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HISTAMINE H_1, H_3 AND H_4 RECEPTORS ARE INVOLVED IN PRURITUS

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Abstract—Histamine has long been recognised as a classical inducer of pruritus. However, the specific mechanism of histamine-induced itch has still not been fully understood. The H_1 and H_4 receptor appear to be key components in the induction of itch. The specific role of the H_3 receptor in histamine-induced itch remains unclear. The aim of our study was to investigate the role of the four known histamine receptors ($H_{1-4}R$) in acute itch in mice. Intradermal injection of the selective H_3R inverse agonist pitolisant induced strong itch in mice. Pitolisant (50 nmol/injection)-induced pruritus could be completely blocked by a combined treatment with the H_1R antagonist cetirizine (15 mg/kg) and the H_4R antagonist JNJ 7777120 (15 mg/kg), whereas the H_2R antagonist ranitidine (15 mg/kg) failed to inhibit the scratch response. Next, expression and function of histamine receptors on sensory neurons isolated from dorsal root ganglia of mice were investigated. As the itch sensation results from the excitation of sensory nerves in the skin, we further focused on skin specific sensory neurons. Therefore, neurons were retrograde labelled from the skin by means of a fluorescent tracer. Expression of H_1R, H_3R and H_4R on skin innervating sensory neurons was detected. By single-cell calcium imaging, it was demonstrated that histamine induces a calcium increase in a subset of (skin-specific) sensory neurons via activation of the H_1R and H_4R as well as inhibition of the H_3R. It is assumed that the decreased threshold in response to H_3R antagonism activates H_1R and H_4R on sensory neurons, which in turn results in the excitation of histamine-sensitive afferents and therefore elicits the sensation of itch.

Key words: pruritus, histamine, H_1 receptor, H_3 receptor, H_4 receptor, dorsal root ganglia neurons.

Several mediators have been implicated for the induction of itch (Steinhoff et al., 2006). Among these various substances histamine is the best known endogenous agent. Histamine acts via four G protein-coupled receptor subtypes. The H_1 receptor (H_1R) and H_4 receptor (H_4R) seem to play key roles in the mediation of histamine-induced itch, as H_1R and H_4R agonists provoke itch in mice and blocking these receptors inhibits histamine- and allergen-induced itch (Bell et al., 2004; Rossbach et al., 2009). The H_2R appears to play only a minor role in the induction of itch (Bell et al., 2004). The specific role of the H_3R is still debated in literature. The H_3R antagonists thioperamide and clobenpropit were found to increase scratching behaviour in mice, but it has to be considered that these compounds also exhibit affinities at the H_4R (Hossen et al., 2003; Sugimoto et al., 2004; Tiligada et al., 2009; Baumer and Rossbach, 2010). On the one hand, intradermally injected thioperamide induced itch in mice, systemically administered thioperamide on the other hand, dose-dependently reduced histamine- or clobenpropit-induced scratching behaviour (Bell et al., 2004). Interestingly, itch inducing properties of 4-methylhistamine and inhibitory effects of the H_4R antagonist JNJ 7777120 on histamine-induced itch were unaltered in mast cell-deficient mice (Dunford et al., 2007). Based on these findings, a possible presence of the H_4R on sensory neurons was postulated (Dunford et al., 2007). Strakhova et al. (2009) reported the expression of the H_4R in peripheral nervous tissue; high levels of H_4R mRNA were detected in human and rat dorsal root ganglia (DRG). As DRG neurons contain cell bodies of sensory afferents, these findings support the hypothesis that the H_4R is involved in neural transmission of itch. In humans, histamine-induced itch is transmitted via specific mechanoinsensitive C fibers. These "itch" fibers are preferentially activated by pruritogens like histamine and respond to histamine application with a time course of excitation that reflects the sensation of itch (Schmelz et al., 1997). Besides the histaminergic pathway, electrophysiological studies in humans suggest the existence of a second peripheral pathway for the transmission of itch (Schmelz, 2010). The nerve fiber population of this

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Abbreviations: CPS, capsaicin; DiI, 1,1′-dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine perchlorate; DMSO, dimethyl sulphoxide; DNCB, 2,4-dinitrochlorobenzene; DRG, dorsal root ganglia; FBS, fetal bovine serum; GAIP, Gα-interacting protein; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; $H_{1-4}R$, histamine $_{1-4}$ receptor; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; NK_1 receptor, neurokinin 1 receptor; PBS, phosphate-buffered saline; PPT-A, preprotachykinin A; RGS-19, regulator of G protein signalling 19; TRPV1, transient receptor potential V1; 12-HETE, 12-hydroxyeicosatetraenoic acid.

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pathway consist of mechano-heat-responsive units that are more responsive to cowhage than to histamine (Johanek et al., 2008).

This study was performed to investigate the involvement of H_1R, H_2R, H_3R and H_4R in acute itch in mice. To elucidate the mechanism of histamine-induced itch, histamine receptor expression on skin-labelled single neurons isolated from DRG was determined. Furthermore, functional activity of H_1R, H_3R and H_4R on sensory neurons was studied by means of calcium imaging studies.

EXPERIMENTAL PROCEDURES

Measurement of scratching behaviour

As an indicator of pruritus, the scratching behaviour of mice was determined. The rostral part of mice back was clipped and depilated with chemical depilatory 24 h before starting the experiments. The animals were placed in macrolone cages for approximately 30 min for acclimatisation before each experiment. 50 μl of the H_3R inverse agonists pitolisant (5–500 nmol/injection site), ciproxifan (5–500 nmol/injection site) or ST 889 (50 nmol/injection site) were injected intradermally via a 26 G needle into the rostal part of the back. Additionally, the H_4R agonist 4-methylhistamine (50 nmol/injection site) was tested. The rostral part of the back was chosen as it is only reachable by the mouse's hind paws. Scratching bouts by the hind paws of other sites such as head were disregarded. Vehicle-treated mice received a phosphate-buffered saline (PBS) injection instead or PBS+2.5% dimethyl sulphoxide (DMSO) in case of experiments involving JNJ 7777120. Immediately after the injection of the drugs, mice were recorded on video over 1 h and afterwards scratching bouts with the hind paws on the injection site were analysed. In experiments involving the H_1R, H_2R and H_4R antagonists cetirizine (15 mg/kg), ranitidine (15 mg/kg) and JNJ 7777120 (15 mg/kg) or the H_3R agonist immethridine (30 mg/kg), the substances were administered intraperitoneally (i.p.) 30 min before the intradermal injection of pitolisant (50 nmol/injection site) or ST 889 (50 nmol/injection site).

Hapten-induced scratching

Mice were sensitised to the hapten 2,4-dinitrochlorobenzene (DNCB) as described previously (Rossbach et al., 2009). In brief, the abdominal skin of the mice was shaved, depilated and the epidermis was stripped with adhesive tapes. For active sensitisation, 100 μl DNCB (1% solution) was administered to the skin and 50 μl Freund's complete adjuvant was injected intradermally to induce a Th1 response. On the next two consecutive days, 50 μl DNCB (1% solution) was administered onto the skin. The mice rested for 3 weeks followed by a repeated sensitisation with 20 μl DNCB (0.5% solution) onto the rostral part of the mouse back twice a week until the mice showed a stable itch reaction. To elucidate if H_3R inverse agonism further enhances allergen-induced pruritus, mice received an intradermal injection of pitolisant (50 nmol/injection site) or vehicle (PBS) directly before topical administration of the hapten. Scratching bouts were recorded for 1 h immediately after administration of the hapten.

Generation of recombinant baculoviruses, cell culture and membrane preparation

Recombinant baculoviruses encoding human H_1R or a fusion protein of the human H_2R with $G_{s\alpha S}$ were prepared as described previously (Kelley et al., 2001; Houston et al., 2002). Baculoviruses for the human H_3R, human H_4R fused to Gα-interacting protein (GAIP) (RGS-19), canine, rat and murine H_4R were prepared in analogy to the procedures for the H_1R and H_2R using the BaculoGOLD transfection kit according to the manufacturer's instructions (Schneider et al., 2009; Schnell et al., 2010, 2011).

Steady-state GTPase activity assay with Sf9 insect cell membranes expressing $H_{1-4}R$

Human $H_{1-4}R$-regulated GTP hydrolysis was determined in analogy to the procedures described in previous publications (Houston et al., 2002; Kelley et al., 2001; Schneider et al., 2009; Schnell et al., 2010, 2011). In the case of murine, rat and canine H_4R, GTP hydrolysis was determined by using Sf9 cell membranes co-expressing $G_{\alpha i2}$, $G_{\beta 1\gamma 2}$ and GAIP (RGS-19). NaCl was not present in assays measuring GTP hydrolysis regulated by these receptors. Stimulatory potencies and efficacies of histamine at the human H_1R, H_2R and H_3R and at the human, murine, rat and canine H_4R are already published (Houston et al., 2002; Kelley et al., 2001; Schneider et al., 2009; Schnell et al., 2010, 2011).

Human H_1R and H_4R radioligand competition binding assay for selectivity determination

The human H_1R and H_4R radioligand competition binding assays were performed in analogy to the procedures described in previous publications (Sander et al., 2010; Kottke et al., 2011) with some slight modifications.

Human H_1R radioligand competition binding assay. Prior to the experiment, cell membranes were thawed, homogenised with sonication at 4 °C and suspended in ice-cold HEPES binding buffer (20 mmol/L HEPES, 10 mmol/L $MgCl_2$, 100 mmol/L NaCl, pH=7.4).

Radioligand competition binding experiments were carried out incubating membranes (35 μg/well) in a final volume of 200 μl containing binding buffer and the radioligand [^{3}H] pyrilamine (1.0 nmol/L) on a 96-well plate. Assays were run in triplicates with four concentrations from 1 nmol/L to 100 μmol/L of the test compound. Incubations were performed for 120 min at 25 °C and shaking at 250 rpm. 10 μmol/L chlorpheniramine was used for the determination of non-specific binding. Free radioligand and bound radioligand on the receptor proteins were separated by filtration through GF/B glass fiber filter mats which were pre-treated with 0.3% (m/v) polyethyleneimine using an Inotech cell harvester. Unbound radioligand was removed by washing the filters in four steps with 5 ml ice-cold HEPES buffer. The amount of radioactivity collected in the filter mats was determined by liquid scintillation counting.

Human H_4R radioligand competition binding assay. Prior to the experiments cell membranes were thawed, sedimented by a 16000×g/10 min/4 °C centrifugation and resuspended in H_4R binding buffer (12.5 mmol/L $MgCl_2$, 1 mmol/L EDTA, 75 mmol/L Tris–HCl, pH=7.5). Radioligand binding competition assay were performed by incubating membranes, 35 μg/well (prepared from Sf9 cells expressing human H_4R and co-expressed with G protein $G_{\alpha i2}$ and $G_{\beta 1\gamma 2}$ subunits) in a final volume of 200 μl containing H_4R binding buffer and the radioligand [^{3}H] histamine (10 nmol/L) on a 96-well plate. Assays were run in triplicates with four appropriate concentrations between 1 nM and 100 μM. Incubation time was 60 min at 25 °C and shaking at 250 rpm. 100 μM JNJ 7777120 was used for determination of non-specific binding. Free radioligand was separated from bound radioligand by filtration through GF/B glass fiber filter mats (pre-treated with 0.3% (m/v) polyethyleneimine) using an Inotech cell harvester. Filter mats were washed three times with 5 ml of ice-cold binding buffer. The amount of radioactivity