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Analysis of the histamine H_2-receptor in human monocytes

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ABSTRACT

Histamine receptors are G-protein-coupled receptors (GPCRs). Canonically, the histamine H_2-receptor (H_2R) couples to G_s-proteins and activates adenylyl cyclases (ACs) with subsequent adenosine-3′,5′-cyclic monophosphate (cAMP) generation. Recently, the classic two-state model describing GPCR activation has been extended to a multiple-state model implying that any ligand stabilizes a ligand-specific receptor conformation, also referred to as functional selectivity. In our present study we pharmacologically characterized the H_2R in human monocytes. We found dissociations in the effects of histamine (HA) and H_2R agonists on cAMP accumulation and inhibition of formyl peptide-induced production of reactive oxygen species (ROS). In addition, we excluded participation of protein kinase A (PKA) in HA-induced ROS inhibition. Comparison of the potencies and efficacies of H_2R agonists in monocytes, neutrophils and eosinophils unmasked cell type-specific pharmacological profiles of individual ligands. Taken together, our data extend the concept of functional selectivity to the H_2R in human monocytes and demonstrate striking cell-type specificity in the pharmacological profile of the H_2R.

1. Introduction

Monocytes are blood cells of the innate immune system. During infection, generation of cytokines or microbial products leads to migration of monocytes into peripheral tissue and subsequent differentiation into macrophages or dendritic cells (DCs) [1,2]. Moreover, monocytes are phagocytes and play a crucial role in the defense against microbial pathogens [3]. Upon exposure to specific stimuli, monocytes release reactive oxygen species (ROS) produced by NADPH oxidase [4,5]. The bacterial peptide N-formyl-L-methionyl-L-leucyl-L-phenylalanine (fMLP) is a well-known stimulus for NADPH oxidase activation in monocytes [6,7]. Following binding to the G_i-protein-coupled formyl peptide receptor (FPR) [8], fMLP activates phospholipase C (PLC), hydrolyzing phosphatidylinositol-4,5-bisphosphate (PIP_2) into inositol-1,4,5-trisphosphate (IP_3) and 1,2-diacylglycerol (DAG) [9]. DAG activates protein kinase C (PKC) [9] followed by NADPH oxidase activation [10].

Histamine (HA) inhibits fMLP-induced ROS release in myeloid cells *via* H_2R [11,12] and is available as post-consolidation therapy for patients suffering from acute myeloid leukemia (AML) [13]. The mechanism of action of HA is assumed to involve activation of the H_2R in myeloid cells. Targeting the H_2R results in inhibition of ROS generation and prevents T-cells and natural killer (NK)-cells from undergoing apoptosis [14]. HA mediates its effects *via* four histamine receptor subtypes: H_1R, H_2R, H_3R and H_4R [15]. The H_2R is expressed on human monocytes [16,17]. Canonically, the H_2R couples to G_s-proteins. Stimulation of G_s leads to activation of adenylyl cyclases (ACs) and subsequent accumulation of adenosine-3′,5′-cyclic monophosphate (cAMP) [18]. Additionally, in some cell systems the H_2R couples non-canonically to G_q-proteins resulting in PLC activation and increases in intracellular calcium concentration ($[Ca^{2+}]_i$) [19,20]. The H_2R can also mediate non-canonical activation of ERK1/2 *via* ß-arrestin [21].

The two-state model of GPCR activation describes two distinct receptor conformations where the receptor exists either in an

Abbreviations: AC, adenylyl cyclase; AM, amthamine; AML, acute myeloid leukemia; ATP, adenosine 5′-triphosphate; $[Ca^{2+}]_i$, intracellular calcium concentration; cAMP, adenosine-3′,5′-cyclic monophosphate; DC, dendritic cell; DI, dimaprit; EPAC, exchange protein directly activated by cAMP; FAM, famotidine; fMLP, N-formyl-L-methionyl-L-leucyl-L-phenylalanine; FSK, forskolin; GPCR, G-protein-coupled receptor; HA, histamine; H_2R, histamine H_2-receptor; IBMX, 3-isobutyl-1-methylxanthine; IM, impromidine; JNJ, 1-[(5-chloro-1H-indol-2-yl)carbonyl]-4-methylpiperazine (JNJ7777120); MEP, mepyramine; PKA, protein kinase A; ROS, reactive oxygen species; Rp-cAMPS, adenosine-3′,5′-cyclic monophosphorothioate, Rp-isomer; Rp-8-Br-cAMPS, 8-bromoadenosine-3′,5′-cyclic monophosphorothioate, Rp-isomer; Sp-cAMPS, adenosine-3′,5′-monophosphorothioate, Sp-isomer; Sp-cAMPS-AM, adenosine-3′,5′-monophosphorothioate acetoxymethyl ester, Sp-isomer; Sp-8-Br-cAMPS-AM, 8-bromoadenosine-3′,5′-cyclic monophosphorothioate acetoxymethyl ester, Sp-isomer; THIO, thioperamide; 5-MHA, 5-methylhistamine; 8-pCPT-2′-O-Me-cAMP, 8-(4-chlorophenylthio)-2′-O-methyladenosine-3′,5′-cyclic monophosphate..

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inactive (R) or active (R*) state. Binding of agonists stabilize the R* state facilitating GPCR activation [22,23]. This concept has been replaced by a more complex multiple-state model. In terms of the extended model, each ligand stabilizes a ligand-specific receptor conformation resulting in distinct activation of G-protein-dependent as well as G-protein-independent signaling pathways. This concept of ligand-specific GPCR conformations is also designated as functional selectivity or biased agonism [24,25].

The H_2R in human monocytes has only been poorly studied so far. We already reported functional selectivity of the H_2R in human eosinophils and neutrophils [11]. Our present study has three aims. First, we pharmacologically characterized the H_2R in human monocytes with respect to cAMP accumulation and inhibition of fMLP-induced ROS generation. In this context, we studied functional selectivity in monocytes. Second, we analyzed the role of cAMP in the H_2R-mediated inhibition of NADPH oxidase. Third, we investigated cell type-specific differences in pharmacology between eosinophils, neutrophils and monocytes.

2. Materials and methods

2.1. Materials

CD14 MicroBeads human, Pan Monocyte Isolation Kit human, autoMACS Pro Running Buffer and MACSQuant Running Buffer were purchased from Miltenyi Biotec (Bergisch-Gladbach, Germany). Impromidine (IM) was provided by Prof. Dr. A. Buschauer (Department of Pharmaceutical and Medicinal Chemistry II, University of Regensburg, Germany). Histamine dihydrochloride (HA), amthamine dihydrobromide (AM), dimaprit dihydrochloride (DI), 5-methylhistamine dihydrochloride (5-MHA), mepyramine maleate (MEP), JNJ7777120 (JNJ), thioperamide (THIO) and Fura-2AM were obtained from Tocris Bioscience (Bristol, UK). Propidium iodide, *N,N′*-dimethyl-9,9′-biacridinium dinitrat (lucigenin), N-formyl-L-methionyl-L-leucyl-L-phenylalanine (fMLP), 3-isobutyl-1-methylxanthine (IBMX), adenosine 5′-triphosphate (ATP), forskolin (FSK) and famotidine (FAM) were supplied by Sigma–Aldrich (St. Louis, MO, USA). Stock solutions (10 mM) of IM, HA, AM, DI, 5-MHA, THIO, ATP, FAM and IBMX were prepared in distilled water and stock solutions (10 mM) of MEP, JNJ, FSK and fMLP in DMSO. Adenosine-3′,5′-cyclic monophosphorothioate, Rp-isomer (Rp-cAMPS), 8-bromoadenosine-3′,5′-cyclic monophosphorothioate, Rp-isomer (Rp-8-Br-cAMPS), 8-(4-chlorophenylthio)-2′-O-methyladenosine-3′,5′-cyclic monophosphate (8-pCPT-2′-O-Me-cAMP), adenosine-3′,5′-monophosphorothioate, Sp-isomer (Sp-cAMPS), adenosine-3′,5′-monophosphorothioate acetoxymethyl ester, Sp-isomer (Sp-cAMPS-AM) and 8-bromoadenosine-3′,5′-cyclic monophosphorothioate acetoxymethyl ester, Sp-isomer (Sp-8-Br-cAMPS-AM) were obtained from BIOLOG (Bremen, Germany). Stock solutions (100 mM) of cAMP analogs were prepared in distilled water apart from Sp-cAMPS-AM and Sp-8-Br-cAMPS-AM which were dissolved in DMSO. H89 dihydrochloride and SQ 22536 were from Merck (Darmstadt, Germany). KT5720 was supplied by Enzo Life Sciences (Farmingdale, NY, USA). H89 and KT5720 were prepared as 10 mM stock solutions in DMSO. 10× Dulbecco's PBS without Ca^{2+} and Mg^{2+} was from PAA Laboratories (Pasching, Austria). Biocoll separation solution was purchased from Biochrom (Berlin, Germany). The FITC Mouse Anti-Human CD14 antibody was supplied by Becton Dickinson (Franklin Lakes, NJ, USA). Tenefovir was from the National Institutes of Health (Bethesda, MD, USA). RevertAid M-MuLV Reverse Transcriptase (200 U/μl), oligo$(dT)_{18}$ primer, random hexamer primer, dNTP mix (10 mM) and 5× Reaction Buffer were purchased from Fermentas (St. Leon-Rot, Germany). RiboLock RNAse Inhibitor (40 U/μl) was supplied by Thermo Scientific (Wilmington, DE, USA). TaqMan probes (Hs00939627_m1 (GUSB) LOT#1191192, Hs00299832_m1 (ADCY1) LOT#860374, Hs00392747_m1 (ADCY2) LOT#866042, Hs00269618_m1 (ADCY3) LOT#845604, Hs00328499_m1 (ADCY4) LOT#910032, Hs00766287_m1 (ADCY5) LOT#873551, Hs00209600_m1 (ADCY6) LOT#845608, Hs00936808_m1 (ADCY7) LOT#851741, Hs00181588_m1 (ADCY8) LOT#817574, Hs00181599_m1 (ADCY9) LOT#967631, Hs00218262_m1 (ADCY10) LOT#964687) and TaqMan Gene Expression Master Mix were purchased from Applied Biosystems (Carlsbad, CA, USA).

2.2. Methods

2.2.1. Isolation of human monocytes

The present study was approved by the Ethics Committee of the Hannover Medical School. For the isolation of human monocytes, venous blood of healthy human volunteers was drawn in EDTA blood collection tubes containing 1.6 mg EDTA/ml blood. Nine ml of blood were diluted with 10 ml of 1× phosphate buffered saline (Dulbecco's PBS without Ca^{2+} and Mg^{2+}). Subsequently, 15 ml of Biocoll separation solution (density: 1.077 g/ml) were slowly overlaid with the diluted blood and centrifuged at room temperature at 400 × *g* for 30 min without brake. The layer of peripheral blood mononuclear cells (PBMCs) was transferred into a new tube and 1× PBS was added to a final volume of 45 ml followed by a centrifugation at 300 × *g* for 10 min. The cell pellet was resuspended in 20 ml of 1× PBS and centrifuged at 200 × *g* for 10 min to remove thrombocytes. Afterwards, the cell number was determined using a Neubauer counting chamber.

PBMCs were incubated with CD14 MicroBeads or Pan Monocyte Isolation Kit following the manufacturer's instructions. Monocytes were isolated using the autoMACS Pro Separator (Miltenyi Biotec). Cell purity was assessed by CD14 staining and flow cytometry. For CD14 staining, monocytes (1×10^4 cells) were suspended in 100 μl of MACSQuant Running Buffer and 2 μl of FITC-conjugated CD14 antibody were added. Cells were incubated at 4 °C for 20 min and CD14-positive cells were detected using MACSQuant Analyzer (Miltenyi Biotec). For cell viability, 100 μg/ml propidium iodide was used. Purity and viability of monocytes were evaluated with the MACSQuantify Software (Miltenyi Biotec).

2.2.2. Production of reactive oxygen species (ROS)

Measurement of ROS production in monocytes was performed *via* the lucigenin chemiluminescence assay [26,27]. The experiments were conducted in white 96-well plates to avoid disturbances of scattered light from adjacent wells. For this purpose, monocytes (1×10^6 cells/ml) were resuspended in Hank's Balanced Salt Solution (HBSS). 100 μl of cell suspension and 100 μl of 250 μM lucigenin were incubated in each well at 37 °C and 5% (v/v) CO_2 for 45 min. Subsequently, 50 μl of ligands at different concentrations were added to a volume of 250 μl. After preincubation at 37 °C for 5 min, reactions were started by addition of 25 μl of fMLP (1 μM final). To determine ROS production, chemiluminescence was recorded at 37 °C for 60 min by a Synergy 4 microplate reader (BioTek, Winooski, VT, USA). The chemiluminescence maxima between 10 and 30 min were used for data analysis.

2.2.3. Quantification of cAMP levels

PBS, supplemented with 200 μM IBMX and 2 mM $CaCl_2$, was preincubated with ligands at different concentrations to a total volume of 50 μl at 37 °C for 5 min. Monocytes (1×10^7 cells/ml) were resuspended in PBS and 50 μl of cell suspension were added to 2 ml safe-lock tubes followed by incubation at 37 °C for 10 min. In order to stop the reactions, tubes were placed on ice and subsequently heated at 95 °C for 10 min. After cooling down the samples on