ELSEVIER

Immunopharmacology 30 (1995) 71–78

Immunopharmacology

The influence of betahistine on the dynamics of the cutaneous hypersensitivity reaction in patients with grass pollen allergy

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Received 17 May 1994; revised 14 November1994; accepted 17 January 1995

Abstract

Histamine has been well documented as an immune modulator, but the dynamics of a number of histamine receptor agonists and antagonists have not been similarly established. The aim of this study was to determine the effect of betahistine (an H_3-receptor blocker with partial H_1- and H_2-agonism) on the dynamics of the cutaneous hypersensitivity reaction. The skin blister technique was used to collect inflammatory cells after intradermal (i.d.) administration of grass pollen antigen, histamine and betahistine to 11 atopic volunteers. In this open, cross-over study, volunteers were randomly allocated to five treatment protocols i.e. (a) histamine 1 μg i.d.; (b) betahistine 57, 114 and 285 μg i.d.; (c) i.d. grass pollen antigen; (d) (c) plus oral betahistine; (e) (c) plus oral betahistine, cetirizine, (H_1-blocker) and cimetidine (H_2-blocker). Blister fluid containing cells were collected on microscope slides at 6 and 24 h after i.d. injections. The areas of the wheal and flare and of induration were measured, respectively, at 0.25, and, 1, 6 and 24 h. Combined oral therapy with cetirizine, cimetidine and betahistine reduced the area of grass pollen-induced induration significantly at all time periods, but caused a significant increase in eosinophil and neutrophil vacuolisation during the late phase reaction. This did not occur with orally administered betahistine alone. Intradermal betahistine induced significantly more neutrophil and eosinophil vacuolization than histamine and, in contrast to the latter, also mediated a concentration-dependent late phase induration. The results of this study suggest that the H_3-receptor regulates a feedback system in conjunction with that previously proven for the H_2-receptor. Consequenly H_2- and H_3-blockade control the release of different mediators.

Keywords: Betahistine; Cimetidine; Cetirizine; Grass pollen allergy; Eosinophil vacuolisation; Neutrophil vacuolisation; H_3-receptor

1. Introduction

Histamine has been well-documented as an immune modulator (Beer et al., 1984; Rocklin and Beer, 1983). However, its specific effects and receptors have not been similarly established. Certain regulatory functions mediated by histamine receptors on inflammatory cell membranes have been demonstrated in vitro, but their in vivo relevance needs to be clarified.

Selective histamine-1 (H_1) receptor blockers such as cetirizine only partially suppress hypersensitivity reactions in atopic individuals as several other mediators of inflammation are involved (Charlesworth et al., 1989; Simons, 1989; Snyman et al., 1992).

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SSDI 0162-3109(95)00007-0

Some of these newer agents (e.g. cetirizine) not only reduce immediate wheal and flare, but also partially inhibit the late phase of the cutaneous hypersensitivity reaction in atopic individuals. It is, however, unlikely that the latter effect is due solely to H_1-antagonism (Charlesworth et al., 1992, Snyman et al., 1992). Almost complete suppression of the late phase cutaneous induration occurs when patients with schistosomiasis are pretreated with both H_1- and H_2-blockers before i.d. antigen challenge. The addition of an H_2-blocker after H_1 blocker pretreatment, however, has no additional effect on cell dynamics (Snyman et al., 1993). However, the addition of betahistine, an H_3-receptor antagonist with partial H_1- and H_2-agonistic effects (Arrang et al., 1985), to this regimen enhances cellular activity but not the vascular reaction: i.e. cellular vacuolisation occurrs, possibly due to an effect of H_3-blockade on cytokine release (Snyman et al., 1993) without a simultaneous increase in induration, probably because of concurrent H_1- and H_2-blockade.

Intradermal (i.d.) administration of histamine is known to cause a typical wheal and flare reaction (with a $t_{\frac{1}{2}}$ of only 87 min) which is not followed by a typical late phase reaction (Cook and Shuster, 1980). Similarly betahistine i.d. causes a dose-related immediate wheal and flare reaction (Gater et al., 1986). Little is, however, known of in vivo late reactions and associated cellular reactions with both these agents.

The study attempted to establish whether i.d. betahistine mediates a late phase cutaneous hypersensitivity reaction, and whether, when orally administered, it modifies the reaction elicited by the i.d. administration of allergen to atopic individuals. This data could help to clarify the involvement of histamine receptors.

2. Materials and methods

Eleven atopic volunteers, four female and seven male, aged 20 to 22 years were recruited for this study. Strict inclusion criteria were applied and only volunteers allergic to grass pollen, and who gave a history of allergic rhinitis, conjunctivitis or asthma during the pollen season were admitted to the trial. Participants had taken no medication for a minimum of one month prior to testing, which occurred outside the pollen season. All volunteers selected reacted with a wheal not less than 20 mm in diameter to intradermally administered antigen but showed no reaction to saline control. The antigen was the Southern Grass Mix® (Bayer Miles) containing extracts of Kentucky-Blue, Orchard, Redtop, Timothy, Sweet Vernal, Bermuda and Johnson grass varieties. The protocol was approved by the Ethical Committee of the University of Pretoria and the volunteers gave their written informed consent.

In this open, cross-over study the volunteers were randomly allocated to five different treatment regimens. A washout period of at least 21 days separated them. The regimens comprised:

(a) histamine i.d.
(b) betahistine i.d.
(c) grass pollen antigen i.d.
(d) (c) plus oral betahistine (Keatings)
(e) (c) plus oral betahistine, cetirizine (UCB) and cimetidine (Lennon)

The orally administered test drugs were chosen for their receptor specificity and in each case were administered for two consecutive days. Cetirizine is a selective H_1-receptor antagonist (Snyder and Snowman, 1987) and a 10 mg dose was taken twice daily. Cimetidine, an H_2-receptor antagonist, was administered in doses of 400 mg three times a day. Betahistine is an H_3-receptor antagonist with only weak H_1- and H_2-agonistic activity (Arrang et al., 1985) and an 8 mg dose was administered four times a day. During regimens (d) and (e) skin tests commenced on the morning of the second day 1 h after the first oral drug dose of that particular day.

The skin blister technique is a non-invasive in vivo method used to study the effects of the various regimens on inflammatory cell dynamics (Kiistala et al., 1967). Two suction blisters, each with an 8 mm diameter base, were simultaneously induced on the volar aspect of one forearm. Alternate forearms were used each time. The blisters were induced over approximately 2 h by vacuum suction with a negative pressure of 300 mm Hg. Thereafter 0.05 ml; alliquots of either 10 PNU of the Southern Grass Mix, or histamine (1 μg in phosphate buffered saline) or betahistine (57 μg in phosphate buffered saline) were injected i.d. at the base of these blisters in accor-

dance with the various treatment regimens. As an elicitor of an immediate wheal and flare reaction betahistine is known to be at least 65 times less potent on a molar basis than histamine (Gater et al., 1986).

In order to establish whether the late reaction was dose dependent, betahistine was administered in two additional concentrations (respectively, 114 and 285 μg i.d.) to the volunteers with no induration at 24 h.

The blisters were left intact and covered with a small Petri-dish for protection until blister fluid was collected at 6 and 24 h. The blister fluid was applied to glass microscope slides and after air-drying, May-Grünwald-Giemsa solution was used for staining. Subsequently a differential cell count was performed to determine the percentage of each type of inflammatory cell from a total count of 500 cells. These cells were differentiated by generally accepted criteria (Fawcett, 1986). To estimate eosinophil and neutrophil activity, the percentage vacuolated cells (minimum of three vacuoles in the cytoplasm) were counted as this correlates with the physiological function of the cell at the site of antigen administration (Dikeakou et al., 1970, Kay et al., 1989).

The surface areas of the immediate (i.e. the wheal and flare) and late phase skin reactions (i.e. the induration) were calculated by marking the perimeters of these reactions; the former at 0.25 and the latter at 1, 6 and 24 h after intradermal administration of either grass-pollen antigen, histamine or betahistine. The marked areas were then transferred to transparent plastic film for later measurement by computerised planimetry.

Cell differentiation and evaluation of immediate and late phase reaction areas were performed by two independent examiners, ignorant of the treatment protocol.

For purposes of statistical analysis Friedmans two-way analysis of variance, which allows for multiple comparisons between variables on the different regimens was applied (Miller, 1986). This method permits comparisons within each individual as opposed to between them. When only two groups were compared or when deemed appropriate the Wilcoxon matched pairs test was used. The Spearman rank correlation test was used to establish correlations between eosinophil recruitment and the area size of induration. Throughout changes in data were taken to be significant at the 5% level.

3. Results

3.1. The surface areas of the immediate and late phase skin reactions (Figs. 1 and 2)

Pretreatment with a combination of cetirizine, cimetidine and betahistine caused a significant mean reduction in the areas of grass pollen-induced wheal and flare and of induration; at 0.25 h wheal and flare were reduced by 46% and 65%, respectively, while induration was reduced by 73.5%, 31.6% and 58.1% at, respectively, 1, 6 and 24 h. Oral betahistine pretreatment alone did not significantly change the areas of induration after i.d. grass pollen antigen. However, in two of the four volunteers whose reactions had already disappeared at 24 h with i.d. antigen administration alone, these were still clearly visible at this time after pretreatment with oral betahistine. The wheal and flare reactions after i.d. grass pollen antigen, betahistine (57 μg) and histamine did not differ significantly from each other. However, after i.d. antigen alone these reactions were generally larger.

At 6 h there was no visible induration after i.d. histamine, while five volunteers still showed induration after i.d. betahistine (57 μg). The areas of induration after i.d. antigen were significantly larger than those after i.d. betahistine (57 μg) at 1, 6 and 24 h. The seven volunteers who exhibited no induration after i.d. betahistine (57 μg) at 24 h were subsequently given the two other concentrations of betahistine i.d. The induration was clearly dose related, but with both 114 μg and 285 μg two volunteers still had no reaction at 6 and 24h, and a further two did not show induraton with 114 μg at 24 h (Fig. 2).

The correlation between the areas of induration and eosinophil accumulation were as follows:

(a) i.d. antigen without pretreatment: at 6 h, $r = 0.54$ ($p = 0.9$) and at 24 h, $r = 0.64$ ($p = 0.04$)
(b) i.d. antigen with oral betahistine therapy: at 6 h, $r = 0.64$ ($p = 0.04$) and at 24 h, $r = 0.81$ ($p = 0.003$)

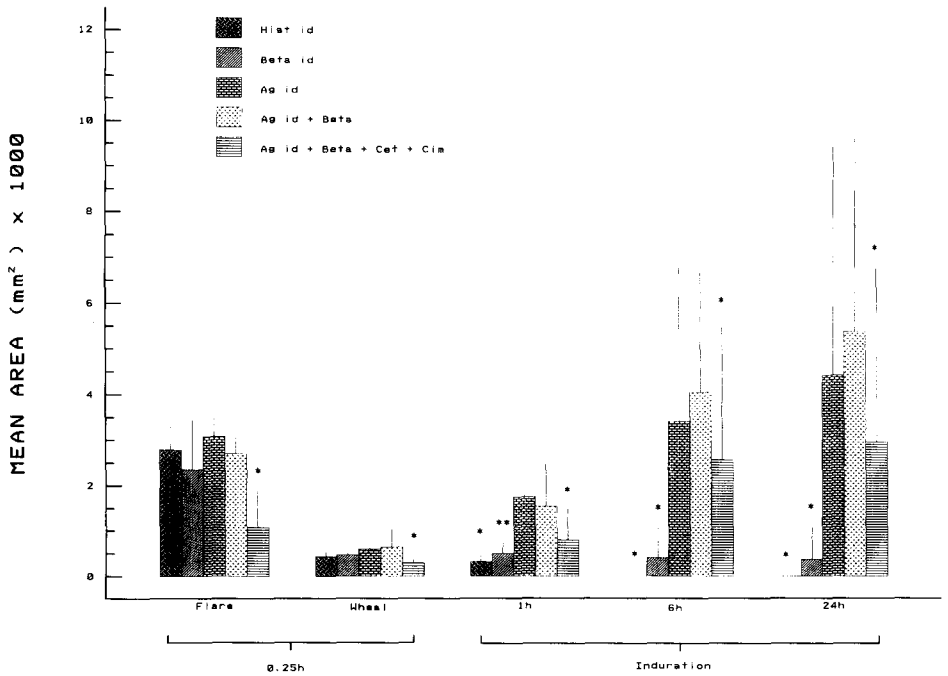

Fig. 1. Mean area (mm^2) (standard deviation) of wheal, flare and induration after i.d. administration of histamine, betahistine and antigen ($n = 11$) * Differs significantly from i.d. antigen alone and from i.d. antigen after betahistine pretreatment. ** Differs significantly from i.d. histamine alone; i.d. = intradermal; Ag = grass pollen antigen; Hist = histamine; Beta = betahistine; Cet = cetirizine; Cim = cimetidine.

(c) i.d. antigen with oral cimetidine, cetirizine and betahistine combination therapy: at 6 h, $r = 0.88$ ($p = 0.0004$) and at 24 h, $r = 0.7$ ($p = 0.02$)

There was, however, no correlation between cell vacuolisation and area of induration.

3.2. Analysis of cell dynamics (Table 1)

Except for a reduction in lymphocyte accumulation oral betahistine alone did not significantly change any of the cellular parameters after i.d. administration of grass pollen antigen. This reduction was already noticeable at 6 h ($p = 0.08$) but only became significant after 24 h ($p \leq 0.05$). Pretreatment with the combination of cimetidine, cetirizine and betahistine caused a similar pattern of reduced lymphocyte accumulation.

The main findings of these experiments were that in combination, cimetidine, cetirizine and betahistine (i.e. H_1- + H_2- + H_3-blockade) caused an increase in neutrophil and eosinophil vacuolisation after i.d. antigen administration and that vacuolisation of these cells with oral betahistine alone (i.e. H_3-blockade and H_1- and H_2-stimulation) was significantly less than that observed with combination therapy with the latter (i.e. H_1- + H_2- + H_3-blockade). Significant neutrophil vacuolisation occurred at both 6 ($p \leq 0.004$) and 24 h ($p \leq 0.04$), whereas eosinophil vacuolisation was only significantly increased at 6h ($p \leq 0.05$).

Contrary to previous findings in patients with schistosomiasis (Snyman et al., 1993) monocyte and basophil accumulation in these atopic individuals were already clearly evident with i.d. antigen administration alone; pretreatment with oral betahistine or the triple combination, did not modify the incidence significantly. Furthermore, eosinophil accumulation was seemingly not affected by the combination.

I.d. administration of betahistine (57 μg) caused significantly more neutrophil vacuolisation than i.d. antigen or histamine. Although eosinophil vacuoli-

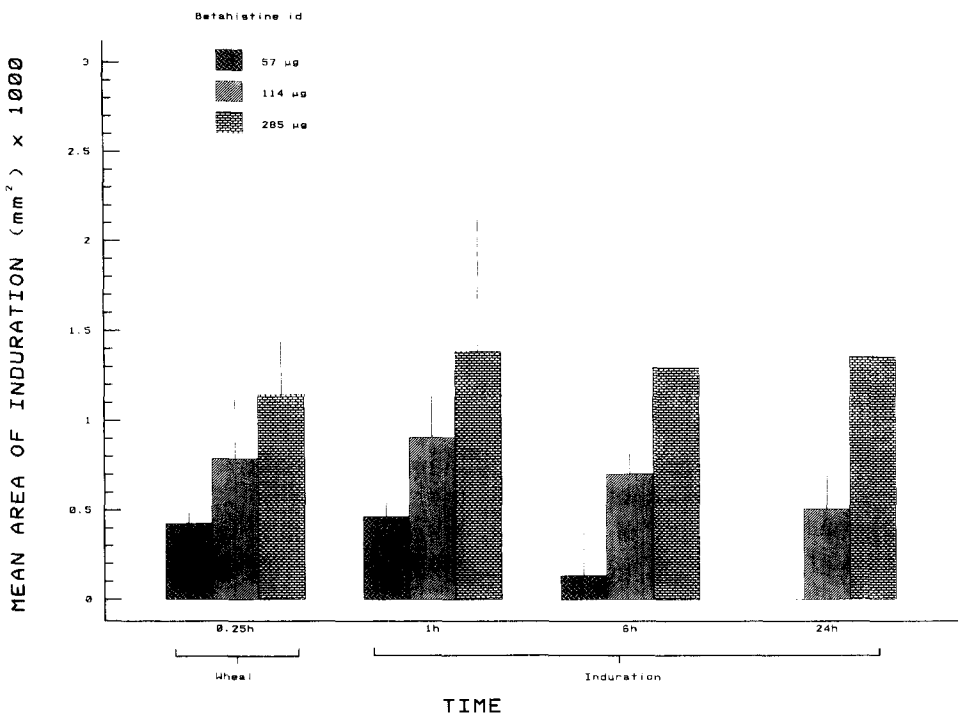

Fig. 2. Mean area (mm^2) (standard deviation) of induration after intradermal administration of betahistine to 7 volunteers not responding with induration at 24 h.

Table 1

Mean percentage (standard deviation) of total number of cells recruited (**R**) and percentage of these cells vacuolised (**V**) at the different observation periods, i.e. 6 h and 24 h after antigen administration

	Histamine i.d.		Betahistine i.d.		Antigen i.d.		Antigen i.d. + Bet		Antigen i.d. + Bet + Cim + Cet	
	6 h	24 h	6 h	24 h	6 h	24 h	6 h	24 h	6 h	24 h
Eos(**R**)	1.5[a]	1.2[a]	4.6[a]	2.7[a]	8.3	8.0	14.2	7.3	9.2	13.1
	(1.1)	(1.3)	(10.5)	(5.3)	(9.0)	(7.8)	(17.3)	(6.6)	(8.8)	(13.7)
(**V**)	16.4	3.7	10.7	25.2[c]	17.5	25.5	21.3	37.7	25.5[a]	37.6
	(12.3)	(6.5)	(15.2)	(26.2)	(18.7)	(15.7)	(20.3)	(24.9)	(20.3)	(25.3)
Neu(**R**)	62.0	31.8	51.3	32.4	62.1	54.9	59.6	51.6	57.9	52.4
	(17.5)	(16.7)	(19.7)	(20.9)	(17.9)	(17.9)	(14.3)	(19.2)	(20.8)	(23.2)
(**V**)	11.5	23.7	45.0[d]	36.1[d]	25.4	29.2	25.3	21.7	52.0[b]	40.9[b]
	(6.4)	(15.1)	(20.9)	(20.5)	(21.7)	(13.5)	(15.9)	(11.6)	(18.3)	(17.5)
Lym(**R**)	10.2	16.1	11.3	9.8	12.8	18.0	11.8	11.7	10.7	6.9[a]
	(8.1)	(7.3)	(7.4)	(4.7)	(9.5)	(14.7)	(13.6)	(11.6)	(9.7)	(4.2)
Mon(**R**)	25.4[a]	48.8[a]	32.0[a]	55.5[a]	12.6	18.8	14.0	28.0	21.0	23.7
	(12.9)	(12.9)	(13.7)	(23.0)	(10.8)	(9.3)	(9.0)	(13.3)	(15.5)	(15.6)
Bas(**R**)	0.2	0.3	0.1	0.1	0.4	0.8	0.5	1.1	0.6	1.2
	(0.2)	(0.3)	(0.1)	(0.1)	(0.5)	(0.6)	(0.5)	(1.1)	(0.9)	(2.2)

Eos, eosinophil; Neu, neutrophil; Lym, lymphocyte; Mon, monocyte; Bas, basophil; Bet, betahistine; Cim, cimetidine; Cet, cetirizine.

[a] Differs significantly from intradermal (i.d.) antigen alone.

[b] Differs significantly from reactions after i.d. antigen alone as well as those after oral betahistine.

[c] Differs significantly from i.d. histamine.

[d] Differs significantly from i.d. histamine as well as i.d. antigen alone.

sation was increased significantly by i.d. betahistine (57 μg) when compared to histamine, no difference was evident when compared to antigen. The significance of these findings is, however, lessened by the fact that accumulation was very low after i.d. betahistine (57 μg) and histamine, i.e. significantly less than with i.d. antigen.

Furthermore i.d. betahistine (57 μg) and histamine increased monocyte accumulation significantly compared to i.d. antigen.

4. Discussion

The cutaneous hypersensitivity reaction is an extensively used model system for the evaluation of drug effects on both the early and late phases of allergy-related diseases (Deshazo et al., 1983). In patients with atopy, cutaneous challenge with the allergen causes the release of those mast cell and basophil mediators responsible for both phases of the hypersensitivity reaction, e.g. histamine, platelet activating factor, prostaglandin (PG) D_2, leukotriene (LT)C_4 and eosinophil and neutrophil chemotactic factors etc. These induce an accumulation of the inflammatory cells typical of this reaction, mainly eosinophils and neutrophils, and they then release secondary mediators, causing an inflammatory process to evolve over hours (Gleich, 1983; Kay, 1988). Drugs able to reduce this late phase reaction ought to have real therapeutic potential in the treatment of allergies (Altounyan, 1980). As some H_1-antagonists e.g. cetirizine, have documented effects on both phases of the cutaneous hypersensitivity reaction (Snyman et al., 1992), histamine receptor blockers are contenders in this regard. Some, however, suppress only the immediate wheal-and-flare response, e.g. chlorpheniramine (Snyman et al., 1992). Combined therapy with H_1- and H_2-blockers has been demonstrated to mediate total clinical suppression of the late phase reaction in atopic patients (Smith et al., 1980).

Although H_3-receptors have been implicated in urticaria and bronchospasm (Ichinose and Barnes, 1990; Theoharides, 1989), they have only been proven to be present presynaptically on neurons. Consequently their involvement in cell dynamics, and bronchial and vascular changes are only speculative (Van der Werf and Timmerman, 1989). However, previous findings by our group, suggests a regulatory function for H_3-receptors in the immediate hypersensitivity reaction (Snyman et al., 1993). The results of the present study suggest that all three known histamine receptors (i.e. H_1, H_2 and H_3) play important roles in the in vivo regulation of the cutaneous hypersensitivity reaction.

Pretreatment with oral betahistine (i.e. H_3-antagonism and partial H_1- and H_2-agonism) did not significantly modify any of the parameters of the reaction, except for a slight, but significant, reduction in lymphocyte accumulation at the area of antigen administration. The latter was also significantly suppressed by pretreatment with the triple combination, cetirizine, cimetidine and betahistine (i.e. $H_{1\_} + H_{2\_} + H_3$-blockade). Betahistine alone seemingly also prolonged the duration of the late phase reaction, as it caused allergen-induced reactions to persist longer in some volunteers and also tended to increase the size of the 24 h reactions in all volunteers. The reduced lymphocyte accumulation is thus probably not just due to a general suppression of plasma leakage. Histamine receptors are not randomly distributed on all lymphocyte subsets (Beer et al., 1984) but as the various T cell subsets were not identified in the present study, speculation on receptor involvement and T cell function is not possible. However, H_3 receptor involvement seems probable.

Simultaneous H_1-, H_2- and H_3-blockade significantly reduced induration at all observation periods and mediated significant changes in cell activity. The increase in eosinophil and neutrophil vacuolisation corroborated our previous findings in patients with schistosomiasis (Snyman et al., 1993). However, the mean reduction of induration at 24 h in the present study was 44%, compared to 97% in the schistosomiasis study. Simultaneous H_1- and H_2-blockade can almost completely inhibit late phase oedema (Smith et al., 1980) and the attenuated inhibition of the induration in atopic individuals could possibly be ascribed to H_3-blockade. Other factors could, however, be responsible, as the two studies differed in important respects, e.g. (a) in the schistosomiasis study H_1-and H_2-blockade had been established for three days before betahistine was added to the regimen, and (b) the atopic patients exhibited a higher

effector cell activity at baseline; the respective mean eosinophil vacuolisation for the atopic and schistosomiasis groups were 17.5% and 3.0% respectively, at 6 h; and 25.5% and 12.3%, respectively, at 24 h. Similarly the mean monocyte accumulations were 12.6% and 0.0% respectively, at 6 h and 18.8.% and 0.1% respectively, at 24 h. Another finding in the present study relates to the fact that the number of eosinophils recruited to the area of i.d. antigen administration (but not eosinophil vacuolisation) correlated with area size of the late phase reaction. Felacra and Lowell (1971) have previously described the correlation between local eosinophil recruitment and the intensity of the atopic reaction. Histamine is unlikely to be the sole contributor to both the vascular (induration) and cellular components of these reactions and Ting et al. (1980) has demonstrated that local histamine release does not correlate with eosinophil recruitment.

The immediate wheal and flare reactions after i.d. antigen, 57 μg betahistine and 1 μg histamine did not differ significantly, but while histamine caused no visible late phase induration, betahistine caused a dose-dependent reaction, 114 μg and 285 μg causing larger and more prolonged reactions than 57 μg. Betahistine's relatively long elimination half-life of 3.5 h (Dollery, 1991) is not sufficient to serve as explanation because the drug distributes quickly in tissue. As the appearance and disappearance of histamine-induced areas of induration have been shown to be linearly related to the concentration injected (Cook and Shuster, 1980), the prolongation of effect with betahistine can probably not only be ascribed to betahistine's weak H_1- and H_2-agonistic effects.

The cellular reactions differed between i.d. regimens. Both histamine and betahistine caused recruitment of more monocytes but fewer eosinophils than i.d. antigen. Betahistine significantly increased neutrophil vacuolization compared to both histamine and antigen, probably due to its effect on the third histamine receptor subtype. Low concentrations of histamine inhibit eosinophil chemotaxis (Clark et al., 1975), presumably caused by secretion of eosinophil imobilising factor by monocytes, known to be regulated by histamine H_1- and H_2-receptors (Kownatzki et al., 1977). This factor is also responsible for entrapment of eosinophils in an area of antigen administration (Kownatzki et al., 1977). Differences in cell accumulation could be due to a stimulus-dependent differential release of mediators from mast cells and basophils; granular and lipid mediators (e.g. of histamine, serotonin, LTC_4, PGD_2 etc) being released by antigen, but only histamine by incomplete stimuli such as complement and histamine (Bull et al., 1993; Massey and Lichtenstein, 1992). This could explain both eosinophil accumulation and late phase induration after antigen administration, but only explains induration after i.d. betahistine, and explains neither eosinophil accumulation nor induration after histamine. H_3-receptors are presumably situated on mast- or other cells as basophils seemingly do not have them (Bull et al., 1993). In lung tissue preparations the H_3-receptor has been shown to be involved in a negative feedback system for histamine release (Schwarts et al., 1989). However, the H_3-receptor may be autoinhibitory for several mediators as the blockade of H_3-receptors by betahistine caused an increase in both neutrophil and eosinophil vacuolisation.

In conclusion, we suggest that the H_3-receptor regulates a feedback system in conjunction with that previously shown for the H_2-receptor (Bull et al., 1993), and that H_2- and H_3-blockade possibly thus control the release of different mediators.

Acknowledgements

The authors would like to acknowledge Keatings, Lennon and UCB for the supply of test drugs, the Medical Research Council of South Africa for partial financial support and Mrs M. Kemp for technical and Mrs J. Bekker for secretarial assistance.

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