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Short communication

The human histamine H_3 receptor couples to GIRK channels in *Xenopus* oocytes

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Abstract

The histamine H_3 receptor mediates inhibitory responses in the nervous system. Here, we demonstrate the coupling of the human histamine H_3 receptor to G protein-coupled inward rectifier potassium (GIRK) channels in *Xenopus* oocytes, using voltage-clamp. The histamine H_3 receptor agonist (*R*)-α-methylhistamine increased GIRK currents with an EC_{50} of 2.5 nM. The response to (*R*)-α-methylhistamine was inhibited by the specific antagonist/inverse agonist clobenpropit. GIRK channels represent a novel effector pathway for the histamine H_3 receptor, also suggesting the use of electrophysiology assays in histamine H_3 receptor drug screening, allowing for the resolution of G protein activation kinetics.

Keywords: Histamine H_3 receptor; GIRK channel; *Xenopus* oocyte; (*R*)-α-methylhistamine; Clobenpropit; Voltage-clamp

1. Introduction

Originally identified on a pharmacological basis as an inhibitory histamine autoreceptor in the central nervous system (Arrang et al., 1983), the human histamine H_3 receptor was first cloned in 1999 using database sequence homology searches for G-protein coupled receptors and was found to negatively regulate the activity of adenylate cyclase when heterologously expressed (Lovenberg et al., 1999). The receptor coupling to adenylate cyclase and other effectors is pertussis toxin-sensitive, suggesting the involvement of $G_{\alpha i/o}$ subunits (see, e.g., Wieland et al., 2001).The histamine H_3 receptor has also been shown to activate mitogen-activated protein kinase, phosphatidylinositol 3-kinase and phospholipase A2, to inhibit the Na^+/H^+ exchanger and to decrease the intracellular calcium concentration, most likely via the inhibition of N-type calcium channels (see Leurs et al., 2005). However, the regulation of G protein-coupled inward rectifier potassium channels, (GIRKs, also known as Kir3) has not yet been reported for this receptor.

GIRK channels are gated by binding to the βγ subunits of large G proteins (Logothetis et al., 1987). Although the βγ subunits are responsible for activating the channel, mainly G proteins in which the α subunit is of the $G_{i/o}$ class mediate coupling of G protein-coupled receptors to GIRKs (Leaney et al., 2000). However, $G_{q/11}$-protein coupling to GIRKs has also been reported (Saugstad et al., 1996).

The human histamine H_3 receptor mRNA can undergo extensive splicing, and multiple isoforms of the receptor protein, differing in terms of ligand binding properties, constitutive activity (see below) and effector coupling have been identified (see Hancock et al., 2003). We have chosen to investigate the 445-residue isoform, since it is recognized as the full-length protein and is the most thoroughly characterized (Hancock et al., 2003; Leurs et al., 2005).

In the present study, coupling of the heterologously expressed human histamine H_3 receptor to channels composed of human GIRK1 (Kir3.1) and GIRK4 (Kir3.4) subunits is demonstrated by the activation of GIRK currents by the H_3-selective agonist (*R*)-α-methylhistamine. This activation could be counteracted via the concurrent application of clobenpropit, a histamine H_3 receptor ligand previously described as a potent antagonist/inverse agonist at the H_3-receptor (Wieland et al., 2001). However, although the full-length histamine H_3 receptor has been described to display constitutive (agonist-independent)

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activity (Wieland et al., 2001), we could not detect any effect of clobenpropit on GIRK currents in the absence of agonist.

2. Materials and methods

All studies were performed in accordance with guidelines from the Swedish National Board for Laboratory Animals. Human GIRK1 and GIRK4 cDNA were provided in pCDNA3 (Invitrogen) by Dr. Terence Hebert, University of Montreal, whereas cDNA encoding the full-length human histamine H_3 receptor (sequence described in Lovenberg et al., 1999; GenBank accession no. AF140538) in pCI-neo (Promega) was obtained from the laboratory of Dr. Rafael Franco, University of Barcelona. The plasmids were linearized with the restriction enzymes NdeI (GIRK1 and GIRK4) and BamHI (histamine H_3 receptor) and transcribed *in vitro* using the T7 mMessage mMachine kit (Ambion, Austin, TX, USA). mRNA concentration and purity were determined using a spectrophotometer. Oocytes were removed surgically from *Xenopus laevis* toads and injected with cRNA as described earlier (Persson et al., 2005). The amount of cRNA injected was 1 ng for each of the GIRK subunits and 13 ng for the histamine H_3 receptor. The oocytes were incubated at 12 °C for 4 to 5 days in modified Barth's solution (88 mM NaCl, 1 mM KCl, 2.4 mM $NaHCO_3$, 0.015 mM HEPES, 0.33 mM $Ca(NO_3)_2$, 0.41 mM $CaCl_2$, 0.82 mM $MgSO_4$, adjusted to pH 7.5) supplemented with 10 μg/ml pyruvate, 10 units/ml penicillin and 10 μg/ml streptomycin.

The electrophysiological experiments were performed using a two-electrode voltage-clamp setup (CA-1 amplifier, Dagan, Minneapolis, MN, USA; Digidata 1200 analogue/digital converter, Molecular Devices). Micropipettes were pulled from borosilicate glass capillaries (GC150-10, Harvard Apparatus LTD. Edenbridge, U.K.) to have a resistance of 0.3–1 MΩ when filled with 3 M KCl. pCLAMP (Molecular Devices) software were used for data collection and analysis. Experiments were carried out at room temperature (20–22 °C). The oocytes were placed in a 20 μl recording chamber perfused by gravity flow at ~0.5 ml/min with a high-potassium recording solution (64 mM NaCl, 25 mM KCl, 0.8 mM $MgCl_2$, 0.4 mM $CaCl_2$, 15 mM HEPES, adjusted to pH 7.4), giving a K^+ reversal potential of about −40 mV. The oocytes were clamped at this potential, and any "leak" current was compensated for by the leak subtraction circuitry of the amplifier, so that the current at −40 mV was taken to be zero. Two types of pulse protocols were used to evoke GIRK currents from a holding potential of −40 mV; either 20 increasingly negative 800 ms-pulses from +50 to −140 mV (used to visualize the voltage dependence of channel opening, in order to confirm GIRK expression) or single −80 mV pulses of varying duration (to study current responses to histamine H_3 receptor ligand application). The histamine H_3 receptor agonist (*R*)-α-methylhistamine (provided by the laboratory of Dr. Gilberto Fisone, Karolinska Institutet) and the selective antagonist/inverse agonist clobenpropit (Sigma-Aldrich) were diluted and, when appropriate, mixed to the desired concentrations in the high-potassium recording solution before experiments. In order to avoid contamination of the perfusion system and to reduce the amount of drug used, drugs were injected manually into the recording chamber at ~0.5 ml/min using a small syringe attached to a short piece of tygon tubing connected to the chamber separately from the gravity flow perfusion system (which was stopped before drug application). Volumes 10 times that of the recording chamber were injected. After the response hade reached its peak value, the gravity flow perfusion system was used for washout. Receptor activation-evoked current increase was determined by subtracting the basal (agonist-independent) current from the peak current amplitude following drug application.

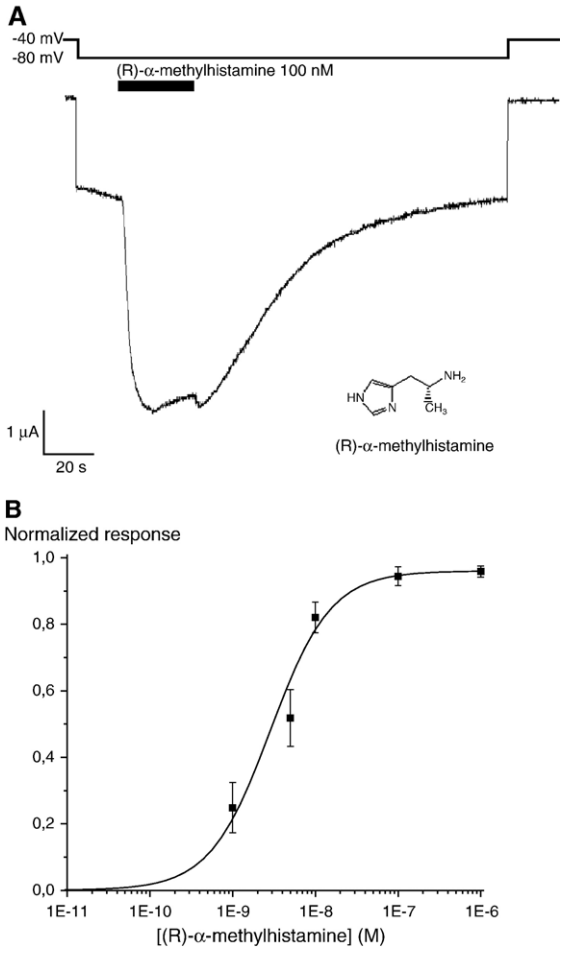

Fig. 1. Effects of (*R*)-α-methylhistamine on oocytes coinjected with GIRK1/4 and human histamine H_3 receptor cRNA. (a) Time course of GIRK current activation and deactivation following drug application and washout, respectively. (b) Concentration–response relationship for peak GIRK current activation via H_3 receptor activation by (*R*)-α-methylhistamine. The fitted curve is described by a modified Hill equation; $y=y_{max}\times x^n/(k+x^n)$, where y_{max} is set to 1, n is also set to 1 (corresponding to a reaction with a single binding site) and k=2.5 nM, corresponding to the EC_{50}. For each oocyte used, the evoked current response for each concentration of drug tested was normalized to the maximum current response obtained in that oocyte. Means±s.e.m. of the normalized response at the indicated concentrations are shown, representing data from 12 oocytes from 6 different batches.

3. Results

In order to increase the inward electrochemical driving force for potassium ions, thus enhancing the GIRK currents evoked

upon hyperpolarization, recordings of these currents were carried out in a high potassium (25 mM) solution. Under these conditions, GIRK1/4 channel conductance reaches its maximum at potentials of about −80 mV, whereas the potassium reversal potential is around −40 mV. In order to obtain as exact a measure of the basal (agonist-independent) GIRK current amplitude as possible, the oocytes were voltage-clamped to a holding potential of −40 mV, at which GIRK currents should be minimal, and basal currents evoked by a hyperpolarizing step to −80 mV. This potential was maintained throughout application and washout of histamine H_3 receptor ligands. Inward currents in uninjected oocytes or oocytes injected with the histamine H_3 receptor alone were consistently lower than 200 nA under these conditions (not shown), whereas only oocytes displaying basal GIRK currents exceeding 800 nA were considered for further experiments.

3.1. (R)-α-methylhistamine activates GIRK currents via the histamine H_3 receptor

Application of nanomolar concentrations of the histamine H_3 receptor agonist (*R*)-α-methylhistamine to oocytes coinjected with cRNA encoding the human histamine H_3 receptor and human GIRK1 and GIRK4 subunits induced a prominent increase in GIRK current amplitudes (Fig. 1A). Maximum current activation was seen at a concentration of about 100 nM, and the EC_{50} was estimated to 2.5±0.49 nM (Fig. 1B), close to that reported by Lovenberg et al. (1999) for the inhibitory action of (*R*)-α-methylhistamine on forskolin-induced cAMP formation (1 nM).

Application of 10 μM (*R*)-α-methylhistamine to oocytes coexpressing GIRK1/4 with the human dopamine D_2 receptor, or to oocytes only expressing the histamine H_3 receptor, failed to elicit any increase in current amplitudes, supporting the notion that the response is indeed elicited via the histamine H_3 receptor and dependent on GIRK channel activation (not shown).

3.2. Clobenpropit antagonises the action of (R)-α-methylhistamine, but does not modify basal GIRK current amplitudes

Clobenpropit, a compound previously described as an antagonist/inverse agonist at the human histamine H_3 receptor (e.g., Lovenberg et al., 2000; Wieland et al., 2001), potently antagonised the response to 100 nM (*R*)-α-methylhistamine when coapplied with the agonist (Fig. 2). The IC_{50} of clobenpropit in the presence of 100 nM (*R*)-α-methylhistamine was estimated to 19.8±4.00 nM, which compares well to earlier reports on cAMP-based experiments where half-maximal antagonism of the response to 100 nM (*R*)-α-methylhistamine occurred between 10 and 100 nM of clobenpropit (Lovenberg et al., 2000).

However, we were unable to detect any change in basal GIRK current amplitudes when clobenpropit was applied alone. Although constitutive activity has been reported for the histamine H_3 receptor action on adenylate cyclase, in which case clobenpropit acts as an inverse agonist, the receptor

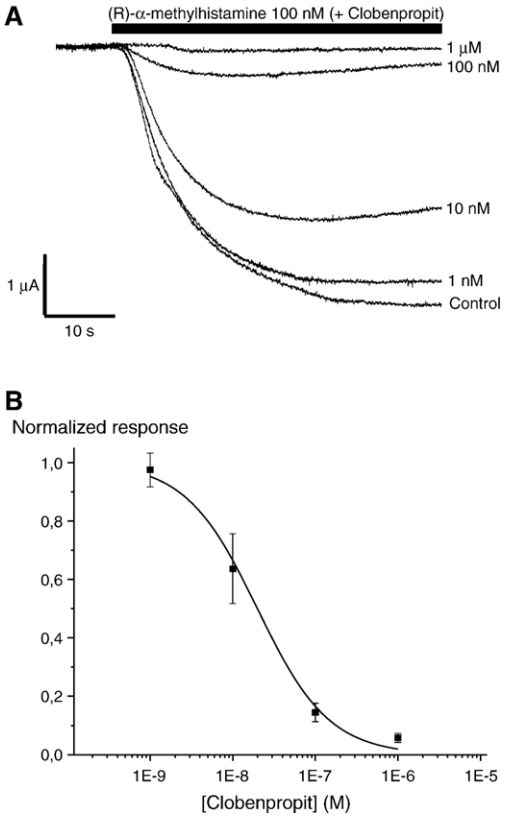

Fig. 2. Antagonism of (*R*)-α-methylhistamine-induced current activation by clobenpropit. (a) Representative current traces showing the effects of increasing concentrations of clobenpropit, as indicated in the figure, on the current response induced by 100 nM (*R*)-α-methylhistamine. (b) Concentration–response relationship for clobenpropit when coapplied with 100 nM (R)-α-methylhistamine. The fitted curve is described by a modified Hill equation; $y=y_{max}\times x^{-n}/(k+x^{-n})$, where y_{max} is set to 1, n is set to −1 (corresponding to a reaction with a single binding site) and k=19.8 nM, corresponding to the IC_{50} of clobenpropit in the presence of 100 nM (*R*)-α-methylhistamine. For each oocyte used, the peak evoked current response for each combination of drugs tested was normalized to the maximum current response obtained in that oocyte. Means±s.e.m. of the normalized response at the indicated concentrations are shown, representing data from 8 oocytes. Mean peak current amplitude under control conditions was 3.7±0.57 μA.

apparently lacks agonist-independent activity when coupling to certain other effector systems, e.g., mitogen-activated protein kinase (Gbahou et al., 2003). Thus, our inability to detect any effect of clobenpropit on basal GIRK currents might reflect an absence of constitutive histamine H_3 receptor activity that is related to the specific effector system, or perhaps, to the non-mammalian *Xenopus* expression system. Also, we cannot exclude the possibility that there might exist a small degree of constitutive activity which we are unable to resolve due to insufficient expression of the receptor.

4. Discussion

The role of the histamine H_3 receptor in the nervous system appears to be one of an inhibitory auto-and heteroreceptor: Activation of presynaptic histamine H_3 receptors has been shown to inhibit the release of histamine (Arrang et al., 1983), dopamine (Schlicker et al., 1993), serotonin (Threlfell et al., 2004),

glutamate (Molina-Hernandez et al., 2001), GABA (Arias-Montano et al., 2001) and noradrenaline (Schlicker et al., 1994) in various brain regions, and radioligand binding and mRNA *in situ* hybridisation data agree with the localization of the histamine H_3 receptors on neuronal presynaptic terminals (Pillot et al., 2002). The presence of GIRK channels has also been demonstrated on axon terminals in the brain (Ponce et al., 1996) and GIRK activation has been implicated in autoreceptor inhibition of neurotransmission (see, e.g., Davila et al., 2003). Although closing of N-type calcium channels has been suggested as the main mechanism underlying the down-regulation of neurotransmitter release mediated by histamine H_3 receptors, (see, e.g., Schlicker et al., 1994; Arias-Montano et al., 2001), it is not unlikely that GIRK channels also play a physiological role as effectors coupled to histamine H_3 receptor activity.

For example, the dopamine D_2 receptor, another $G_{i/o}$-coupled receptor implicated in autoreceptor functions, has been shown to activate GIRK channels in dopaminergic neurons, an effect at least partially responsible for the inhibitory autoreceptor influence on transmitter release and firing rates (Davila et al., 2003; Congar et al., 2002). Likewise, the $G_{i/o}$-coupled cannabinoid CB1 receptor exerts inhibitory presynaptic actions via GIRKs (see Chevaleyre et al., 2006). There are also actions of the histamine H_3 receptor other than on the release of transmitters; e.g., histamine H_3 receptor activation has been shown to decrease the firing rates of "waking-selective" neurons of the posterior hypothalamus (Vanni-Mercier et al., 2003) and to hyperpolarize GABAergic neurons of the substantia nigra pars reticulata (Zhou et al., 2006). In agreement, histamine H_3 receptors are not found exclusively on nerve terminals, but appear also to be located on the dendrites and somata of neocortical and hippocampal pyramidal cells (Pillot et al., 2002), where GIRK channels are present (Ponce et al., 1996). The exact function of the histamine H_3 receptor at these postsynaptic sites remains to be determined.

Experimental compounds targeting the histamine H_3 receptor are being investigated for the relief of a diverse range of disorders including obesity, insomnia, migraine, Alzheimer's disease, schizophrenia, attention-deficit hyperactivity disorder, narcolepsy, myocardial ischemia, inflammatory diseases (see Leurs et al., 2005) and Parkinson's disease (Gomez-Ramirez et al., 2006). The *in vitro* techniques used in the characterization of histamine H_3 receptor pharmacology have so far mainly been biochemical assays, employing measurement of cAMP levels, arachidonic acid release, GTP-γ-S-and radioligand binding (see Hancock et al., 2003; Leurs et al., 2005). Because electrophysiological studies of the coupling of G protein-coupled receptors to GIRK channels in heterologous systems afford higher temporal resolution compared to the corresponding biochemical assays, the use of the former system is of particular advantage when studying the time course of rapid signalling events such as the kinetics of G protein activation upon agonist binding to the receptor (Doupnik et al., 2004). Thus, the present findings demonstrating the coupling of the histamine H_3 receptor to GIRK channels could be of physiological significance, and might also be of interest to researchers investigating the pharmacodynamics of histamine H_3 receptor-modulating substances.

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