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Molecular and functional profiling of histamine receptor-mediated calcium ion signals in different cell lines

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ABSTRACT

Calcium ions (Ca^{2+}) play a pivotal role in cellular physiology. Often Ca^{2+}-dependent processes are studied in commonly available cell lines. To induce Ca^{2+} signals on demand, cells may need to be equipped with additional proteins. A prominent group of membrane proteins evoking Ca^{2+} signals are G-protein coupled receptors (GPCRs). These proteins register external signals such as photons, odorants, and neurotransmitters and convey ligand recognition into cellular responses, one of which is Ca^{2+} signaling. To avoid receptor cross-talk or cross-activation with introduced proteins, the repertoire of cell-endogenous receptors must be known. Here we examined the presence of histamine receptors in six cell lines frequently used as hosts to study cellular signaling processes. In a concentration-dependent manner, histamine caused a rise in intracellular Ca^{2+} in HeLa, HEK 293, and COS-1 cells. The concentration for half-maximal activation (EC_{50}) was in the low micromolar range. In individual cells, transient Ca^{2+} signals and Ca^{2+} oscillations were uncovered. The results show that (i) HeLa, HEK 293, and COS-1 cells express sufficient amounts of endogenous receptors to study cellular Ca^{2+} signaling processes directly and (ii) these cell lines are suitable for calibrating Ca^{2+} biosensors in situ based on histamine receptor evoked responses.

Immortalized cell lines are an established system to study a plethora of cellular signaling processes. Usually foreign genes are transfected into such cell lines, allowing transient or constitutive expression of the encoded protein(s). To examine the functional properties of heterologously expressed proteins, sophisticated biochemical, biophysical, and pharmacological methods have been developed. Typical cellular signaling events start on the cell surface, where membrane receptors interact with external physical or chemical ligands (for reviews, see Refs. [1–5]). Once they are recognized by their receptors, ligand binding is transduced to the intracellular compartment and results in changing the activity profile of various downstream targets, including enzymes, transcription factors, and ion channels (for reviews, see Refs. [6–9]). Membrane receptor-mediated signaling often results in transient changes of second messenger concentrations such as 3′,5′-cyclic adenosine monophosphate (cAMP),[2] 1,4,5-inositol-trisphosphate (IP_3), and calcium ions (Ca^{2+}). It is these small molecules that participate in various aspects of cellular physiology, including cytoskeletal rearrangement, secretory processes, and gene regulation [10–14]. During recent years, much effort has been put forth to exploit the dynamics of second messengers with high temporal and spatial resolution. To this end, immortalized cell lines are a versatile source to set up experimental conditions. However, the endogenous repertoire of membrane receptors and downstream effectors is often unknown or ill-defined in commonly used cell lines. Our research is focused on the dynamics of receptor-evoked Ca^{2+} signaling. To overcome gene delivery to cells, we decided to uncover the presence of endogenous receptors evoking Ca^{2+} signals in a series of six commonly used laboratory cell lines. From our previous studies, we already knew that a human embryonic kidney cell line (HEK 293) expresses histamine receptors (HR) [15]. Histamine is a biogenic amine with important gastrointestinal, immune, cardiovascular, and neuronal functions [16]. In mammals, four genes have been identified encoding histamine receptors, which belong to the super gene family of G-protein coupled receptors (GPCRs). Except for HR subtype 2 (HRH2), which activates adenylyl cyclase, HRH1, HRH3, and HRH4 evoke IP_3 production that leads to Ca^{2+} release from intracellular stores [17].

Here we report the molecular and functional characterization of histamine receptors expressed in six established laboratory cell lines. MDCK cells, HeLa cells, two HEK 293 cell lines from different

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[2] *Abbreviations used:* cAMP, 3′,5′-cyclic adenosine monophosphate; IP_3, 1,4,5-inositol-trisphosphate; Ca^{2+}, calcium ion; HR, histamine receptor; GPCR, G-protein coupled receptor; HRH1/2/3/4, HR subtype 1/2/3/4; ECACC, European Collection of Cell Cultures; ATCC, American Type Culture Collection; FCS, fetal calf serum; AB/AM, antibiotics/antimycotics; DMEM, Dulbecco's modified Eagle's medium; h, human; mRNA, messenger RNA; cDNA, complementary DNA; RT, reverse transcriptase; PCR, polymerase chain reaction; ECS, extracellular solution; $[Ca^{2+}]_i$, intracellular Ca^{2+} concentration.

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cell collection resources, COS-1 cells, CHO-K1 cells, and 3T6 Swiss Albino fibroblasts were monitored for the presence of histamine receptors by Ca^{2+} fluorimetry. From these lines, HeLa cells, one HEK 293 cell line, and COS-1 cells showed Ca^{2+} signals on histamine stimulation. Using a set of receptor-specific primers, we identified HRH1 as the most likely receptor being expressed in these lines. Our results provide a molecular fingerprint that facilitates studying receptor-evoked Ca^{2+} signaling in model cell lines and simultaneously makes introducing exogenous genes superfluous. The data also allow for selecting cell lines where potential cross-talk to (in this case) histamine receptors must be excluded.

Materials and methods

Origin and propagation of cell lines

Cell lines were obtained from the European Collection of Cell Cultures (ECACC): (i) HEK 293 (85120602, human embryonic kidney), (ii) HeLa (93021013, human cervix carcinoma), (iii) CHO-K1 (85051005, Chinese hamster ovary), (iv) COS-1 (88031701, African green monkey), (v) 3T6 Swiss Albino (86120801, Swiss Albino mouse embryo fibroblast), and (vi) MDCK (84121903, canine cocker spaniel kidney). An independent batch of HEK 293 cells (vii) was obtained from American Type Culture Collection (ATCC, CRL-1573). Cells were grown in the following media. HEK 293 cells from ECACC were adapted to a low glucose-containing medium (HEK 293^{M10}, M10 = minimal essential medium [MEM] + Glutamax, Life Technologies, Carlsbad, CA, USA), 10% (v/v) fetal calf serum (FCS, Life Technologies), 1% (v/v) antibiotics/antimycotics (AB/AM, Life Technologies), and 1% (v/v) nonessential amino acids (Life Technologies). HeLa cells were grown in DH10 medium (Dulbecco's modified Eagle's medium [DMEM] + Glutamax, high glucose, Life Technologies), 10% (v/v) FCS, and 1% (v/v) AB/AM. CHO-K1 cells were grown in F12 medium (Ham's F-12, Life Technologies), 10% (v/v) FCS, and 1% (v/v) AB/AM. COS-1 cells and 3T6 Swiss Albino cells were grown in DH10 medium. MDCK cells were grown in M10 medium. The HEK 293 cells obtained from ATCC were grown in DH10 medium (HEK 293^{DH10}). Cells were kept in 9-cm petri dishes at 37 °C, 5% CO_2, and approximately 95% relative humidity. Cells were trypsinized twice a week when they reached confluence and were seeded at densities of 8 to 14 × 10^5 cells onto new petri dishes.

Molecular identification of expressed histamine receptors

In previous studies, we observed that HEK 293^{M10} cells possess endogenous histamine receptors that cause an increase of intracellular Ca^{2+} [15]. To identify the molecular identity of histamine receptors expressed in HEK 293^{M10} and the other cell lines, we designed specific primers against histamine H1, H3, and H4 receptors. These isoforms have been described to mediate Ca^{2+} signals on activation, whereas histamine H2 receptors were shown to specifically activate adenylyl cyclase and thereby cause cAMP production [16]. For amplification of receptor-specific fragments, for each receptor regions were chosen that are well conserved across species. For the H1 receptor, the forward primer was 5′-CATCTTC TTCATGGTCATTGCC-3′ and the reverse primer was 5′-TTGAATGT CTTCTTGAAGTTCTC-3′. Amplification results in a 145-bp fragment covering residues 1295 to 1439 in the open reading frame of human (h)HRH1 (accession no. NM_001098213). For the H3 receptor, the forward primer was 5′-CTCGCCATCTCCGACTTCC-3′ and the reverse primer was 5′-GCGCCTCTGGATGTTCAGG-3′. Amplification results in a 461-bp fragment covering residues 225–685 in the open reading frame of hHRH3 (accession no. NM_007232). For the H4 receptor, the forward primer was 5′-GACA(G/A)AAACCTTA GACATCGAAG-3′ and the reverse primer was 5′-CAG(C/T)AC(C/T) CCAAAC(G/A)GCCACCAT-3′. Amplification results in a 300-bp fragment covering residues 127–426 in the open reading frame of hHRH4 (accession no. AY136745). All primers were synthesized by Eurofins MWG/Operon (Ebersberg, Germany).

From all cell lines, messenger RNA (mRNA) was prepared with the AllPrep RNA/Protein Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. RNA was immediately transcribed into first-strand complementary DNA (cDNA) using oligo$(dT)_{12–18}$ primers and reverse transcriptase (M-MLV–RT, both from Life Technologies). Briefly, RNA was incubated with 0.8 μg of oligo$(dT)_{12–18}$ for 10 min at 65 °C. For hybridization, primers and mRNA were incubated for 2 min on ice. Finally, the reaction volume was adjusted to 50 μl by the addition of dNTPs (1 mM), RNaseOUT (40 U, Life Technologies), First-Strand Buffer (250 mM Tris–HCl [pH 8.3], 375 mM KCl, and 15 mM $MgCl_2$), dithiothreitol (DTT, 10 mM), and M-MLV–RT (400 U). After 1 h at 37 °C, M-MLV–RT was inactivated for 10 min at 65 °C. The cDNA was stored at −80 °C.

To test for the presence of HR subtypes in different cell lines, 0.5 μl of cDNA products or 5 ng of plasmid DNA harboring m*hrh*1, -3, or -4 cDNA were used as template for amplification employing HR subtype-specific primers and 0.4 U of KOD Hot Start Polymerase (Merck–Millipore, Nottingham, UK). In addition, β-actin primers were used to amplify a reference gene. The polymerase chain reaction (PCR) protocol was as follows: denaturation for 2 min at 94 °C (1 cycle), followed by 35 cycles with denaturation at 95 °C for 20 s, annealing for 20 s at a temperature that depended on the lowest melting point (T_m) of the two primers, and elongation at 72 °C for 2 min. Reactions to amplify *hrh*4 fragments were supplemented with dimethyl sulfoxide (DMSO) to a final concentration of 4% (v/v). PCRs with double-distilled water (ddH_2O) instead of DNA served as controls.

Monitoring functional histamine receptor activity

The ability of histamine to induce Ca^{2+} signals in the different cell lines was monitored with the Ca^{2+}-sensitive fluorescence dye Fluo-4. Cells were grown in 96-well dishes to a density of approximately 2 × 10^4 cells per well. Cells were loaded at room temperature with Fluo-4 AM as described previously [18]. After 90 min, the loading solution was substituted for dye-free ECS (extracellular solution; 120 mM NaCl, 5 mM KCl, 2 mM $MgCl_2$, 2 mM $CaCl_2$, 10 mM Hepes, and 10 mM glucose, pH 7.4 [NaOH]). The plate was transferred into a fluorescence reader (FLUOstar Omega, BMG Labtech, Offenburg, Germany) to monitor Fluo-4 fluorescence. The excitation wavelength was 485 nm. Fluorescence emission was detected at 520 nm. A concentration series of histamine (10^{-9} to 10^{-4} M) was added once Fluo-4 fluorescence had reached a stable value in each well. The changes in Fluo-4 fluorescence were recorded automatically. Concentration–response curves were established from at least two independent experiments.

Alternatively, Ca^{2+} signals were monitored in single cells using an upright laser-scanning fluorescence microscope (BX51, Olympus, Hamburg, Germany) with 2-photon excitation (Mai Tai HPDS, 80-fs pulse length, 75-MHz repetition frequency, Newport Spectra-Physics, Darmstadt, Germany). Cells were seeded on poly-l-lysine-coated glass coverslips. Cells were loaded with Fluo-4. For measurements, a coverslip was transferred into a measuring chamber filled with ECS. Cells were superfused with ECS via a perfusion system that was connected to the measuring chamber. To stimulate cells, the perfusion system was switched to ligand containing ECS. Excitation was at 800 nm. Images were collected and processed using the software FV300 (Olympus Optical) at 1 Hz (256 × 256 pixels). Data were analyzed and displayed by using Prism 5.04 software (GraphPad, San Diego, CA, USA).