP was measured.

Calcium mobilization with ATP in 293H cells

CAMP measurements
- cAMP measurements were performed via the HTR® technology (CaltiaBio).
- 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.

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 cAMP 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 expression 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.

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