Evidence for bidirectional histamine transport by murine hematopoietic progenitor cells

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Received 21 May 1996

Abstract Murine hematopoietic progenitor cells synthesize substantial amounts of histamine in response to IL-3 or calcium ionophore. They also take up extracellular histamine by an active transport system. In the present study we demonstrate that this system mediates both influx and efflux of histamine. Indeed, MR16155 and thioperamide, the two H_3 antagonists which are most effective in inhibiting histamine uptake, likewise diminish the release of preloaded histamine from bone marrow cells. These compounds also inhibit the release of histamine which has been newly synthesized by hematopoietic progenitors in response to IL-3 or calcium ionophore, as assessed by the accumulation of the mediator inside the cells in the presence of the antagonists. The potency of different histamine receptor antagonists as inhibitors of histamine release increases with their capacity to block histamine uptake.

Key words: Hematopoietic progenitor; Histamine; Bidirectional transport; Interleukin-3

1. Introduction

Histamine, a ubiquitous messenger in cell to cell communication, has been mainly studied for its role in hypersensitivity and in neural transmission [1,2]. The notion that it might also affect hematopoiesis originated from experiments showing that exogenous histamine could induce proliferation and/or differentiation of hematopoietic progenitors [3,4]. In support of the physiological relevance of these findings, we have demonstrated several years ago that large amounts of histamine are generated by murine hematopoietic progenitors upon stimulation with interleukin-3 and granulocyte-macrophage-colony stimulating factor [5–7]. Furthermore, we established that endogenous histamine is requisite for IL-3-induced CFU-S cell cycling [8].

Most of the biological activities exerted by histamine are mediated by either of the three membrane receptor subtypes H_1, H_2 and H_3, characterized so far [9,10]. More recently, two additional binding sites, termed intracellular histamine receptors (H_{ic}), have been identified in the microsome and in the nucleus [11,12]. The existence of these receptors, together with our recent evidence for histamine uptake by hematopoietic progenitors [13], is consistent with the idea that histamine does indeed play a physiological role inside hematopoietic progenitor cells.

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Abbreviations: BMC, bone marrow cells; αFMH, α-fluoromethylhistidine; IL-3, Interleukin-3; CFU-S, colony forming units in spleen

Histamine uptake is temperature-dependent and partially requires sodium exchange [13]. Although we have provided evidence that this phenomenon is not mediated by classical histamine receptors, H_3 receptor antagonists, such as thioperamide and MR16155, are potent inhibitors. Conversely to the polyamine transport system, which exists in numerous cell types and has been extensively studied [14,15], histamine transport seems to be restricted to astrocytes, endothelial and glial cells, and is still poorly understood [16–18]. It has been suggested that astrocytes could use the same means of transport for histamine uptake and release [18]. Herein, we provide evidence for a bidirectional transporter in bone marrow cells (BMC) and in the IL-3-dependent cell line FDCP1, as assessed by the finding that the inhibitors of histamine uptake likewise decrease release of preloaded histamine. We also show that this system is involved in the release of endogenous histamine which is newly synthesized in response to IL-3 or calcium ionophore since we found a decrease in histamine release accompanied by an increase in intracellular histamine contents in the presence of H_3 receptor antagonists.

2. Materials and methods

2.1. Animals

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

2.2. Chemicals and drugs

Histamine dihydrochloride was purchased from Sigma (St. Louis, MO). Thioperamide and MR16155 were donated by Bioprojet (Marne la Coquette, France). α-Fluoromethylhistidine (αFMH) was a gift from Merck Sharp and Dohme Research Laboratories (Rahway, NJ).

2.3. Cell preparations

Bone marrow cells were removed from femurs and tibiae by flushing with ice-cold Hanks' balanced salt solution (HBSS; Gibco, Grand Island, NY). After centrifugation, all cells were suspended in minimum essential medium (MEM; Gibco) containing 10% horse serum (Flobio, Courbevoie, France), 1% sodium pyruvate 100×, 1% nonessential amino acids, 100 IU/ml penicillin, and 100 μg/ml streptomycin (all from Gibco; referred to as culture medium). The IL-3-dependent FDCP1 cell line was grown in RPMI containing 10% fetal calf serum (Gibco) and 10% WEHI-conditioned medium as a source of IL-3.

2.4. Histamine uptake

Total bone marrow cells (10^7/ml) were suspended in MEM, containing 10^{-4} M histamine together with 5×10^{-5}M α-FMH (irreversible inhibitor of histidine decarboxylase (HDC: EC 4.1.1.22), which prevents endogenous histamine synthesis) with or without various compounds at indicated concentrations. They were incubated for 3 h in polystyrene tubes (Falcon 2003), centrifuged, and washed twice in HBSS. Pelleted cells were lysed in 0.4 N perchloric acid, and intracellular histamine was measured in lysates by an automated continuous flow fluorometric technique previously described [19]. The lower limit of sensitivity of this assay is about 0.5 ng/ml.

PII S0014-5793(96)00741-7

2.5. Histamine efflux

Total bone marrow cells (10^7/ml) or FDCP1 cells (5×10^6 /ml) suspended in culture medium were preloaded for 3 h or 30 min, respectively, with 10^{-4} M histamine in the presence of 5×10^{-5} M α-FMH. After two successive washings, cells were incubated at 2.5×10^6 per ml with various chemicals. At indicated times cells were centrifuged, washed, and intracellular histamine contents determined.

2.6. Histamine synthesis

Total bone marrow cells (2.5×10^6/ml) were incubated for 24 h in culture medium in the presence of 1 ng/ml IL-3 (R&D, Abingdon, UK) or 5×10^{-7} M calcium ionophore (Boehringer, Mannheim, Germany) with or without the indicated compounds. Histamine concentrations in supernatants and pellets were determined as mentioned above.

2.7. Statistical analysis

Results are presented as means ± S.E.M. of n experiments. Statistical significance was assessed by Student's t-test for paired variants.

3. Results and discussion

We have previously established that hematopoietic progenitor cells from murine bone marrow have the capacity to take up substantial amounts of extracellular histamine, which accumulates inside the cells [13]. This histamine transport is partially dependent on the presence of an electrochemical Na^+ gradient and strongly inhibited by MR16155 and thioperamide. These compounds have been described as specific H_3 histamine receptor antagonists. Yet, we could demonstrate that they do not inhibit histamine uptake via H_3 receptor blockade [13]. It was therefore plausible that hematopoietic progenitor cells possessed a means of histamine transport, as described for a few other cell types such as astrocytes, endothelial and glial cells [16–18].

In the present study, we addressed the question of whether the histamine transporter present in bone marrow cells could function in two directions, i.e. take up histamine from the extracellular space when required and mediate its release from hematopoietic progenitors when it is generated inside the cells. To this end, we preloaded bone marrow cells for 3 h in the presence of 10^{-4} M histamine. Under these conditions the intracellular histamine contents rose from 100 ± 10 to 2080 ± 220 pg/10^6 cells ($n=5$). We then washed the cells extensively and incubated them in histamine-free medium. As shown in Fig. 1A, the amine underwent rapid efflux since within 60 min its contents decreased from 2080 ± 220 to 900 ± 80 pg/10^6 cells and continued to decrease more slowly after 2 h. Concomitantly, histamine levels were augmented in the extracellular compartment (data not shown). Like histamine uptake [13], histamine release was abolished at low temperatures and inhibited by MR16155 (1 μM) or thioperamide (1 μM), the most potent inhibitors of histamine uptake (Fig. 1A).

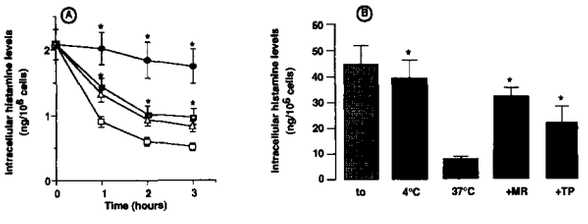

Fig. 1. Changes in intracellular histamine contents in the presence of MR16155 or thioperamide. (A) Total bone marrow cells, preloaded with 100 μM histamine for 3 h, were suspended in fresh medium and intracellular histamine levels were determined at different time points under control (37°C) conditions (□), low temperature conditions (●), with 1 μM MR16155 (▣) or 1 μM thioperamide (△). (B) FDCP1 cells were exposed for 30 min to 100 μM histamine. After washing, intracellular histamine levels were determined (t_o). Changes in histamine contents were then evaluated after a 30 min incubation in histamine-free culture medium at 4°C, 37°C, with 1 μM MR16155 (MR) or 1 μM thioperamide (TP). $^*p<0.05$ vs control at 37°C ($n=5$).

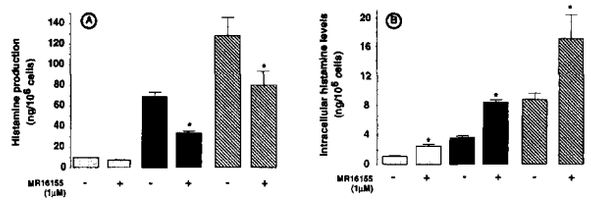

Fig. 2. Effect of MR16155 on extra- and intracellular histamine levels generated by BMC. Total bone marrow cells were incubated for 24 h in medium alone (stippled bars), with IL-3 (1 ng/ml) (cross-hatched bars) or calcium ionophore (5×10^{-7} M) (hatched bars) with or without MR16155 (1 μM). (A) Histamine production in supernatants. (B) Intracellular histamine levels in BMC. $^*p<0.05$ vs columns without MR16155 ($n=5$).

Knowing that the cells involved in histamine uptake belong to the immature compartment of the bone marrow, we verified whether similar results could be obtained using the IL-3 dependent cell line FDCP1. The latter represents a homogeneous population of hematopoietic progenitors arrested at a relatively early stage of differentiation, in contrast to the heterogeneous bone marrow cells. As shown in Fig. 1B, these cells are also capable of histamine uptake and release, both inhibited by MR16155, thioperamide, as well as low temperatures. Yet, the kinetics are more rapid since influx and efflux are maximal within 30 min.

These data support the notion that hematopoietic progenitors possess a histamine transport system with bidirectional functions, the inward and outward currents being specifically blocked by the same inhibitors (MR16155 and thioperamide). With regard to the mechanism involved, it is conceivable that these compounds have a better affinity for the transporter than histamine itself, thus preventing it from crossing the membrane. Among the few reports on histamine uptake by other cell types [16–18,20,21], only astrocytes seem to be capable of releasing histamine by a common mechanism related to ionic exchanges. The effect of known histamine receptor antagonists in this context has not been studied [18].

We have previously shown that IL-3 promotes increased histamine synthesis by hematopoietic progenitors. Most of the newly generated histamine is released, though the intracellular histamine contents is also consistenly increased [5]. The fact that these cells are identical with those capable of histamine uptake [13] prompted us to verify whether inhibitors of histamine uptake also affected the release of endogenous histamine from bone marrow cells. We found that MR16155 strongly inhibited IL-3-induced histamine production, as shown in Fig. 2A. Similar inhibition was observed when bone marrow cells were stimulated with the calcium ionophore A23187, which is also a potent inducer of histamine production, as we have shown before [22]. In both situations, the decrease in histamine levels in bone marrow supernatants in the presence of MR16155 was accompanied by a striking

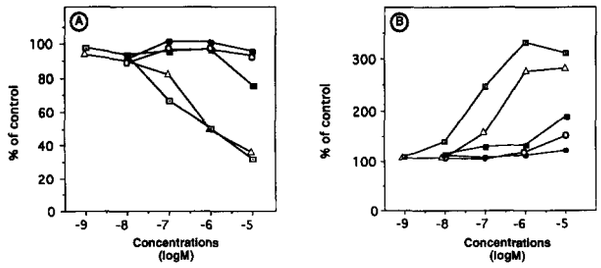

Fig. 3. Effect of histamine receptor antagonists on IL-3-induced histamine production by hematopoietic progenitors. Total bone marrow cells were incubated with IL-3 for 24 h in the presence of the following antagonists: MR16155 (▣), thioperamide (△), cimetidine (○), lupitidine (□) and cetirizine (●). Histamine was assayed in the supernatants (A) and inside the cells (B). Data represent one typical experiment out of two.

increase in intracellular histamine contents (Fig. 2B). It should be noted that intracellular histamine levels were already significantly increased in cells cultured in medium alone. Furthermore, IL-3-induced histamine concentrations in cell supernatants were even more strikingly decreased by MR16155 or thioperamide when histamine-producing cells were enriched from total bone marrow by fractionation on a discontinous Ficoll gradient (data not shown). This decrease was also concomitant with increased intracellular histamine contents.

In addition to MR16155 and thioperamide, various other histamine receptor antagonists inhibit histamine uptake by bone marrow cells with low efficiency [13]. We assayed them on histamine efflux and found a good correlation between the capacity of a given compound to inhibit histamine uptake and its ability to decrease the release of histamine synthesized in response to IL-3. Indeed, as shown in Fig. 3, the most potent inhibitors of histamine uptake (MR16155, $IC_{50} = 14 \pm 3.6$ nM and thioperamide, $IC_{50} = 78.9 \pm 17$ nM) were also the best blockers of histamine release, as assessed by the significant increase in intracellular histamine contents. Lupitidine, which inhibits histamine uptake at very high doses ($IC_{50} = 7 \pm 0.3$ μM), also required higher concentrations to inhibit histamine release, while cimetidine and cetirizine, which have very little effect if any on histamine uptake, did not modify IL-3-induced histamine production whatever the dose used (up to 10^{-5} M). These findings argue in favor of a common mechanism accounting for histamine influx and efflux in hematopoietic progenitors. It is also noteworthy that the total histamine production which comprises both extracellular and intracellular histamine, was significantly diminished in the presence of MR16155 (41.69 ± 2.22 vs 72.33 ± 4.90 ng/10^6 cells, $n = 9$). This diminution might be the result of a feed-back mechanism regulating histamine synthesis when high intracellular levels are attained.

This is reminiscent of the very sophisticated regulation of polyamine biosynthesis. Indeed, polyamines which are important modulators of cell proliferation and differentiation [23], are taken up by the cells similarly to histamine. It has been demonstrated that the activity of ornithine decarboxylase (ODC), the rate-limiting enzyme of the polyamine cascade, is regulated by intracellular polyamine concentrations in various ways, namely inhibition of translation, acceleration of ODC degradation and induction of an ODC-inhibitory protein named antizyme [24,25]. Knowing that both polyamines and histamine may play an important role during cell proliferation and/or differentiation, and that their synthesis by ODC and HDC respectively, is induced in hematopoietic organs by the same growth factors [7,26–28], we are actually investigating whether similar mechanisms regulate HDC and ODC activities.

Acknowledgements: The authors wish to thank Dr. E. Schneider for critical reading of the manuscript. They also thank A. Arnould and F. Machavoine for their excellent technical assistance. S.C. is a recipient of a fellowship from the 'Ligue Nationale contre le Cancer'. This work has been partially supported by the Association pour la Recherche contre le Cancer ARC N° 6365.

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