Binding of histamine H_3-receptor antagonists to hematopoietic progenitor cells

Evidence for a histamine transporter unrelated to histamine H_3 receptors

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Abstract Hematopoietic progenitor cells can take up histamine or release IL-3-induced histamine through a bi-directional transport system that is blocked by H_3-receptor antagonists. In the present study we demonstrate a correlation between the affinity of various H_3-receptor antagonists and their potency as inhibitors of histamine uptake. All compounds that blocked histamine uptake also inhibited IL-3-induced histamine release. Yet, classical H_3 receptors are not involved in this biological activity, since highly specific histamine H_3-receptor agonists neither alter histamine uptake nor affect the release of endogenous histamine synthesized in response to IL-3. Furthermore, the inhibitory effect of H_3-receptor antagonists on histamine uptake was not reversed by the agonists. Unlike H_3-receptor antagonists, the agonists did not displace the binding of the labeled antagonist iodoproxyfan.

Key words: Histamine; Hematopoiesis; Transport; Histamine H_3-receptor

1. Introduction

Histamine, an ubiquitous mediator of cellular communication, is produced by a variety of tissues [1]. Most of its biological functions are exerted through the three membrane receptor subtypes, H_1, H_2 and H_3 [2,3]. Intracellular histamine-binding sites, termed H_{IC}, have been more recently identified in microsomes and nuclei [4,5]. Histamine can also be taken up by a specific transport system, similar to the one described for other bioamines, such as serotonin and dopamine [6]. This take up is temperature-dependent and requires sodium exchange for optimal function. It was observed in astrocytes, endothelial and glial cells [7–9].

We have shown that histamine is also taken up by murine hematopoietic progenitor cells. The bone marrow cells (BMC) involved are at least partially identical with those synthesizing histamine in response to the hematopoietic growth factors IL-3 or GM-CSF, and calcium ionophore [10–13]. They have been characterized as progenitors with partially restricted differentiation potential and purified on the basis of their high retention of the fluorescent dye rhodamine-123 [10]. Their histamine transporter is bi-directional, i.e. the blockade of histamine uptake also inhibits the release of newly synthesized, IL-3-induced histamine from BMC, while intracellular histamine levels increase. Il-3-dependent cell lines composed of immature myeloid cells arrested at a particular stage of the differentiation scheme have a similar transport system [14].

Based on our previous evidence that the two highly specific H_3-receptor antagonists carboperamide (MR 16155) and thioperamide are very potent inhibitors of histamine uptake by hematopoietic progenitor cells [10], our present study addressed the relationship between classical H_3 receptors and this histamine transporter. To this end, we tested a number of well-defined histamine H_3-receptor antagonists and agonists and evaluated their effect on histamine uptake and IL-3-induced histamine production by murine BMC.

2. Materials and methods

2.1. Animals

Male or female 6–8-week-old C57BL/6 mice were used in all experiments. They were bred under specific pathogen-free conditions in our own facilities.

2.2. Chemicals and drugs

Histamine dihydrochloride was purchased from Sigma (St. Louis, MO) and imetit from RBI (Natick, MA). Carboperamide and (R)α-methylhistamine (RαMeHa) were donated by Bioprojet (Paris, France). All other H_3 antagonists were synthesized by two of us (H.S. and W.S.) as described [15–17]. Labeled iodoproxyfan was synthesized according to the method described by Krause (manuscript submitted). IL-3 was purchased from R&D (Abingdon, UK).

2.3. Cell preparations

BMC were prepared as previously described [10]. The IL-3-dependent FDCP-2 cell line, was grown in RPMI medium (Gibco, Grand Island, NY) containing 10% foetal calf serum and 10% WEHI-conditioned medium as a source of IL-3.

2.4. Membrane preparation

FDCP-2 cells were harvested and centrifuged. The cell pellet was washed in phosphate-buffered saline (PBS) without Ca/Mg (Gibco), resuspended in the same buffer and homogenised with a Polytron. After centrifugation at $140\times g$ for 10 min, the supernatant was recentrifuged at $23\,000\times g$ for 30 min. The pellet constitutes the membrane fraction. It was resuspended in ice-cold buffer and used for all binding assays. The protein concentration was determined by using the BCA assay (Pearce Chemical, Rockford, IL).

2.5. Histamine uptake

The binding experiments on total BMC were performed as previously described [10]. Each sample was assayed in triplicate and histamine binding, expressed as fmol/10^6 cells, was calculated from total cpm after substraction of non-specific binding determined with 1 μM carboperamide.

2.6. [^{125}I]iodoproxyfan binding assays

Aliquots of the membrane suspension (20 μg in 100 μl) were incu-

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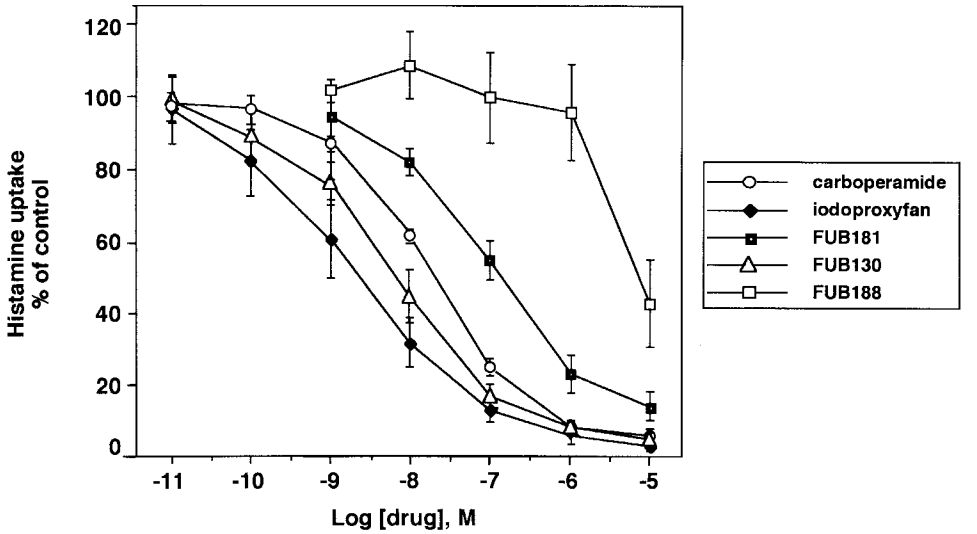


Fig. 1. Effect of histamine H_3-receptor antagonists on [^{3}H]histamine uptake by bone marrow cells (BMC). Total BMC were incubated for 2 h with [^{3}H]histamine (100 nM) with or without indicated antagonists at increasing concentrations. Radioactivity was measured after extensive washing. Data represent means ± SEM from 5 to 8 separate experiments.

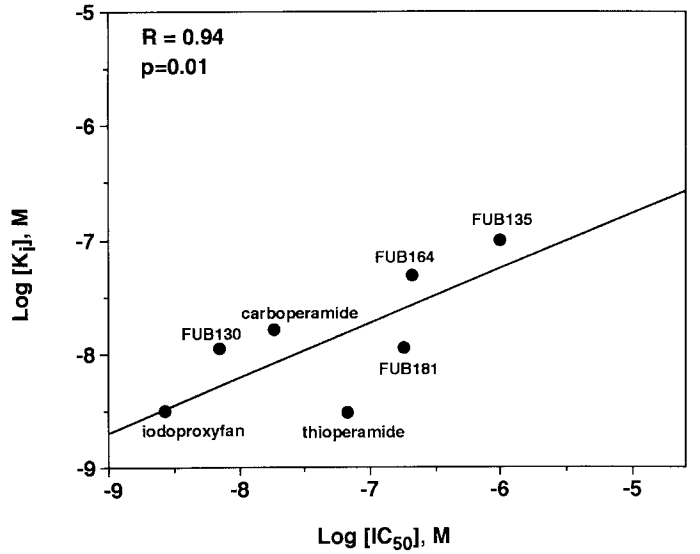
bated for 120 min at 37°C with [^{125}I]iodoproxyfan (30 pM) with or without competing drugs dissolved in the same phosphate buffer to a final volume of 200 μl. Incubations were stopped by repeated (4 times) addition of 5 ml of ice-cold medium, followed by rapid filtration through glass microfiber filters (GF/C Whatman, Clifton, NJ), presoaked in 0.3% polyethylene-imine. Radioactivity trapped on the filters was measured by using an LKB (Rockville, MD) gamma-counter. Non-specific binding was determined with 1 μM carboperamide.

2.7. Histamine assay

Total BMC (2.5×10^6/ml) were suspended in culture medium containing 1 ng/ml IL-3, with or without the indicated compounds. They were incubated for 24 h in polystyrene tubes (Falcon 2003), centrifuged and washed twice in Hanks' balanced salts solution (HBSS, Gibco). Histamine concentrations were determined both in supernatants and inside the cells lysed by 0.4 N perchloric acid, by using an automated continuous flow fluorometric technique previously described [18].

3. Results and discussion

The observation that thioperamide and carboperamide, two specific histamine H_3-receptor antagonists, are potent inhibitors of [^{3}H]histamine uptake by BMC [10], led us to investigate the effect of various other compounds chemically related to these antagonists. We found that all those having a potent antagonistic effect on H_3 receptors also inhibited the interaction between histamine and BMC (Fig. 1). The high-affinity H_3-receptor antagonists iodoproxyfan, FUB 130 and carboperamide were the most potent inhibitors of tritiated histamine uptake (dose inducing 50% of inhibition $IC_{50} = 4 \pm 3$, 8 ± 4 and 30 ± 6 nM, respectively), while low-affinity compounds, like FUB 188 and FUB 135 (data not shown) were also less effective in our model. As shown in Fig. 2, the IC_{50}



	K_i	IC_{50}
iodoproxyfan [15]	5 nM	4 nM
FUB181 [15]	13 nM	250 nM
FUB130 [16]	11 nM	8 nM
FUB188	> 500 nM	9 μM
FUB132 [21]	>>1 μM	2 μM
FUB164 [15]	64 nM	400 nM
FUB135	100 nM	1 μM
thioperamide [15]	4.3 nM	79 nM
carboperamide [15]	20 nM	30 nM
FUB515	>> 1μM	> 100 μM

Fig. 2. Affinity of histamine H_3-receptor antagonists for their receptor (K_i) is correlated with their inhibitory effect on [^{3}H]histamine uptake (IC_{50}). The coefficient of correlation was determined by a computing program. References between brackets. FUB 515, FUB 135 and FUB 188: X. Ligneau, personal communication.

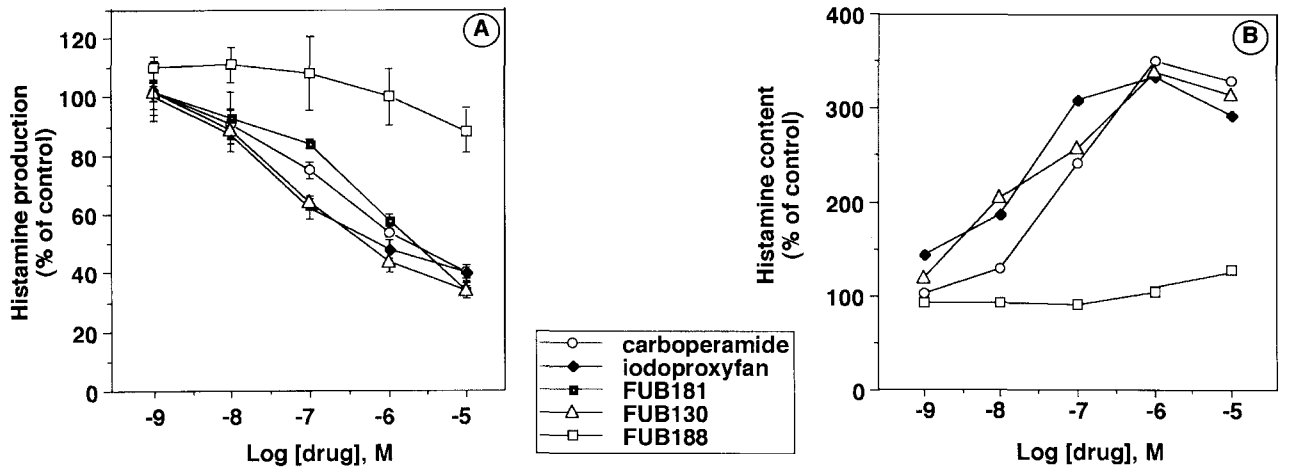


Fig. 3. Effect of histamine H_3-receptor antagonists on histamine production by BMC stimulated with IL-3. Total BMC were incubated with IL-3 for 24 h in the presence of the indicated antagonists. A: Histamine was determined in the supernatants. Data represent means ± SEM from 5 to 8 separate experiments. B: Histamine assay in pellets. Data represent one typical experiment out of two.

determined in our study and the K_i value, established by H_3-receptor classical test ([^{3}H]histamine release from rat cerebral cortex synaptosomes [19]), were well correlated. However, some discrepancy could be noted: indeed, FUB 181 and FUB 130 displayed a similar K_i for H_3 receptors, while their IC_{50} were quite different. The same observation applies to iodoproxyfan and thioperamide.

Since histamine transport by hematopoietic progenitors is

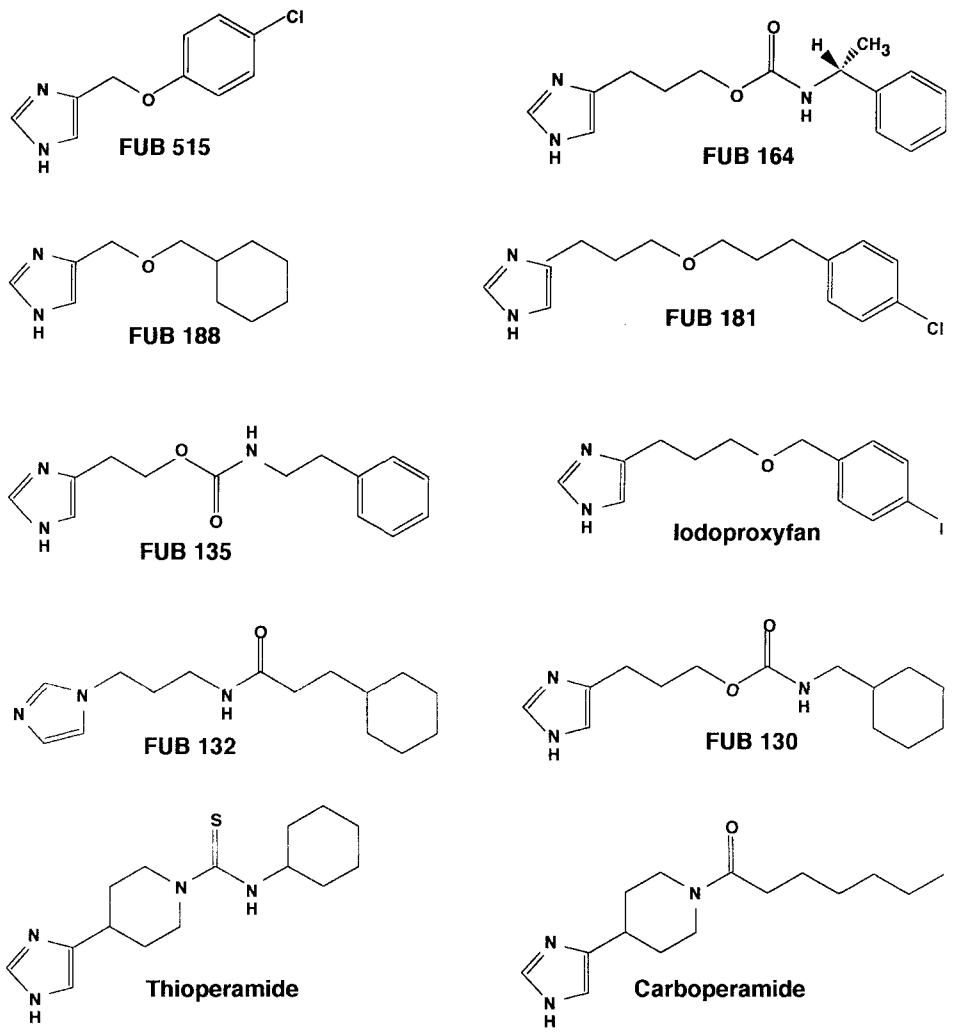


Fig. 4. Chemical structure of various compounds used in competition experiments.

bi-directional [14], we examined whether the compounds that blocked histamine uptake also inhibited its release from IL-3-induced BMC. We found that in the presence of iodoproxyfan, carboperamide, FUB 181 or FUB 130 at optimal concentration (10 μM), bone marrow supernatants contained about 60% less histamine than controls (Fig. 3A), while intracellular histamine levels significantly increased under these conditions (Fig. 3B). In contrast, a compound like FUB 188, that did not inhibit histamine uptake, did also not modify its secretion in response to IL-3 (Fig. 3A,B). The same observation applies to a number of other lower-affinity antagonists of histamine H_3 receptors, such as FUB 132 and FUB 135 (data not shown). The imidazole moiety may be placed in a suitable position only by long spacers between the ring and the lipophilic part, as shown before for antagonists interacting with H_3 receptors [15].

One possible explanation for the strong inhibitory effect of histamine H_3-receptor antagonists on histamine uptake by BMC could be the fixation of the lipophilic moiety of the molecular structure which is common to all antagonists. This is, however, not true for all compounds since FUB 515 (Fig. 4), a lipophilic compound with an imidazole ring substituted in 4-position which has no H_3-antagonist activity does neither block histamine binding nor inhibit its IL-3 induced release (data not shown).

Up to this point our data supported the notion that classical H_3 receptors could be involved in the uptake of histamine by hematopoietic progenitor cells. This conclusion could not be maintained since the H_3-receptor agonists imetit [20] and (R)α-methylhistamine [19] whose affinity for histamine H_3 receptors is as high as that of the inhibitory antagonists, do not modify histamine uptake [10]. In the same line of evidence, we observed that (R)α-methylhistamine could not reverse the inhibitory effect of iodoproxyfan or carboperamide. Indeed, a 1 h preincubation with 1 μM (R)α-methylhistamine neither modified histamine uptake (100 nM) nor altered the inhibitory effect of carboperamide or iodoproxyfan (Fig. 5). Similar results were obtained with imetit instead of (R)α-methylhistamine (data not shown). In addition, the pretreatment with H_3-receptor agonists did not affect the inhibition of IL-3-induced histamine production by H_3-receptor antagonists (data not shown). These findings strengthen the assumption that the histamine transport system is not mediated by a known classical H_3 receptor. It seems nonetheless that antagonists of the histamine transport system may possess structure–activity relationships comparable to those of histamine H_3-receptor antagonists.

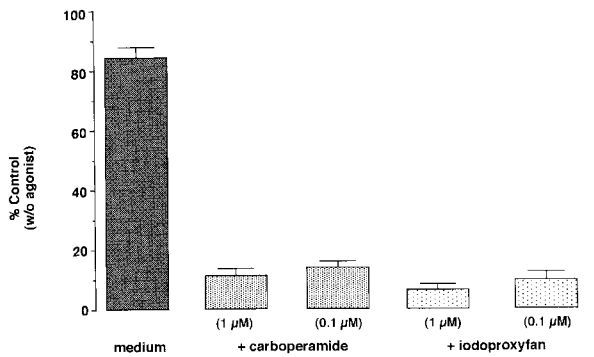


Fig. 5. (R)α-methylhistamine does not reverse the inhibition of [^{3}H]histamine uptake induced by carboperamide or iodoproxyfan. Total BMC were first incubated for 1 h with (R)α-methylhistamine (1 μM) and [^{3}H]histamine (100 nM) was then added with or without indicated antagonists at two concentrations. Radioactivity was measured after extensive washing. Data represent means ± SD from two separate experiments done in triplicate.

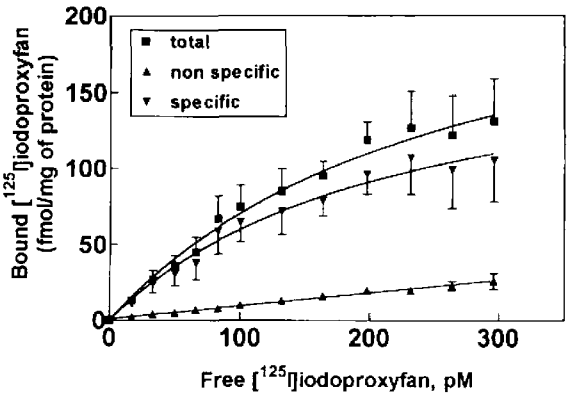


Fig. 6. Concentration-response curve of [^{125}I]iodoproxyfan binding on FDCP-2 cell membrane preparation. 20 μg of total crude membranes were incubated for 2 h with increasing concentrations of radioligand. The non-specific binding was determined in the presence of 1 μM carboperamide. Radioactivity was measured after extensive washing on fiber glass membranes. Data represent means ± SD from three separate experiments.

Among all H_3-receptor antagonists tested, iodoproxyfan was the most potent inhibitor of histamine uptake by BMC. Using the radiolabeled form of this compound we could indeed demonstrate that it did actually bind both to BMC and to the myeloid cell line FDCP-2 which is also capable of histamine uptake [10] (data not shown). Similarly, [^{125}I]iodoproxyfan (30 pM) binds to crude FDCP-2 membrane preparations (19.3 ± 3.8 fmol/mg protein) and this binding is not inhibited by 1 μM (R)α-methylhistamine (20.6 ± 2.6 fmol/mg protein; data are means ± SEM from three separate experiments), a concentration which displaces H_3-receptor antagonist binding on classical H_3 receptors [21]. In contrast, carboperamide or thioperamide (1 μM) are potent inhibitors of [^{125}I]iodoproxyfan (30 pM) binding on FDCP-2 membrane preparations (3.8 ± 0.9 and 4.5 ± 1.6 fmol/mg protein, respectively). As illustrated by Fig. 6, saturation analysis-specific [^{125}I]iodoproxyfan binding, defined using 1 μM carboperamide for non-specific binding, led to a K_d of 212 ± 39 pM and a B_{max} of 188 ± 19 fmol/mg of protein (means ± SD from three separate experiments).

In conclusion, our data provide evidence for the existence of a histamine transporter in murine hematopoietic progenitor cells which is clearly distinct from histamine H_3 receptors. Indeed, histamine uptake was not affected by highly specific agonists of this receptor subtype. In contrast the binding sites of the transporter were recognized with high affinity by histamine H_3-receptor antagonists, such as iodoproxyfan. This interesting feature, together with the occurrence of histamine uptake by IL-3-dependent myeloid cell lines, will be very useful for further characterization of this new transport system.

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