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Ligands of histamine receptors modulate acid-sensing ion channels

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ABSTRACT

Recently we found that synthetic compounds containing amino group linked to hydrophobic or aromatic moiety are potent modulators of the proton-gated channels (ASICs). These structures have clear similarity with ligands of histamine receptors. We have also demonstrated that histamine potentiates homomeric ASIC1a by shifting its activation dependence to less acidic conditions. In the present work the action of a series of histamine receptors ligands on recombinant ASIC1a and ASIC2a was characterized. Two types of action were found for ASIC1a. 1-methylhistamine, N-alpha-methylhistamine, dimaprit and thioperamide caused significant potentiation, which was pH-dependent and voltage-independent. The H4R antagonist A943931 caused inhibition, which is likely due to voltage-dependent pore block. ASIC2a were virtually insensitive to the drugs tested. We conclude that ligands of histamine receptors should also be considered as ASIC modulators.

1. Introduction

Histamine is involved in the immune and inflammatory response, and it also plays a role of a neurotransmitter in the central nervous system. The cell bodies of histamine neurons are found in the posterior hypothalamus, in the tuberomammillary nuclei; they send projections to various brain areas [1–3]. Action of histamine is mediated by H1-H4 histamine receptors. These metabotropic receptors regulate numerous physiological functions and are involved in many CNS pathologies such as epilepsy, Alzheimer's disease, narcolepsy, attention-deficit hyperactivity disorder, schizophrenia, multiple sclerosis [4–6]. A large number of ligands was developed for histamine receptors H1-H4 [7]. They are rather small molecules that can potentially affect other targets, thus leading to the non-specific interactions, which in turn may result in the side effects of histamine receptor ligands. For instance, diphenhydramine also acts as an intracellular sodium channel blocker, which is responsible for its action as a local anesthetic [8].

Recently we found that synthetic amine-containing compounds affect the proton-gated ion channels of ASIC family [9]. Structure of some of these compounds, which contain an aromatic or hydrophobic moiety connected to amino group, is markedly similar to those of histamine receptor ligands [10]. This similarity has inspired the idea, that histamine and synthetic ligands of histamine receptors can target ASICs. Subsequent study demonstrated that histamine selectively potentiates recombinant homomeric ASIC1a [11]. Histamine shifts the activation curve of ASIC1a to less acidic values, thus allowing for the receptor activation even by modest acidifications. Under such conditions even 10 μM histamine produces significant potentiation. This finding seems important for understanding of physiological mechanism of ASIC function, because these channels generate transient response to pH drops, while slow long-lasting acidifications result in steady-state desensitization. The pH drops can take place during synaptic transmission since the content of synaptic vesicles is acidic. However, ASIC-mediated component of synaptic responses revealed in recent studies [12,13] is very small. It is possible that some endogenous ligand(s) potentiates ASICs in physiological conditions of weak synaptic acidifications.

The finding of histamine action on ASICs also raises the question about possible action of other histamine receptor ligands. In the present work we selected several compounds and performed electrophysiological testing of their action on recombinant homomeric ASIC1a and ASIC2a. Nα-methylhistamine is a H3R agonist [14]. This compound together with stable histamine metabolite 1-methylhistamine [15] represents minor modifications of the histamine structure. The H3R agonist imetit and H2R agonist dimaprit [16] were selected to test how replacement of the amino group and imidazole ring by the isothiourea moiety affects the action on ASICs. The inverse agonist of H1R diphenhydramine and antagonist of H1R tripelenamine were selected because of their structural

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similarity with recently found ASIC ligand IEM-2044. In contrast, H3R antagonist thioperamide [17] and H4R antagonist A943931 [18] were selected to test if these distinct structures can affect ASICs. Thus, the series of drugs tested in the present study includes structurally different compounds that exhibit different selectivity for histamine receptors.

2. Methods

ASIC1a and ASIC2a homomers were expressed in Chinese hamster ovary (CHO) cells. CHO cells were cultured at 37 °C in a humidified atmosphere of 5% CO_2. Cells were maintained with standard culture conditions (Dulbecco's modified Eagle's medium DMEM plus 10% fetal bovine serum and 5% gentamycin). Plasmids encoding ASIC subunits were transfected using Lipofectamine 2000 (Invitrogen, USA) following the manufacturer's protocol. Expression vectors encoding rat ASIC1a and ASIC2a were a kind gift from Dr. A. Staruschenko. For the expression of the channels, cells were transfected with 0.5 μg rASIC1a or rASIC2a cDNA per 35 mm^2 dish together with 0.5 μg GFP. Electrophysiological experiments were performed 48–72 h after transfection. Transfected cells were identified by green fluorescence using the Leica DMIL LED microscope.

Whole cell currents were recorded using patch clamp technique at holding potential of −80 mV. For this purposes EPC-8 amplifier (HEKA Electronics, Lambrecht, Germany) was used. The signal was filtered in the frequency band of 0–5 kHz, digitized at the sampling rate of 1 kHz and recorded on a personal computer using the Patchmaster software. Series resistance of about 10MΩ was compensated by 70–80% and monitored during the experiments. Only recordings where access resistance and capacitance changed less than 10% were used. All experiments were performed at room temperature (23–25 °C). The pipette solution contained (in mM):100CsF, 40CsCl, 5NaCl, 0.5$CaCl_2$, 5EGTA and 10HEPES adjusted to 7.2 by adding CsOH. Cells were continuously perfused with an extracellular solution contained the following (in mM):143NaCl, 5KCl, 2.5$CaCl_2$, 2 $MgCl_2$, 18D-glucose, 10HEPES and 10MES adjusted to 7.4 by adding NaOH. Test solutions were prepared from this extracellular solution by addition of the drugs studied and final adjusting of pH by to the required value. All solutions were filtered through micropore cellulose membranes using a vacuum glass filter (Sartorius AG, Germany). For fast drug application the ALA-VM8 micromanifold system (ALA Scientific Instruments, USA) was used. Compounds were purchased from Sigma, Tocris and Abcam.

All data are presented as a "mean ± standard deviation" calculated from at least five experiments. The statistical significance of the effects was evaluated using the paired *t*-test with $P < 0.05$ (the value of the response amplitude in the presence of a compound relative to the control).

3. Results

Action of histamine receptor ligands on ASIC1a. Recombinant homomeric ASIC1a were activated by 20 s pH drops from 7.4 to 6.5. Interval between activations was 30 s to ensure full recovery from desensitization. After recording of at least three control responses both neutral and activating solutions were replaced by the ligand-containing solutions, so the ligand perfused the cell continuously. At least three responses were recorded in the presence of the ligand to prove the stability of the effect. Next, the ligand was washed out and another series of responses was recorded to demonstrate the effect reversibility. In the case of a response rundown during the experiment, the drug effect was measured relative to the averaged response amplitude of control series and the responses after wash out.

Fig. 1 summarizes the results for 500 μM concentration of the drugs. Action of histamine is presented for comparison. Both 1-methylhistamine and Nα-methylhistamine strongly potentiated ASIC1a responses. The concentration-dependence of action of Nα-methylhistamine was measured (Fig. 1B). Fitting by Hill equation suggests that Nα-methylhistamine has larger efficacy than histamine has maximal potentiation reaches 340% the EC_{50} values were estimated as 350 ± 90 μM. At 1 mM concentration both 1-methylhistamine and Nα-methylhistamine were significantly more potent than histamine (paired *t*-test, $n = 7$, $P < 0.05$).

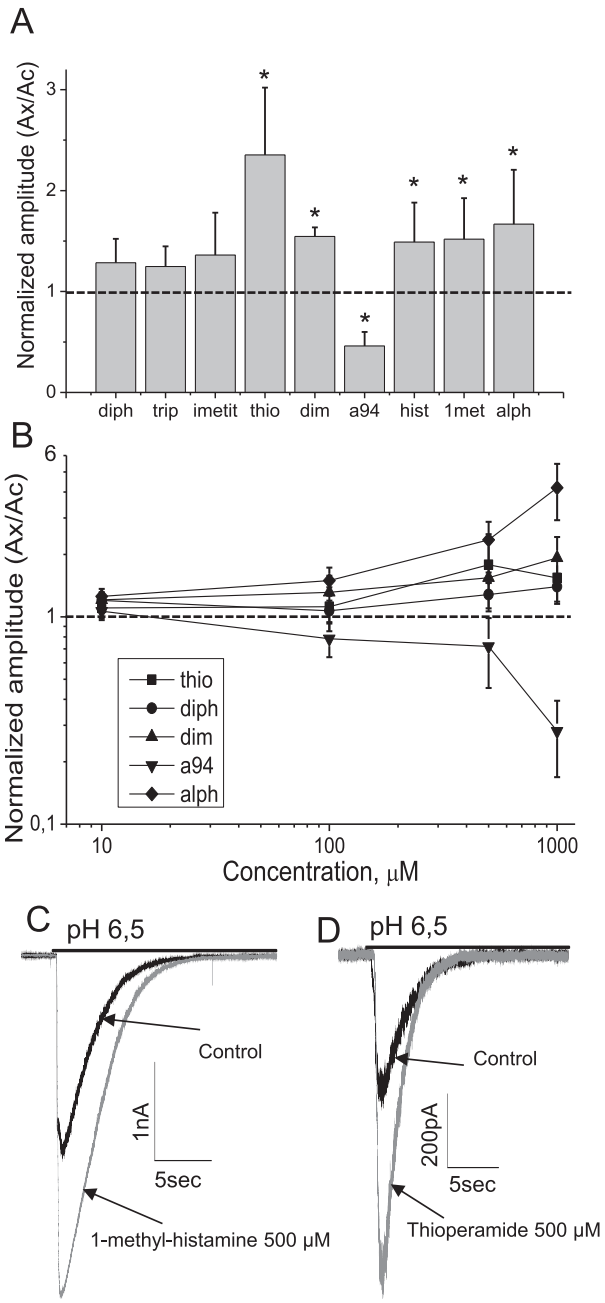

Fig. 1. Action of histamine receptor ligands on recombinant ASIC1a. A, summary data on action of compounds (500 μM) on the response amplitude. B, concentration dependencies of blocking action of A943931 and potentiating action of thioperamide, diphenhydramine, dimaprit and Nα-methylhistamine. Low concentrations do not cause any effect. Increase of concentration to 1 mM strongly enhances potentiation by Nα-methylhistamine. C and D, representative examples of ASIC1a-mediated currents in control (black) and in the presence of 1-methylhistamine (C) and thioperamide (D).

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