Histamine H_2 receptor activity during the differentiation of the human monocytic-like cell line U-937

Comparison with prostaglandins and isoproterenol

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Histamine H_2 receptor activity (cAMP generation) has been characterized in U-937 cells before and after retinoic acid-induced differentiation into monocyte-/macrophage-like cells. The differentiation is associated with a decreased capacity of U-937 monocytes to generate cAMP under basal conditions or after cell surface receptor stimulation by histamine, isoproterenol and PGE_1. In contrast, the potencies of the hormones are unchanged during monocytic maturation (EC_{50} values = $3.2-4.6 \times 10^{-6}$ M histamine, $4.6-7 \times 10^{-9}$ M isoproterenol, $2-4.6 \times 10^{-6}$ M PGE_1). The data support the view that histamine and cAMP-inducing agents may control the proliferation and differentiation of normal and leukemic cells committed to monocytic maturation in man. They also raise the possibility that normal human monocytes also possess functional H_2 receptors and that histamine may be implicated in the regulation of monocyte/macrophage functions.

Histamine H_2 receptor *Monocytic differentiation* *U-937 cell* *Monocyte-Macrophage-like cell*

1. INTRODUCTION

We have recently demonstrated that the human promyelocytic leukemic HL-60 cells possess typical histamine H_2 receptors [1] with pharmacological properties similar to those established in mature human peripheral neutrophils [2]. The HL-60 cells can be induced to differentiate into granulocyte-like cells by retinoic acid [3] or into monocyte-/macrophage-like cells in the presence of leucocyte-conditioned medium [4]. The human histiocytic lymphoma cell line U-937, which has the characteristics of immature monocytoid cells with monoblast-like characters [5] can also be primed for differentiation into monocyte-/macrophage-like cells by treatment with retinoic acid [6]. This morphological maturation is accompanied by the apparition of biochemical and functional characteristics of mature monocytes, including nonspecific esterase staining, phagocytosis, chemotaxis, hexose monophosphate shunt activity and the capacity to reduce nitroblue tetrazolium (NBT). The U-937 system thus provides a model for the study of plasma membrane development and cell surface receptor expression during human monocytic differentiation. Since monoblastic and promyelocytic progenitors originate from the common hemoblastic precursor, we have investigated the presence of histamine receptors in U-937 cells before and after monocytic differentiation by retinoic acid. Histamine receptor activity and specificity were evaluated by measurements of cAMP levels in U-937 cells after adding histamine or its H_1 and H_2 agonists and antagonists. Changes in cAMP production after histamine addition were compared to those obtained with the ubiquitous adenylate cyclase activators, isoproterenol and prostaglandins. A portion of this work has been described in [7].

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2. MATERIALS AND METHODS

Histamine dihydrochloride, diphenhydramine (DPH), prostaglandins E_1 (PGE_1), L-isoproterenol, all-*trans*-β-retinoic acid, NBT, 3-isobutyl-1-methylxanthine (IBMX) and cyclic adenosine 3′-5′-monophosphate (cAMP) were purchased from Sigma (St. Louis, MO), bovine serum albumin (fraction V) was from Miles Laboratories (Elkhart, IN). RPMI-1640 medium, fetal calf serum and antibiotics were from Flow Laboratories. Highly purified natural porcine VIP and crystallized porcine glucagon (lot 421306) were, respectively, purchased from Professor V. Mutt (GHI-Laboratory, Stockholm, Sweden) and from Novo Research Institute (Bagsvaerd, Denmark). Impromidine, (4-methyl)histamine (4-MH), 2-(2-aminoethyl)-thiazole (AET), 2-(2-pyridyl)ethylamine (PEA) and cimetidine were generous gifts from Dr R. Brimblecombe of Smith, Kline and French Laboratories (Welwyn Garden City, Hertfordshire, England). All other chemicals used were of the highest purity available.

2.1. *Cell culture*

The U-937 cells, generously provided by Dr Breitman (National Cancer Institute, Bethesda, USA) were grown in continuous liquid suspension culture in RPMI 1640 medium supplemented with 10% heat-inactivated fetal calf serum, 2 mM L-glutamine, streptomycin (100 μg/ml) and penicillin (100 IU/ml). The cells are passaged at starting densities of $1-2 \times 10^5$ cells/ml and were incubated at 37°C in a humidified atmosphere of 5% CO_2 in air. Cells were harvested by centrifugation (200 × *g*, 5 min), washed twice in Krebs-Ringer phosphate buffer (KRP, pH 7.4) and finally resuspended in this buffer to a final concentration of $1-2 \times 10^6$ cells/ml for cAMP analysis. Cells were counted in a homocytometer chamber and cell viability was determined by trypan blue exclusion. Differential counts were performed on a minimum of 100 cells for each experiment. Cell viability was 85–95%, and incubation of U-937 cells (37°C for 5–15–30 min) in the presence of 10^{-4} M isoproterenol, prostaglandins E_1, histamine or its analogs did not change this percentage. Differentiation of U-937 cells was induced by culturing the cells in the presence of 1 μM retinoic acid for 6 days. Differentiation of U-937 cells was estimated by a number of criteria [8], including morphological assessment on cytospin slide preparations stained with May-Grunwald-Giemsa, reduction of NBT, chemotaxis towards activated human serum in modified Boyden chambers, hexose monophosphate shunt activity, phagocytosis of heat-inactivated yeast cells and antibody-dependent cellular cytotoxicity.

The HGT-1 cell line, provided by Dr Laboisse (Paris), was routinely cultured in Dulbecco's modified Eagle's medium, as in [9]. Adherent cells cultured in the absence or in presence of 1 μM retinoic acid for 7 days were removed from culture flasks with 0.02% EDTA. Cell viability was about 90–95% after 7 days of cell culture in the absence or presence of 1 μM retinoic acid.

2.2. *Cyclic AMP assay*

In a standard assay, 150 μl from the U-937 cell suspensions ($1-2 \times 10^6$ cells/ml, initial concentration) were preincubated for 10 min at 37°C in 250 μl Krebs Ringer phosphate buffer (pH 7.5) containing 1% BSA and 2 mM IBMX. The reaction was initiated by the addition of appropriate hormones or drugs (100 μl). The reaction was stopped after 10 min incubation by adding 50 μl 11 N $HClO_4$ and cAMP was determined by the radioimmunoassay method described in [2]. None of the agents used in the present study interfered with the assay of cAMP.

2.3. *Processing of the data and statistical analysis*

Absolute values are given as pmol cAMP produced by 10^6 cells. The apparent EC_{50} and IC_{50} potency values were the doses required to produce, respectively, 50% of the maximal stimulation or inhibition produced by the test agents. Antagonism by H_1 and H_2 antihistamine was analyzed assuming competitive inhibition, according to the equation [10]:

$$K_i = IC_{50}/(1 + S/EC_{50}),$$

where: K_i = the inhibition constant of the antagonist, and S = the histamine concentration. The data were normalized as the percentage of the response to a given concentration of histamine. Results were analyzed by standard methods using Student's paired *t*-test.

3. RESULTS

3.1. *Influence of time and IBMX on histamine receptor activity in U-937 cells*

As shown in fig.1, basal cAMP levels in U-937 cells, before (left) and after (right) monocytic differentiation by retinoic acid, stay constant (1.4 ± 0.26 and 1.05 ± 0.22 pmol/10^6 cells, respectively) during the 0–15 min incubation period at 37°C, in the absence of phosphodiesterase inhibitor. Addition of 10^{-4} M histamine produced a rapid (1 min) and significant (2.7- to 2.4-fold) production during 5 min (respective peak values: 2.9 ± 0.41 and 2.6 ± 0.43 pmol/10^6 cells), followed by a progressive decrease within differentiated U-937 cells. Addition of the phosphodiesterase inhibitor IBMX from 0.1 to 2 mM potentiated remarkably the stimulatory effect of histamine in undifferentiated U-937 cells. The maximal difference between basal (5.1 ± 0.9 pmol/10^6 cells) and histamine-stimulated cAMP levels (75.4 ± 6.5 pmol/10^6 cells, n = 4) was seen with 1 mM IBMX (fig.2). This concentration of phosphodiesterase inhibitor allowed us to study histamine receptor activity under conditions of equilibrium in U-937 cells since cAMP levels attained a plateau within 3 min, and remained constant for up to 15 min. It was also verified that, in U-937 cells incubated in the absence or presence of 10^{-3} M histamine, cAMP generation was a linear function of cell concentration from 0.08 to 1.2 × 10^6 cells/ml (not shown).

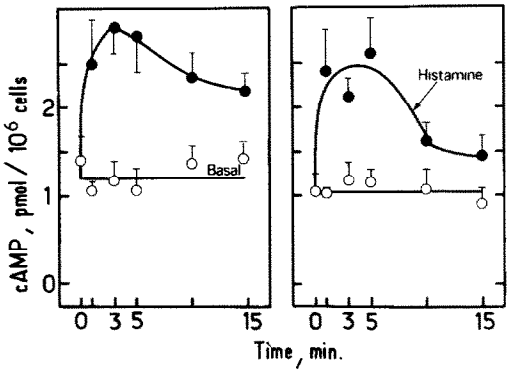

Fig.1. Effect of time on histamine receptor activity in U-937 cells (left) or U-937 monocytes (right), incubated in the absence of phosphodiesterase inhibitor. Cells (0.3–0.6 × 10^6 per ml, final concentration) were preincubated at 37°C for 10 min in KRP buffer containing 1% BSA and then incubated for the time indicated in the absence (basal cAMP levels, ○) or presence (●) of 10^{-4} M histamine. Data are means ± SE of the results from 4–7 experiments performed in duplicate or triplicate.

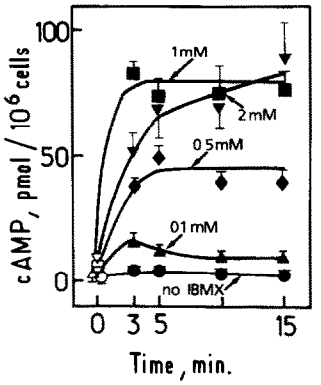

Fig.2. Effect of the phosphodiesterase inhibitor IBMX on histamine receptor activity in U-937 cells. Cells were preincubated for 10 min at 37°C in the standard KRP buffer containing various concentrations of IBMX, and incubated for the time indicated in the presence of 10^{-4} M histamine. Data are means ± SE of the results from 4 experiments performed in duplicate or triplicate.

3.2. *Comparative effect of histamine, isoproterenol and histamine*

Cyclic AMP production in U-937 cells incubated for 10 min at 37°C was stimulated by histamine over a range of 10^{-7} to 10^{-3} M (fig.3). Half-

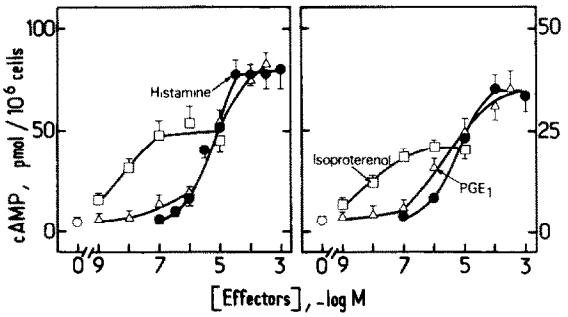

Fig.3. Effect of various concentrations of histamine, L-isoproterenol and PGE_1 on cAMP generation in U-937 cells (left) or U-937 monocytes (right). Cells were preincubated for 10 min at 37°C in the standard buffer containing IBMX and incubated for 10 min in the presence of histamine (●), isoproterenol (□) and PGE_1 (△). Data are means ± SE of the results from 4–12 experiments performed in duplicate or triplicate.

Table 1

Cyclic AMP production in U-937 cells and U-937 monocytic-like cells incubated under basal conditions or exposed to histamine, isoproterenol and prostaglandins

Effectors	cAMP (pmol/10^6 cells)	
	U-937 cells	U-937 monocytes
Control	4.86 ± 0.52 (22)	2.48 ± 0.19 (12)
Histamine (10^{-4} M)	76.4 ± 6.4 (12)	35.4 ± 3.6 (5)
Isoproterenol (10^{-6} M)	54 ± 10 (6)	21 ± 1.5 (4)
PGE_1 (10^{-4} M)	75 ± 5.4 (4)	31.3 ± 3.9 (4)

Cells were incubated at 37°C in the standard solution containing IBMX. After 10 min, the indicated agents were added and the incubation was continued for 10 min. The values are means ± SE of the number of separate experiments indicated in parentheses

maximal stimulations (EC_{50} values) were, respectively, observed at 3.16 or 4.6 × 10^{-6} M histamine in U-937 cells, before (left) and after (right) retinoic acid-induced monocytic differentiation. Basal cAMP levels in U-937 cells were significantly increased by 10^{-9} M isoproterenol or 10^{-7} M PGE_1. The potency of isoproterenol and PGE_1 were similar in U-937 cells before (EC_{50} = 4.6 × 10^{-9} M isoproterenol, 4.6 × 10^{-6} M PGE_1) or after monocytic differentiation (EC_{50} = 7 × 10^{-9} M and 2 × 10^{-6} M, respectively). Table 1 indicates that U-937 cell differentiation into monocyte-like cells is associated with a remarkable reduction of the capacity of cells to generate cAMP under basal or stimulated conditions. In contrast, vasoactive intestinal peptide and glucagon, two peptides having, respectively, specific receptor sites in T lymphocytes [11,12] and monocytes [13], are inactive in U-937 cells and U-937 monocytes. It is therefore possible that functional glucagon receptors are expressed in more mature monocytes during myeloid differentiation.

3.3. *Comparative effect of histamine and synthetic related analogs*

The effect of impromidine and 4-MH, two H_2-selective agonists, and of PEA and AET, two H_1-selective agonists were compared to histamine receptor activity in U-937 cells (fig.4), before (left) and after (right) retinoic acid-induced monocytic differentiation. The H_2 agonist 4-MH and the H_1 agonist AET produced parallel dose–response curves to histamine with a similar extent of cAMP stimulation. In contrast, the H_2 agonist impromidine and the H_1 agonist PEA were partial agonists of histamine, since they produced, respectively, only 22–26% and 34–44% of the maximal stimulation observed with histamine. The relative potencies of the H_1, H_2 agonists studied has been compared in U-937 cells and U-937 monocytes (table 2). Fig.5 shows that the H_1 antagonist DPH and the H_2 antagonist cimetidine completely abolished in a monophasic manner cAMP production induced by 10^{-4} M histamine in U-937 cells and U-937 monocytes. Approximately 50% of the

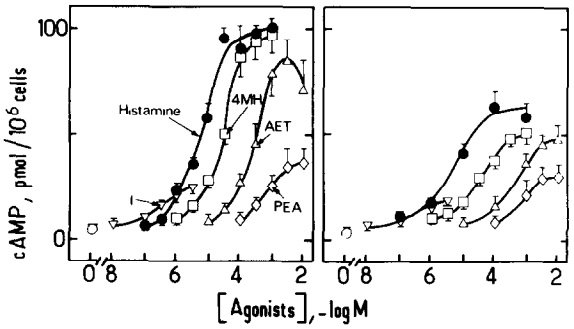

Fig.4. Receptor specificity of histamine-induced cAMP production in U-937 cells (left) and U-937 monocytes (right) toward various histamine H_1 or H_2 agonists. Cells were preincubated for 10 min at 37°C in the standard buffer containing IBMX and incubated for 10 min after addition of histamine (●) or its H_2 agonists impromidine (I, ▽), 4-MH (□) and H_1 agonists AET (△), PEA (◇). Data are means ± SE of the results from 4–6 experiments performed in duplicate or triplicate.

Table 2

Comparison of the relative potencies of histamine and its H_1 or H_2 agonists on cAMP production in U-937 cells and U-937 monocytes

Cells	Agonists				
	Impromidine	H (EC_{50})	4-MH	AET	PEA
U-937 cells	2960	100 (7.4×10^{-6} M)	23	2.3	1.3
U-937 monocytes	2700	100 (4.6×10^{-6} M)	15	1	0.7

The relative potency of each agonist was established as the ratio: EC_{50} for histamine/EC_{50} for agonist × 100. The potency (EC_{50}) of histamine: H in each preparation is indicated in parentheses

response was inhibited with an IC_{50} of 2.15×10^{-5} M cimetidine, giving a K_i value of 0.66×10^{-6} M. The H_1 antagonist DPH also produced a dose-related inhibition, but with 100–200 times higher concentrations ($IC_{50} = 2.5 \times 10^{-4}$ M in U-937 cells and 4.7×10^{-4} M DPH in U-937 monocytes), giving K_i values of 0.76 and 1.44×10^{-4} M DPH, respectively.

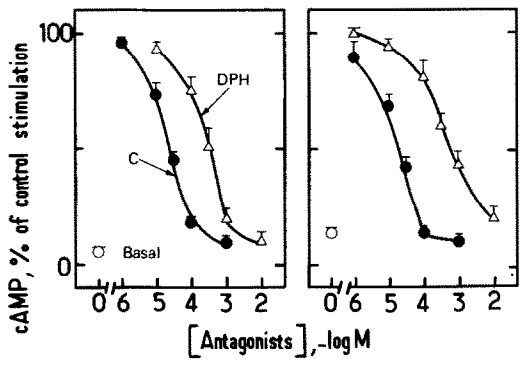

Fig.5. Receptor specificity of histamine-induced cAMP production in U-937 cells (left) and U-937 monocytes (right) toward histamine H_1 or H_2 antagonists. After a 10-min incubation period at 37°C, histamine alone (10^{-4} M, control stimulation) or in combination with the H_2 antagonist cimetidine (C, ●) or the H_1 antagonist DPH (DPH, △) were added and the incubation was continued for 10 min in the presence of IBMX, as indicated in section 2. Cyclic AMP production in control cells (○). Results are expressed as the percentage of cAMP production elicited by histamine alone. Data are means ± SE of the results from 4 experiments performed in duplicate or triplicate.

4. DISCUSSION

Our current studies demonstrate the presence of functional and specific H_2 receptors for histamine in the human monoblastic U-937 cells, before and after retinoic acid-induced differentiation into monocyte-/macrophage-like cells. The pharmacological analysis of the effect of histamine and its H_1 or H_2 agonists in U-937 cells and U-937 monocytes shows a potency sequence (I > H > 4-MH > AET > PEA) characteristic of the H_2 type histamine receptor. The same order of potency ($H_2 > H_1$) has been established for histamine H_2 receptor activity in human promyelocytic HL-60 cells [1], in mature human neutrophils [2] as well as in other tissues bearing H_2 receptors [14,15]. In contrast, an inverse sequence ($H_1 > H_2$) was found for H_1 receptors [16]. The measured IC_{50} and calculated K_i values for the H_2 antagonist cimetidine ($K_i = 0.66 \times 10^{-6}$ M) and the H_1 antagonist DPH ($K_i = 0.76–1.44 \times 10^{-4}$ M) indicate that DPH was approx. $1–2 \times 10^2$ times less potent than cimetidine in inhibiting histamine receptor activity in U-937 cells and U-937 monocytes. The inhibition constants obtained here are comparable to the relative affinities of these two antagonists ($H_2 > H_1$) for H_2 receptors [1,2] in human neutrophils ($K_i = 0.4 \times 10^{-6}$ M cimetidine and 65×10^{-6} M DPH) and human promyelocytic HL-60 cells (K_i = 0.65 and 51×10^{-6} M, respectively).

The relative potencies of histamine and the H_1,H_2 receptor agonists and antagonists studied are remarkably similar in U-937 cells before and after differentiation with retinoic acid, indicating that the structural requirements of the H_2 receptor

for adenylate cyclase activation are unchanged during monocytic/macrophage maturation. The same observation is made for PGE_1 and isoproterenol. In contrast, the differentiation is associated with a decreased capacity of the U-937 monocytes to generate cAMP under basal conditions or after cell surface receptor activation by histamine, isoproterenol and prostaglandins. Such a phenomenon has been described previously during the granulocytic differentiation of the human promyelocytic cell line HL-60 by retinoic acid [17], as well as for the comparison between differentiated HL-60 cells and mature human peripheral neutrophils [1,2]. It is therefore possible that histamine, isoproterenol or prostaglandins may play a role in the regulation of proliferation/differentiation of human monocytic precursor cells since dibutyryl cAMP or the cAMP-inducing agent cholera toxin have been shown to act synergistically with retinoic acid or 1,25-dihydroxyvitamin D_3 to induce differentiation of HL-60 or U-937 cells [6,18,19]. Changes in histamine receptor activity during retinoic acid-induced monocyte differentiation of U-937 cells are well related to cell differentiation since there is no change in H_2 receptor activity [14] and no morphological differentiation [9] in the human gastric cancer HGT-1 cells when they are cultured under the same conditions with retinoic acid.

Histamine receptors (H_1, H_2 and a third type) have been identified in alveolar macrophages [20] as well as in a variety of circulating human blood cells, including lymphocytes [21–23], platelets [24,25], eosinophil [26], basophil [27] and neutrophil [2,28] leukocytes. Since the role of histamine in the inflammatory and immune response is now well accepted [29], the presence of cell surface receptors for histamine in the human monocytic U-937 cells also raises the possibility that normal human monocytes possess functional histamine H_2 receptors linked to a biological response. Studies are now in progress on isolated human peripheral monocytes to attempt to confirm this hypothesis.

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REFERENCES

[1] Gespach, C., Saal, F., Cost, H. and Abita, J.-P. (1982) Mol. Pharmacol. 22, 547–553.
[2] Gespach, C. and Abita, J.-P. (1982) Mol. Pharmacol. 21, 78–85.
[3] Breitman, T.R., Selonick, S.E. and Collins, S.J. (1980) Proc. Natl. Acad. Sci. USA 77, 2936–2940.
[4] Nilsson, K., Anderson, L.C., Gahmberg, C.G. and Forsbeck, K. (1980) in: International Symposium on New Trends in Human Immunology and Cancer Immunotherapy (Serrou, B. and Rosenfeld, C. eds) pp.271–292, Paris, Doin.
[5] Sundström, C. and Nilsson, K. (1976) Int. J. Cancer 17, 565–577.
[6] Olsson, I.L., Breitman, T.R. and Gallo, R.C. (1982) Cancer Res. 42, 3928–3933.
[7] Gespach, C. and Abita, J.-P. (1983) 15th FEBS Meeting, Brussels, Belgium, July 24–29 1983, p.222.
[8] Abita, J.-P., Gauville, C., Balitrand, N., Gespach, C. and Canivet, J. (1984) Leukemia Res. 8, 213–221.
[9] Laboisse, C.L., Augeron, C., Couturier-Turpin, M.-H., Gespach, C., Cheret, A.-M. and Potet, F. (1982) Cancer Res. 42, 1541–1548.
[10] Chen, Y.C. and Prusoff, W.H. (1973) Biochem. Pharmacol. 22, 3099–3100.
[11] O'Dorisio, M.S., Hermina, N.S., O'Dorisio, T.M. and Balcerzak, S.P. (1981) J. Immunol. 127, 2551–2554.
[12] Ottaway, C.A. and Greenberg, G.R. (1984) J. Immunol. 132, 417–422.
[13] Bhathena, S.J., Geraldine, J.L., Schechter, P., Redman, R.S., Wahl, L. and Recand, L. (1981) Diabetes 30, 127–131.
[14] Emani, S., Gespach, C., Forgue-Lafitte, M.-E., Broer, Y. and Rosselin, G. (1983) Life Sci. 33, 415–423.
[15] Gespach, C., Hui Bon Hoa, D. and Rosselin, G. (1983) Endocrinology 112, 1597–1606.
[16] Durant, G.J., Ganellin, C.R. and Parsons, M.E. (1975) J. Med. Chem. 18, 905–909.
[17] Abita, J.-P., Gespach, C., Cost, H., Poirier, O. and Saal, F. (1982) IRCS Med. Sci. 10, 882–883.
[18] Olsson, I.L. and Breitman, T.R. (1982) Cancer Res. 42, 3924–3927.
[19] Olsson, I.L., Gullberg, U., Ivhed, I. and Nilsson, K. (1983) Cancer Res. 43, 4862–4867.
[20] Diaz, P., Jones, D.G. and Kay, A.B. (1979) Nature 278, 454–456.

[21] Birch, R.E. and Polmar, S.H. (1981) Cell. Immunol. 57, 455–467.
[22] Gordon, D., Lewis, G.P. and Nouri, A.M.E. (1981) Br. J. Pharmacol. 74, 137–141.
[23] Weinstein, Y., Melmon, K.L., Bourne, H.R. and Sela, M. (1973). J. Clin. Invest. 52, 1349–1361.
[24] Launay, J.-M., Gespach, C., Pasques, D., Haimart, M. and Dreux, C. (1984) in: Frontiers in Histamine Research, Jouy-en-Josas, France, 25–27 July 1984, p.63.
[25] Klysner, R., Geisler, A., Hansen, K.W., Stahl Skov, P. and Norn, S. (1980) Acta Pharmacol. Toxicol. 47, 1–4.
[26] Clark, R.A.F., Sandler, J.A., Gallin, J.I. and Kaplan, A.P. (1977) J. Immunol. 118, 137–145.
[27] Lichtenstein, L.M. and Gillespie, E. (1973) Nature 224, 287–288.
[28] Busse, W.W. and Sosman, J. (1976) Science 194, 737–738.
[29] Bourne, H.R., Lichtenstein, L.M., Melmon, K.L., Henney, C.S., Weinstein, Y. and Shearer, G.M. (1974) Science 184, 19–28.