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HISTAMINE INCORPORATION INTO MURINE MYELOBLASTS AND PROMYELOCYTES

FORMATION OF A HISTAMINE TRANSPORT SYSTEM

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Abstract—When isolated murine myeloblasts and promyelocytes were treated with ^{3}H-histamine (5×10^{-7} M) in RPMI-1640 medium supplemented with 20% horse serum at 37°, the radioactivity of these cells increased gradually, reaching a maximum after 6 hr. However, when these progenitor cells were pretreated with unlabeled histamine (5×10^{-7} M) for 1 hr, subsequent exposure to ^{3}H-histamine caused prompt incorporation, the extent of which was more than 3.8 times that seen in cells which were not pretreated. This acceleration was prevented by simultaneous addition of cycloheximide (4×10^{-7} M) or actinomycin D (10^{-7} M) in the pre-incubation stage. While the microsomal fraction of progenitor cells pretreated with histamine initially yielded a higher binding capacity, that of the plasma membrane fraction rose significantly after 1 hr. Most of the incorporated ^{3}H-histamine was detected as unmetabolized. Non-histone chromatin protein had a higher affinity to ^{3}H-histamine than did the DNA fraction of progenitor cell nuclei. Histamine inhibited myeloperoxidase activity of myeloid progenitor cells selectively and dose-dependently without affecting eosinophil peroxidase. These findings suggest that histamine incorporated into murine myeloblasts and promyelocytes induces the synthesis of a specific protein(s) through interaction with the nucleus, and that these proteins, in turn, may be combined into the cell membrane, where they act as a transport system for histamine incorporation.

Precursors of various species of blood cells, such as granulocytes, monocytes and lymphocytes, are present in the bone marrow; their proliferation and differentiation are regulated by growth factors. In the case of neutrophils, it is believed that a glycoprotein called colony stimulating factor (CSF) is a growth factor specific to differentiation [1]. Moreover, it has been reported that chronic administration of histamine caused marked leukocytosis in the peripheral blood of dogs, rabbits and guinea-pigs [2, 3].

Histamine promotes the differentiation of granulocytic precursor cells in murine bone marrow. First, it stimulates the differentiation of stem cells: both colony forming units in the spleen (CFU-s) [4] and colony forming units in culture (CFU-c) are activated by H_2 receptors [5]. Second, histamine preferentially acts to differentiate myeloblasts and promyelocytes after it is incorporated into these cells and this incorporation is unrelated to either H_1 or H_2 receptors [6]. Thus far, the process has been observed in the intestines [7], in lymphocytes [8] and in promyelocytic leukemia cells (HL-60) [9], though neither the role nor the fate of histamine incorporated into the cells has been documented. The present experiment was performed to elucidate the mechanism of histamine incorporation into, and the resultant differentiation of, myeloblasts and promyelocytes.

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MATERIALS AND METHODS

Female BALB/c mice (Charles River) 6–8 weeks old were used. Mice were killed by cervical dislocation and subsequently, bone marrow cells were flushed from femurs and tibias into 10 ml of cold phosphate buffered saline (PBS) containing 135 mM NaCl, 2.7 mM KCl, 8.1 mM Na_2HPO_4 and 1.5 mM KH_2PO_4 (pH 7.4). Thereafter, the cells were exposed to an erythrocyte-lysis buffer (32 mosmol) containing NH_4Cl 155 mM and Tris–HCl 17 mM (pH 7.2) at 4° for 2 min. Cells were washed with PBS by repeated centrifugation, and then suspended in 4 ml of solution consisting of RPMI-1640 and 10% fetal calf serum (FCS, Whittaker, M.A. Bioprodukts). Cell suspension was poured into a plastic dish covered with FCS and placed in a humidified atmosphere of 5% CO_2 in air at 37° for 1 hr. Non-adherent cells were collected and fractionated by Percoll (Pharmacia, Sweden) discontinuous density gradient centrifugation (ρ = 1.06–1.08) [6]. Most of the myeloblasts and promyelocytes were gathered from fractions with specific densities of 1.068–1.070. These cells constituted 70–80% of the total cells, and were used in the following experiments.

Histamine incorporation into granulocyte progenitors was investigated in the presence of ^{3}H-histamine (New England Nuclear, Boston, 13.7 Ci/mmol). Approximately 1×10^6 cells suspended in 0.2 ml of RPMI-1640 medium (Nissui Pharmaceutical Co., Tokyo) supplemented with 20% v/v

horse serum (HS, Whittaker M.A. Bioprodukts) were incubated with ^{3}H-histamine (1×10^{-7}–7×10^{-7} M) at 37° in a humidified atmosphere of 5% CO_2 in air. Thereafter, cells were washed three times with PBS and solubilized in 500 μl of 0.1% Triton X-100. Five millilitres of a scintillator (ATOMLIGHT, New England Nuclear, Boston, MA) were added after which radioactivity was measured by means of a liquid scintillation counter (Aloca, LSC). This was taken as the total radioactivity. When the cells were incubated with ^{3}H-histamine in an excess of unlabeled histamine (5×10^{-4} M), the radioactivity similarly determined was regarded as non-specific histamine incorporation. The extent of specific histamine incorporation was calculated as the difference between the total and non-specific radioactivities.

To study protein synthesis, myeloblasts and promyelocytes (1×10^6 cells) were suspended in 1 ml of leucine-free Eagle's minimum essential medium (Nissui) and incubated at 37° in a humidified atmosphere of 5% CO_2 in air. After addition of 1.0 μCi of ^{3}H-leucine (New England Nuclear) into the medium, which corresponds to 0.2 nM leucine, the cells were incubated at 37° for 7 hr, and thereafter, 9 ml of ice-cold PBS was added to the test tubes. After three washings with PBS, the cells were sonicated in 0.4 ml of 5% trichloroacetic acid (TCA) for 1 min by means of a Branson sonifier (Model 12, Branson Inc.). The cells were then passed through a Whatmann GF/c filter and their radioactivity was measured.

Fractionation of intracellular organellae was performed by a modification of the method used by Record *et al.* [10]. Cells were sonicated for 20 sec in 0.1 M Tris–HCl buffer containing 0.3% polyethylene-glycol, 0.25 M sucrose and 1 mM of *p*-amidinophenylmethanesulfonic fluoride (*p*-APMSF, pH 7.4), after which they were centrifuged at 4° for 5 min at 800 *g* to remove the nuclei and cell debris. The supernatant was centrifuged at 15,000 *g* for 10 min and at 140,000 *g* for 60 min, consecutively. The pellet was used as a microsomal fraction. The latter was suspended in 4 ml of buffer and overlaid onto a mixture of 11 ml of Percoll, 2.2 ml of distilled water and 4.8 ml of a buffer consisting of 400 mM KCl, 20 mM $MgCl_2$ and 400 mM Tris–HCl (pH 9.6). After centrifugation at 160,000 *g* for 10 min, Percoll suspension was suctioned, 700 μl at a time, from the bottom of the centrifugation tube. As marker enzymes for the membrane fraction and endoplasmic reticulum fraction, respectively, the activities of 5′-nucleotidase and glucose-6-phosphatase were measured in each fraction. For the one enzyme, a 5′-nucleotidase assay kit was used (Sigma). Glucose-6-phosphatase was measured according to the method of de Duve [11]: the sample was incubated in a solution containing 5 mM of glucose-6-phosphate, 1 mM of ethylenediamine-tetraacetic acid and 20 mM of histidine–HCl (pH 6.5) at 37° for 30 min and the reaction was terminated by the addition of perchloric acid. Released inorganic phosphate was determined using Malachite Green [12].

Chromatin was isolated according to a modification of the method of Huang and Bonner [13] and in all stages of extraction, 1 mM of *p*-APMSF was added as an inhibitor of chromatin protease [14]. The progenitors (5×10^7 cells) were sonicated in 2 ml of a homogenating buffer consisting of 0.25 M sucrose, 0.01 M β-mercaptoethanol, 0.001 M $MgCl_2$ and 0.05 M Tris–HCl (pH 8.0), and were centrifuged at 4000 *g* for 30 min. After the isolated chromatin was washed with a grinding medium (0.25 M sucrose, 0.05 M Tris–HCl, pH 8.0 and 0.01 M β-mercaptoethanol), the final pellet was suspended in Tris-buffered saline. One millilitre of this suspension was overlaid onto 7 ml of 2 M sucrose solution containing 0.01 M β-mercaptoethanol, and the test tubes were centrifuged at 160,000 *g* for 3 hr at 4°. Following final centrifugation, chromatin was obtained from the pellet.

The chromatin was mixed with 4 M CsCl solution and centrifuged at 160,000 *g* for 40 hr. The transparent gelatinous pellet containing DNA was washed with 10 ml of 4 M CsCl. Subsequently, the contaminating RNA was destroyed by incubating with 2 ml of 0.3 N KOH for 12 hr. After neutralization with HCl, insoluble DNA in 7% TCA was precipitated at 800 *g* for 15 min. The protein skin obtained from CsCl centrifugation was extracted with 10 ml of 0.05 M Tris–HCl (pH 8.0) to obtain non-histone chromatin protein (NHCP). From the remainder, histone was further extracted with 10 ml of 2.5 M NaCl containing 0.05 M Tris–HCl (pH 8.0).

DNA content was measured by means of a fluorometric method using etidium bromide (EB) [15]. Precipitated DNA was dissolved with a buffer containing 10 mM of Tris–HCl and 1 mM of EDTA and adjusted to a final concentration of 1 to 10 μg/ml. The solution was mixed with an equal volume (50 μl) of EB solution (5 μg/ml). A glass capillary tube filled with this solution was placed on the table of a fluorescence microscope. The fluorescence derived from EB after exposure to an excitation beam of 365 nm was measured at 580 nm using a fluorescence photon counter (Nikon-XF). When calf thymus DNA was used as a reference, the correlation coefficient between fluorescence intensity and DNA concentration in the standard curve was 0.99. Protein content was measured according to the method of Lowry *et al.* [16].

The binding capacity of histamine to DNA or to chromatin proteins was determined using Scatchard plot analysis. Either isolated chromatin proteins or DNA dissolved in cytosol mimic buffer (110 mM KCl, 10 mM NaCl, 3.3 mM $MgCl_2$, 1 mM EGTA and 10 mM Tris–HCl, pH 6.9) was incubated with various concentrations of ^{3}H-histamine in the presence or absence of an excess amount of unlabeled histamine (1×10^{-4} M) for 12 hr at 4°. After terminating the incubation, the reaction mixture was passed through a Whatmann GF/c filter and the radioactivity of the filter was measured. The extent of specific histamine binding was calculated as described previously. The amount of bound ^{3}H-histamine divided by the free ^{3}H-histamine concentration is plotted versus the amount bound. The dissociation constant (K_d) was calculated as the negative reciprocal of the slope.

Peroxidase activity was measured according to the method of Chance and Mahely [17] with some modifications. Neutrophil progenitors (1×10^5 cells) were disrupted in 1 ml of 0.1% Triton X-100 and 2 ml of a reaction mixture consisting of 13 mM guaiacol,

0.02% acetyltrimethylammonium bromide and 0.1 M phosphate buffer (pH 7.0) were added. The reaction was initiated by adding 50 μl of 20 mM H_2O_2 in the presence or absence of 50 μl of 150 mM 3-amino-1H-1,2,4-triazole (AMT). Peroxidase activity was expressed as moles of tetraguaiacol formed in 1 min per 1 mg of protein. Since the activity of eosinophil peroxidase was diminished in the presence of AMT [18], the remaining enzyme activity was probably derived from the myeloperoxidase.

The histamine metabolites were measured by thin layer chromatography (TLC) as described by Lyons *et al.*, with some modifications [7]. The cells incorporating ^{3}H-histamine (1×10^6 cells) were dissolved by adding 2 ml of 0.1% Triton X-100 and thereafter, 4 ml of cold methanol–acetone mixture (1:1) was added. After vigorous shaking at room temperature for 10 min, these mixtures were refrigerated for 1 hr and centrifuged at 600 *g* for 30 min. The supernatant was kept at 0°, and the precipitate was washed again with the methanol–acetone mixture and centrifuged. The combined supernatant fractions were dried *in vacuo*, and thereafter, 3 ml of acidified methanol (pH 1.0) was added to the residue. After centrifugation, the supernatant was dried *in vacuo* and the residue was dissolved in 1 ml of absolute methanol and applied to a TLC plate (Kieselgel 60, 20 × 23 cm Merck). As authentic substances, 1-methylhistamine, histamine, 1-methylimidazole acetic acid and imidazole acetic acid were used. A TLC plate was spotted with 10 μl of the sample extract and 10 μl of the standard mixture. The plate was developed with a mixed solution of butanol/acetic acid/water (60:22:23). The R_f values of histamine, 1-methylhistamine, 1-methylimidazole acetic acid and imidazole acetic acid were 0.13, 0.21, 0.35 and 0.46, respectively. The corresponding spots were extracted with 500 μl of water for 3 hr and thereafter, 5 ml of a scintillator (ATOMLIGHT) was added to measure the radioactivity.

RESULTS

As shown in Fig. 1a, when ^{3}H-histamine (1×10^{-7}–7×10^{-7} M) was added into the medium containing 1×10^6 cells and incubated for 6 hr at 37°, the radioactivity of myeloblasts and promyelocytes increased dose-dependently, nearly reaching a maximum at 5×10^{-7} M of histamine. Also, the increase in radioactivity of these progenitor cells corresponded to the increment of the cell number in the medium, as shown in Fig. 1b. The linearity between these two parameters was observed up to 4×10^6 cells.

As shown in Fig. 2, after the addition of ^{3}H-histamine (5×10^{-7} M) into the medium, the radioactivity of myeloblasts and promyelocytes (1×10^6 cells) gradually increased and reached a maximum 6 hr later. However, when these cells were pretreated with unlabeled histamine (5×10^{-7} M) for 60 min and washed with PBS repeatedly, the subsequent addition of ^{3}H-histamine (5×10^{-7} M) caused a rapid incorporation of radioactivity without a time lag, so that even after 1 hr, the rate of incorporation was close to a maximum. This acceleration was completely abolished when either cycloheximide (4×10^{-7} M) or actinomycin D (10^{-7} M) was added simultaneously with unlabeled histamine during the pre-incubation period, even though the cells were washed with PBS repeatedly to remove protein synthesis inhibitors. When neutrophil precursors were pretreated with cycloheximide (4×10^{-7} M) or actinomycin D (10^{-7} M) alone for 60 min, the profile of the subsequent ^{3}H-histamine incorporation was almost the same as that of the non-pretreated control. Previous exposure to unlabeled histamine appears to stimulate protein synthesis, which may play an important role in ^{3}H-histamine incorporation.

To evaluate this assumption, the histamine binding of subcellular fractions obtained from neutrophil progenitors was determined with or without unla-

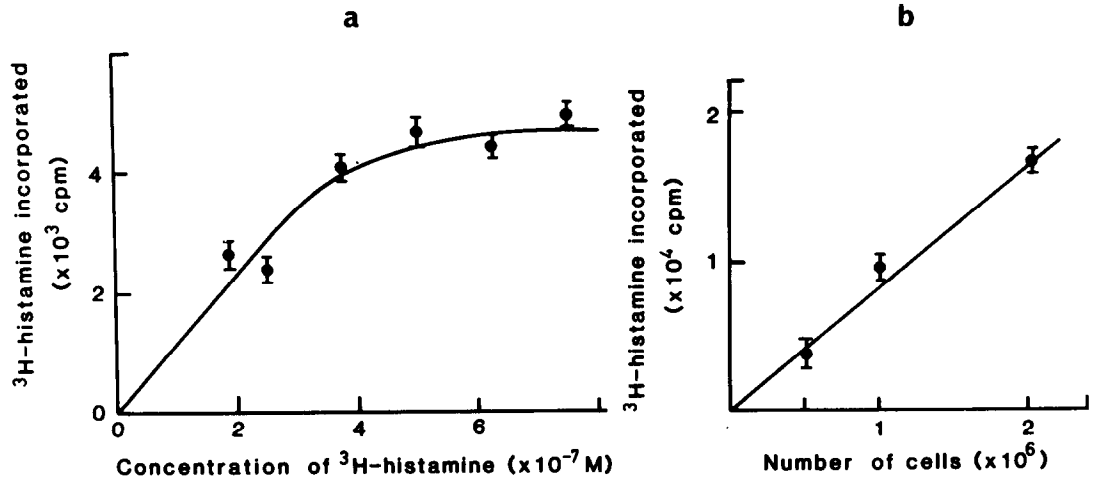

Fig. 1. ^{3}H-histamine incorporation into myeloblasts and promyelocytes. (a) Relationship between the extent of ^{3}H-histamine incorporated and the ^{3}H-histamine concentration. Using 1×10^6 cells, incubation was performed at 37° for 6 hr. (b) Relationship between the amount of incorporated ^{3}H-histamine and the cell number. A 5×10^{-7} M (1 μCi) concentration of ^{3}H-histamine was added and incubation was performed at 37° for 6 hr. Each dot represents the mean ± SEM of six experiments.

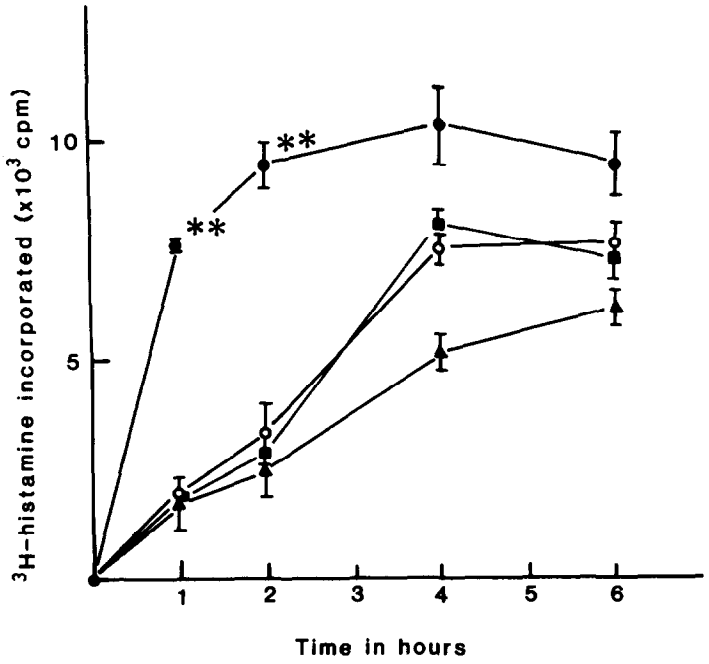

Fig. 2. Time course of ^{3}H-histamine incorporation into myeloblasts and promyelocytes. Cells were incubated in RPMI-1640 medium containing 20% HS with (●–●) or without (○–○) histamine pretreatment (5×10^{-7} M). In another instance, histamine pretreatment was performed in the presence of 4×10^{-7} M of cycloheximide (▲–▲) or 10^{-7} M of actinomycin D (■–■). The radioactivity of 1×10^6 cells after exposure to 5×10^{-7} M ^{3}H-histamine is shown. Each symbol represents the mean ± SEM of six experiments.

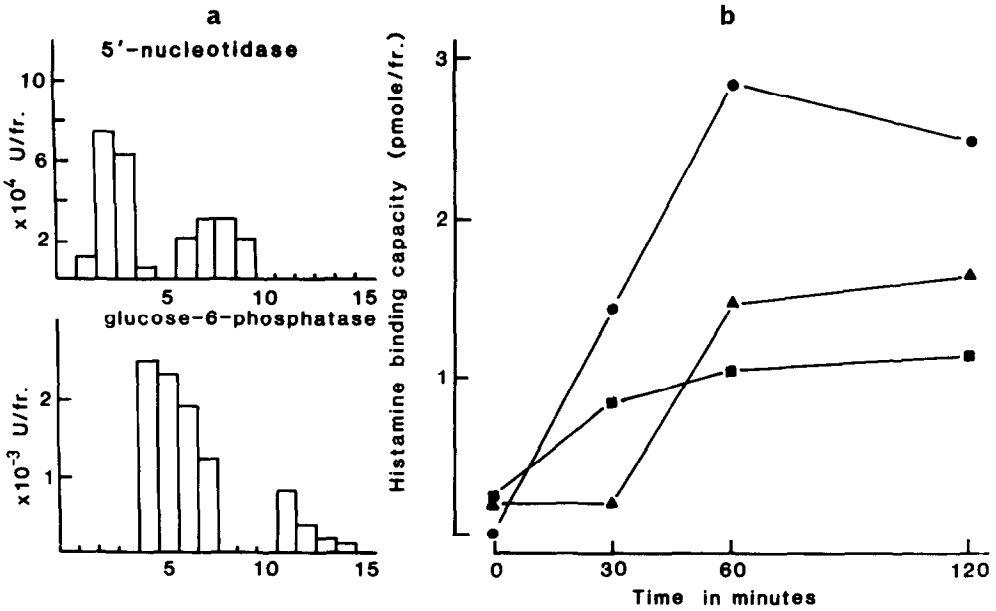

Fig. 3. Biosynthesis of histamine-binding protein obtained from myeloblasts and promyelocytes treated with histamine (5×10^{-7} M). (■–■), binding capacity determined in the endoplasmic reticulum fraction (fractions 4 and 5); (▲–▲), in the plasma membrane fraction; (●–●), in the total microsomal fraction. (a) Profile of 5'-nucleotidase and glucose-6-phosphatase activities in each fraction. (b) Sequential changes in histamine binding capacity in the endoplasmic reticulum, plasma membrane and total microsomal fractions. Abscissa indicates time in contact with cold histamine before ^{3}H-histamine assay performed. N = 6.

beled histamine pretreatment (5×10^{-7} M). As shown in Fig. 3a, 5'-nucleotidase, a marker enzyme of the plasma membrane, appeared mostly from fractions 2 to 3, while the activity of glucose-6-phosphatase was higher in fractions 4–7, indicating that they were endoplasmic recitulum-rich fractions. Although fractions 6 and 7 were high in glucose-6-phosphatase activity, some contamination of membrane fractions was believed to exist. For this reason, fractions 4 and 5 were employed as the endoplasmic reticulum fraction for the histamine binding assay. Sequential changes in ^{3}H-histamine binding in both fractions are shown in Fig. 3b. After 30 min of incubation with unlabeled histamine, the binding capacity of the endoplasmic reticulum increased significantly, while no elevation was observed in the membrane fraction. However, after 60 min of incubation, the binding capacity of the latter surpassed that of the former.

Figure 4 shows the metabolic changes in ^{3}H-histamine incorporated into the progenitor cells. The major metabolite was 1-methylhistamine, while no increase in imidazole acetic acid was observed, even after 6 hr of incubation. The amount of 1-methylhistamine initially increased, but after 1 hr, change was moderate. However, the histamine concentration increased markedly, especially after 4 hr of incubation. At the 6 hr point of incubation, it was determined that 67.0% of the total radioactivity of the cells was histamine and 31.5% was 1-methylhistamine.

In order to investigate the interaction between incorporated histamine and nucleus, the binding capacity of histamine to either DNA or to chromatin proteins was measured (Table 1). When histamine binding to DNA extracted from progenitor cells and to that of calf thymus was compared, no difference was noticed in the dissociation constant. However, comparison of NHCP and DNA, both extracted from neutrophil progenitors, showed that the former has an approximately 10 times higher affinity for histamine than the latter. A negligible amount of histamine was bound to calf thymus histone, histone extracted from progenitor cells or bovine serum albumin.

Table 1. Dissociation constants in the binding of histamine to DNA and to chromatin-related proteins extracted from the nuclei of myeloblasts and promyelocytes

Source	DNA and associated proteins	K_d (M)
progenitor cells	DNA	2.48×10^{-9}
	histone	*
	NHCP	2.54×10^{-10}
	chromatin	5.22×10^{-9}
calf thymus	DNA	3.10×10^{-9}
	histone	*
BSA		*

* No binding with ^{3}H-histamine was detected.

Changes in the protein synthesis of myeloblasts and promyelocytes during incubation in the medium containing histamine is shown in Fig. 5. When the radioactivity of ^{3}H-leucine incorporated into the progenitor cells was measured, the first peak appeared after 1 hr of exposure to histamine and the second after 5 hr of incubation, reaching 219.5% and 188.7% of the control value, respectively. The first peak seems to correspond to the histamine incorporation stage and the second may be related to cell division.

Analysis of myeloperoxidase activity in myeloblasts and promyelocytes revealed that AMT-insensitive enzyme activity decreased according to the histamine concentration, while no significant changes in AMT-sensitive enzyme were observed (Fig. 6).

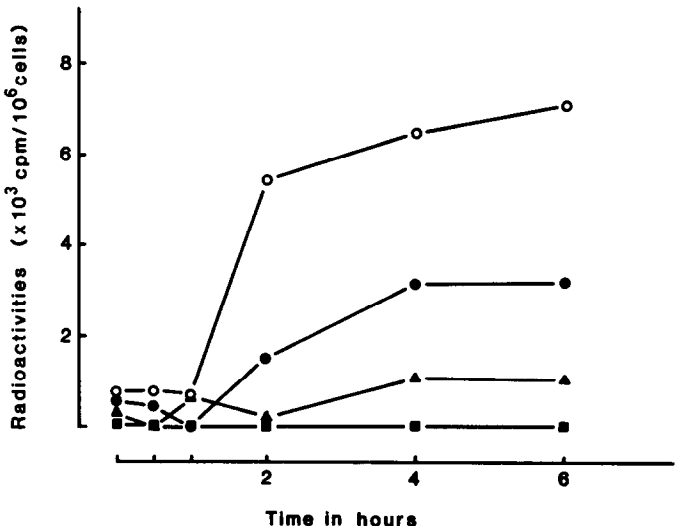

Fig. 4. Metabolism of histamine incorporated into myeloblasts and promyelocytes. Cells were incubated in RPMI-1640 medium containing 20% HS and 5×10^{-7} M ^{3}H-histamine (2.5 μCi), washed three times with PBS and dissolved in 0.1% Triton X-100. $N = 6$. (○—○), histamine; (●–●), 1-methylhistamine; (■–■), 1-methylimidazole acetic acid; (▲–▲), imidazole acetic acid.

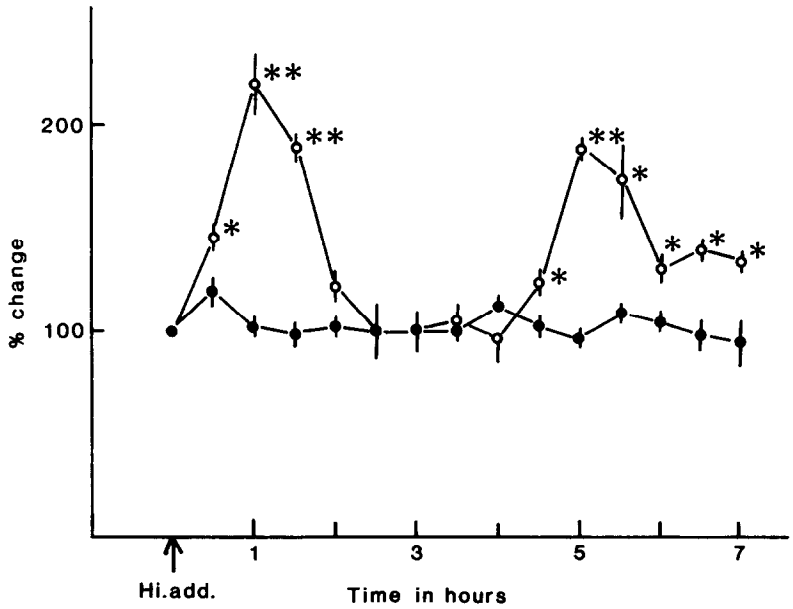

Fig. 5. Time course of ^{3}H-leucine incorporation into myeloblasts and promyelocytes. Cells were incubated in leucine-free Eagles MEM (20% HS) with (○–○) or without (●–●) 5×10^{-7} M histamine. Cells were pulse-labeled with 2×10^{-7} M ^{3}H-leucine (1.0 μCi) for 60 min. N = 6. *, **: $P < 0.05$ and $P < 0.01$, respectively.

Thus, histamine preferentially decreased peroxidase activity in neutrophil progenitor cells dose-dependently without affecting eosinophil peroxidase.

DISCUSSION

In murine bone marrow, neutrophil progenitor cells are present in various stages of differentiation. It is known that CFU-s, the most immature progenitors, have the potential to differentiate into many types of blood cell. Moreover, it has been reported that 4-methylhistamine acts on hematopoietic stem cells to stimulate the cell cycle from the G_0 state to the S-phase [19]. Since this effect was inhibited in the presence of metiamide, it is thought that the process takes place through H_2 receptors as

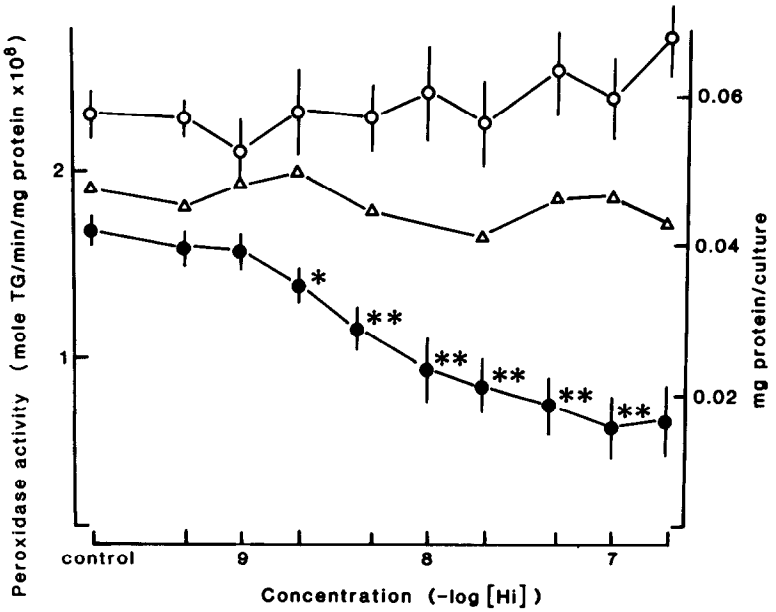

Fig. 6. Changes in the peroxidase activity of myeloblasts and promyelocytes after the addition of histamine. Cells were incubated for 24 hr in RPMI-1640 medium (20% HS) with various concentrations of histamine. Circles (○–○) represent the AMT-sensitive activity indicating the presence of eosinophil peroxidase, dots (●–●) that indicating peroxidase derived from neutrophil progenitors. Triangles (△–△) show the protein contents in each culture. N = 6. *, **: $P < 0.05$ and $P < 0.01$, respectively.

does the marked proliferation of CFU-c's caused by histamine [5]. However, in the myeloblast and promyelocyte stages, histamine stimulates differentiation through a specific receptor not associated with either H_1 or H_2 [6].

We found that histamine was preferentially incorporated into myeloblasts and promyelocytes rather than myelocytes and metamyelocytes, which are the more differentiated in the line of cell maturation [6]. This incorporation reached a maximum when histamine was added to the medium at the concentrations higher than 5×10^{-7} M, as shown in Fig. 1; a similar dose–response relationship was seen in the differentiation of these progenitor cells induced by histamine [6]. However, this raises the question of whether the histamine translocated into these cells acts in an unmetabolized form or not. It is known that histaminase activity is high in the secondary granules of neutrophils and eosinophils but lower in the cytoplasm [20]. In the present experiment, the amount of imidazole acetic acid determined in progenitor cells was almost negligible, while the content of 1-methylhistamine increased to some extent, as shown in Fig. 4. However, it is known that histamine-*N*-methyltransferase activity is high in the cytoplasm of macrophages [21]. It became apparent that a large portion of histamine incorporated into the progenitor cells remained unmetabolized (Fig. 4).

Regarding histamine translocation into human promyelocytic leukemia cell line HL-60, Gespach *et al.* presumed that there exists a high capacity transport system with a specificity for histamine in immature granulocytes [9]. They stated that tricyclic antidepressants which have potent H_1 and H_2 blocking actions do not effectively inhibit the production of this histamine transport system. Previous reports on histamine translocation into neutrophil progenitors have shown that incorporation is highly specific to histamine and is not affected by simultaneous addition of various H_1- and H_2-agonists or antagonists [6]. Histamine incorporation into murine spleen cells has also been reported by Wang *et al.* [8]. Although incorporation was inhibited in presence of diphenhydramine, and not with cimetidine, it seems difficult to relate histamine uptake to the H_1 receptor. In a concentration as high as that employed, diphenhydramine would bind to the lipid bilayer and build up a large positive charge in the cell membrane [8]. The results in the present experiment seem to be in accordance with those described by Gespach *et al.* [9].

When progenitor cells were pretreated with unlabeled histamine for 1 hr, the subsequent ^{3}H-histamine incorporation was markedly accelerated, as shown in Fig. 2. However, in the presence of cycloheximide or actinomycin D, this acceleration was completely inhibited. This evidence strongly suggests that the histamine transport system is produced during the preincubation period and that it consists mainly of proteins. Actually, protein synthesis determined by ^{3}H-leucine incorporation into the neutrophil progenitors reached its first peak about 1 hr after addition of histamine (Fig. 5).

It became apparent that while histamine readily binds to NHCP, its affinity with histone is negligible. It is known that histone consists of many basic proteins, is rich in either lysine or arginine and is covered with a highly positive charge. In the physiological pH range, histamine exists in monocationic form [22], so that histamine binding to histone is quite unlikely (Table 1). There was no histamine binding to either the histone extracted from the nuclei of progenitor cells or to that from calf thymus. By contrast, it is known that NHCP consists of various types of proteins in which high mobility group (HMG) protein 1 and HMG 2 provide negatively charged areas, especially at the *C*-terminals, which contain repeated sequences of acidic amino acids such as glutaminic or aspartic acid [23]. That histamine binding to NHCP takes place initially in these areas seems to be a reasonable assumption. It has also been reported that the *C*-terminal areas of HMGs participate in the complex formation of NHCP with histone [24]. It was found that histamine binds to the DNA of progenitor cell nuclei and of calf thymus to a similar extent; thus, this binding probably arises from physicochemical interaction.

The first stage of the protein synthesis in the cell occurs in the ribosomes which attach to the endoplasmic reticulum. Thereafter, protein synthesis initially takes place in the endoplasmic reticulum, and subsequently, continues in the Golgi apparatus, from which protein vesicles are transferred to the cell membrane. As shown in Fig. 3, the histamine binding capacity of progenitor cells exposed to histamine increased initially in the endoplasmic reticulum fraction. However, after 60 min of exposure, there was a rise in binding capacity in the plasma membrane. This time schedule coincides with that of the rapid ^{3}H-histamine uptake into progenitor cells previously exposed to unlabeled histamine (Fig. 2). This may indicate that the histamine carrier protein synthesized in the endoplasmic reticulum is a proto-type which is later refined, becoming a specific carrier protein. The latter may be transferred to and then combined into the cell membrane. A histamine transport system, thus formed, may serve to increase the histamine uptake into the progenitor cells. In connection with this, it has been reported that histamine has some important involvement in the proliferation of tumor cells [25, 26]. While myeloblasts and promyelocytes are not tumor cells, they are in an immature state and it may be reasonable to assume that a similar process leading to replication may occur in neutrophil progenitor cells.

It has been reported that myeloperoxidase is present in primary granules of granulocytes and in myeloid precursor cells, most abundantly in promyelocytes [27]. The activity of this enzyme, detected in HL-60 cells by histochemical staining, decreased during cell differentiation *in vitro*; it is known that HL-60 cells consist predominantly of promyelocytic cells [28]. As shown in Fig. 6, myeloperoxidase activity in neutrophil progenitors decreased dose-dependently after treatment with histamine. Significant inhibition was induced at concentrations higher than 2×10^{-8} M, reaching a maximum at 10^{-7} M. A similar concentration–effect relationship was seen in the differentiation of murine neutrophil progenitors induced by histamine. Since the primary granules appear at the promyelocyte

stage, the myeloperoxidase activity determined in the present experiment may be derived mainly from promyelocytes rather than myeloblasts.

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