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Rapid communication

Constitutive activity of the histamine H_1 receptor reveals inverse agonism of histamine H_1 receptor antagonists

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Abstract

Transient expression of the wild-type human histamine H_1 receptor in SV40-immortalised African green monkey kidney cells resulted in an agonist-independent elevation of the basal levels of the second messenger inositoltrisphospate. Several histamine H_1 receptor antagonists, including the therapeutically used anti-allergics cetirizine, loratadine and epinastine reduced this constitutive histamine H_1 receptor activity. Inverse agonism, i.e., stabilisation of an inactive conformation of the human histamine H_1 receptor, may therefore be a key component of the anti-allergic mechanism of action of clinically used antihistamines.

Keywords: Histamine H_1 receptor; Constitutive activity; Inverse agonism

1. Introduction

The recent notion of constitutive signalling of G-protein-coupled receptors has fundamentally changed insights in receptor pharmacology and led to a reclassification of antagonists into inverse agonists, agents which display negative intrinsic activitity, and neutral antagonists, which do not affect receptor activity (Milligan et al., 1995; Leurs et al., 1998). The actual therapeutic importance of constitutive G-protein-coupled receptor activity has not been clarified yet; however, for a proper evaluation of drug action this new aspect in receptor pharmacology should not be ignored. In view of the widespread therapeutic use of histamine H_1 receptor antagonists in allergy (Woosley, 1996), we investigated the constitutive histamine H_1 receptor activity and inverse agonistic activity of several histamine H_1 receptor antagonists.

2. Experimental

To determine whether wild-type human histamine H_1 receptors exhibit constitutive activity, we transiently transfected (2.5 μg DNA/1×10^6 cells) SV40-immortalised African green monkey kidney (COS-7) cells with either $pcDEF_3$ or $pcDEF_3$ containing the gene for the wild-type human histamine H_1 receptor ($pcDEF_3hH_1$), using the DEAE–dextran method (Wieland et al., 1999). Transient expression resulted in a high affinity binding site for the histamine H_1 receptor antagonist [^{3}H]mepyramine ($pK_D = 8.8 \pm 0.1$, $n = 4$) with a density of 4.5 ± 0.5 pmol/mg protein. Moreover, incubation of [^{3}H]inositol prelabelled cells in serum-free medium (Wieland et al., 1999) with histamine for 1 h results in a 7.6 ± 0.6-fold increase in [^{3}H]inositolphosphates accumulation, with a pEC_{50} value of 7.5 ± 0.1 ($n = 4$). Interestingly, we observed a significant increase of the basal level of [^{3}H]inositolphosphates production upon histamine H_1 receptor expression (mock: 9430 ± 550 dpm/well; H_1 receptor: 15200 ± 760 dpm/well, $n = 3$), indicating constitutive activity of the wild-type human histamine H_1 receptor. This constitutive signalling is inhibited completely by the histamine H_1 receptor antagonist mepyramine (Fig. 1A) (44 ± 2% inhibition, $pIC_{50} = 8.6 \pm 0.1$, $n = 4$), whereas the histamine H_2 receptor antagonist ranitidine and the histamine H_3 receptor antagonist clobenpropit had no effect on [^{3}H]inositolphosphates accumulation at concentrations up to 10 μM ($n = 2$, data not shown). Incubation of mock transfected COS-7 cells with mepyramine did not affect the

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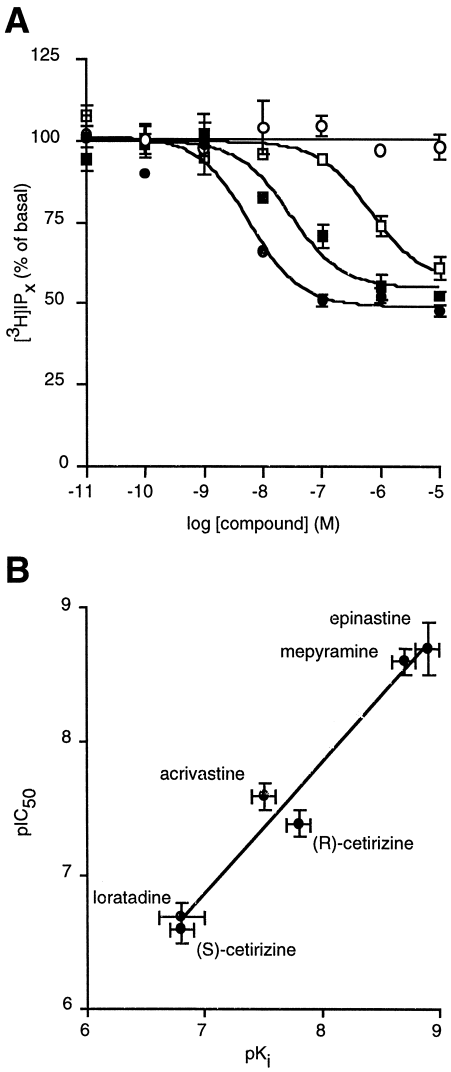

Fig. 1. (A) Effect of mepyramine on the basal [^{3}H]inositolphosphates levels in mock transfected COS-7 cells (○) or COS-7 cells expressing the human histamine H_1 receptor (●, 4.5 ± 0.5 pmol/mg protein). In the same graph, the effects of (*R*)-(■) and (*S*)-cetirizine (□) in COS-7 cells expressing the human histamine H_1 receptor are shown. Representative experiments performed in triplicate are shown, each experiment was repeated at least twice. (B) Correlation graph of the pIC_{50} values obtained for the inverse agonists in the [^{3}H]inositolphosphates accumulation assay versus the pK_i values obtained by [^{3}H]mepyramine displacement in COS-7 cells expressing the human histamine H_1 receptor.

[^{3}H]inositolphosphates accumulation (Fig. 1A), indicating that mepyramine in fact acts as an inverse agonist at the human histamine H_1 receptor. In accordance with the known histamine H_1 receptor stereospecificity (Moguilevsky et al., 1995), the enantiomers of cetirizine display a stereospecific inhibition of the constitutive histamine H_1 receptor activity (Fig. 1A). In fact, all tested histamine H_1 receptor antagonists act as full inverse agonists for the histamine H_1 receptor displaying a pharmacological profile that is expected for the histamine H_1 receptor (Fig. 1B, data not shown). The potencies of the tested inverse agonists correlate well (slope $= 0.98 \pm 0.09$, $r^2 = 0.97$, $n = 6$) with their respective pK_i-values (Fig. 1B), as determined by [^{3}H]mepyramine displacement studies (Wieland et al., 1999).

Despite the general acceptance of constitutive G-protein-coupled receptor signalling, there is considerable concern about the potential interference of endogenously present agonist with the determination of negative intrinsic activity of inverse agonists (Baxter and Tilford, 1995). As our studies were performed in serum-free culture medium histamine contamination via the medium is unlikely (Smit et al., 1996). To exclude a putative endogenous synthesis of histamine by histidine decarboxylase COS-7 cells were treated with 10 or 100 μM *S*-(+)-α-fluoromethylhistidine, an irreversible inhibitor. After pretreatment with *S*-(+)-α-fluoromethylhistidine for 24 h, mepyramine (10 μM) still inhibits basal [^{3}H]inositolphosphate production in COS-7 cells expressing the human histamine H_1 receptor ($43 \pm 3\%$, $n = 2$).

3. Discussion

In this study, we show for the first time constitutive activity of the wild-type human histamine H_1 receptor. Histamine H_1 receptor antagonists are widely used to relieve the symptoms of allergic reactions and have become one of most prescribed drug families in Western countries (Woosley, 1996). We have identified well-known therapeutics as cetirizine (Zyrtec®), epinastine (Flurinol®), loratadine (Claritin®) as inverse agonists, which sheds new light on their presumed mechanism of action. At present no data on the physiological relevance of constitutive histamine H_1 receptor activity are available. Nevertheless, it is tempting to speculate that constitutive histamine H_1 receptor activity can contribute to pathophysiological conditions. In, e.g., patients with allergic rhinitis, histamine H_1 receptor mRNA upregulation has been observed in the nasal mucosa (Iriyoshi et al., 1996). However, the physiological relevance of constitutive histamine H_1 receptor signalling remains to be investigated. In conclusion, we identified constitutive histamine H_1 receptor signalling of the human histamine H_1 receptor and reclassified several histamine H_1 receptor antagonists as inverse histamine H_1 receptor agonists. Our data suggest that stabilisation of the inactive conformation of the histamine H_1 receptor may be a key component of the allergic mechanism of action of histamine H_1 receptor antagonists.

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References

Baxter, G.S., Tilford, N.S., 1995. Endogenous ligands and inverse agonism. Trends Pharmacol. Sci. 16, 258–259.

Iriyoshi, N., Takeuchi, K., Yuta, A., Ukai, K., Sakakura, Y., 1996. Increased expression of histamine H_1 receptor mRNA in allergic rhinitis. Clin. Exp. Allergy 26, 379–385.

Leurs, R., Smit, M.J., Alewijnse, A.E., Timmerman, H., 1998. Agonist-independent regulation of constitutively active G-protein-coupled receptors. Trends Biochem. Sci. 23, 418–422.

Milligan, G., Bond, R.A., Lee, M., 1995. Inverse agonism: pharmacological curiosity or potential therapeutic strategy?. Trends Pharmacol. Sci. 16, 10–13.

Moguilevsky, N., Varsalona, F., Guillaume, J.P., Noyer, M., Gillard, M., Daliers, J., Hénichart, J.P., Bollen, A., 1995. Pharmacological and functional characterisation of the wild-type and site-directed mutants of the human H_1 histamine receptor stably expressed in CHO cells. J. Recept. Signal Transduction Res. 15, 91–102.

Smit, M.J., Leurs, R., Alewijnse, A.E., Blauw, J., Van Nieuw Amerongen, G.P., Van De Vrede, Y., Roovers, E., Timmerman, H., 1996. Inverse agonism of histamine H_2 antagonist accounts for upregulation of spontaneously active histamine H_2 receptors. Proc. Natl. Acad. Sci. U. S. A. 93, 6802–6807.

Wieland, K., Ter Laak, A.M., Smit, M.J., Kühne, R., Timmerman, H., Leurs, R., 1999. Mutational analysis of the histamine H_1 receptor binding site. J. Biol. Chem. 274, 29994–30000.

Woosley, R.L., 1996. Cardiac actions of antihistamines. Annu. Rev. Pharmacol. Toxicol. 36, 233–252.