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POSSIBLE ROLE OF HISTAMINE RECEPTORS IN THE CENTRAL REGULATION OF IMMUNE RESPONSES

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Abstract : The present study was designed to delineate the role of H_1- and H_2- histamine receptors in the neuro-immune regulation in rats. The effects of H_1- and H_2-receptor antagonists on humoral and cell-mediated immune (HI and CMI) responses were investigated after intraperitoneal (ip) and intra-cerebroventricular (icv) administration. HI response was assayed by anti-sheep red blood cell (SRBC) antibody titre in presence and absence of 2-mercaptoethanol (2-ME). The CMI responses were evaluated by delayed type hypersensitivity (DTH) reaction (*in vivo*), i.e., measurement of footpad thickness, and lymphokine activity such as leucocyte migration inhibition (LMI) test (*in vitro*). On ip administration, both H_1- (pheniramine and astemizole) and H_2-receptor antagonists (ranitidine and cimetidine) were observed to produce significant enhancement of anti-SRBC antibody response. However, only H_2- and not H_1-receptor blockers were observed to stimulate CMI response significantly. When administered by icv route, only H_2-receptor antagonists caused a statistically significant increase in both HI and CMI responses, while the H_1-receptor blockers failed to modify the same. Thus, H_2-receptors appear to play a major role in the histaminergic mechanisms involved in immunomodulation both at the level of immunocompetent cells active in the peripheral tissues as well as through the central nervous system structures involved in the central regulation of neuro-immune interaction.

Key words : histamine receptors humoral immune response
neuro-immune interaction cell-mediated immune response

INTRODUCTION

Several studies have pointed out a close link between the brain, endocrine and immune mechanisms (1, 2). Thus, either increasing the synthesis or depleting the central neurotransmitters have affected both humoral- and cell-mediated immune (HI and CMI, respectively) responses (3). Histamine, one of the biogenic amines has been observed to have immunosuppressive effect on both the CMI and HI responses (4). These inhibitory effects are mediated through both the H_1- and/or H_2-histamine receptors in animals and human (5–8). As a neurotransmitter, functions of histamine in various neural processes are well established (9–10). But though the peripheral role of histamine at various levels of immune interactions has been explored extensively,

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its role in the CNS-mediated immune mechanisms remains largely unanswered. The important integrative regions of the brain associated with immune system appear to be the hypothalamus and hippocampus. Most histaminergic fibres, that project to the cortex, originate from cell bodies in these integrative areas (10–12). Various stressful conditions are associated with diminished immunity and central histaminergic mechanisms observed to regulate different stress responses, in experimental animal models, may influence various immune interactions (10–13). In view of the above, the present study was designed to explore the role of histamine in CNS-controlled immune responses and the effects of both H_1- and H_2-receptor blockers on HI and CMI responses after intraperitoneal (ip) and intra-cerebroventricular (icv) administration were investigated.

METHODS

Animals

Adult male Wistar rats (150–200 g) bred in the Central Animal House of the Institution were housed in controlled light (12th light – 12h dark cycle) and temperature (22 ± 2°C) conditions with free access to food and water. The animals were assigned to groups of eight rats/group and two animals were kept in one cage during the whole period of experiment.

Drugs and Doses

Astemizole (Indaca Remedies Ltd, Bombay), pheniramine maleate (Avil, Hoechst, Bombay), ranitidine and cimetidine (Torrent Pharmaceutical Ltd, Ahmedabad) were used. Pilot experiments were performed to find out the effective ip and icv doses based on earlier studies (14–17).

EXPERIMENTAL DESIGN AND TREATMENT SCHEDULE FOR PERIPHERAL (IP) STUDIES:

Assessment of humoral immune response

Haemagglutination titre: Sheep red blood corpuscles (SRBC) washed thoroughly and finally suspended in normal saline (0.9%), were injected in rats ip in 0.1 ml doses (25×10^6 cells) on 1st day. H_1-blockers (pheniramine maleate 10, 20 mg/kg, astemizole 5, 10 mg/kg) and H_2-receptor antagonists (ranitidine 10, 20 mg/kg, cimetidine 10, 20 mg/kg) were administered ip from day 2–6. A control group was maintained with ip normal saline. On 7th day rats were mildly anaesthetized with ether and blood was collected from retro-orbital plexus by using microcapillary technique. The serum was separated and anti-SRBC antibody titre was measured by haemagglutination technique according to Herbert (18). Titration was also carried out with antisera preincubated with 0.1M 2-mercaptoethanol (2-ME) at 37°C for 60 minutes for the estimation of 7S type of antibodies. The antibody titres were expressed as $\log_2$ of the reciprocal of the first dilution where no visible agglutination was observed.

Assessment of cell-mediated immune response

Foot pad thickness: For the determination of delayed type hypersensitivity (DTH), after immunization with 1×10^8 SRBC ip on 1st

day, test groups of rats were treated with the H_1-blockers (pheniramine and astemizole) and H_2-blockers (ranitidine and cimetidine) in the same doses as used for evaluation of HI responses from day 2–6. Control groups treated with ip 0.9% saline were run parallel with the test groups. On 7th day, the rats were challenged with 1×10^8 SRBC in the right hind foot-pad, whereas normal saline was injected in left hind foot pad (19). The increase in foot-pad thickness was measured 24 h after the challenge with the help of dial caliper (Mitutoyo, Japan).

Leucocyte migration inhibition test: Rats were sensitized sc with 0.5 ml of egg albumin (25 mg/ml) mixed with equal volume of Freund's complete adjuvant on 1st day. Test group animals were treated with different doses of H_1-and H_2-receptor blockers from day 2 to 14 after sensitization. Blood was collected on 14th day after cardiac puncture for the test. Viable leucocytes were packed in heparinized capillary tubes and stubs of packed cells were made (20). The tubes were then placed on migration wells (chambers) loaded with growth medium with or without antigen. The wells were finally covered with coverslips and incubated for 20 h at 37°C. Area of migration in control as well as antigen chambers was recorded on a centimeter graph sheet with the aid of an overhead projector and percent leucocyte migration inhibition (% LMI) was calculated by the following formula:

$$\%LMI = (1 - \frac{\text{Area of migration in antigen chamber}}{\text{Area of migration in control chamber}}) \times 100$$

EXPERIMENTAL DESIGN AND TREATMENT SCHEDULE FOR CENTRAL (ICV) STUDIES

A polyethylene cannula was fixed stereotaxically in the right lateral ventricle of rats under pentobarbitone sodium (40 mg/kg, ip) anaesthesia for icv administration of drugs. A post-operative recovery period of seven days was allowed prior to any further experimental procedures. The correct position of cannula in the ventricle was ascertained at the time of termination of experiment by injecting Evan's blue dye (21). Starting from the day of immunization, the number of days of drug treatment and procedures for measurement of immune responses after icv administration were same as described for peripheral administration, except the doses of drugs used for icv administration. The doses for H_1-blockers used were: pheniramine 10, 20 µg/rat, astemizole 5, 10 µg/rat, and for H_2-blockers, ranitidine 10, 20 µg/rat, cimetidine 10, 20 µg/rat.

Statistical Analysis

The results are presented as mean ± S.D. The data were analysed using Student's 't' test, Mann-Whitney U test and Analysis of variance (ANOVA) followed by F-test wherever applicable. P values less than 0.05 were considered significant.

RESULTS

Effect of ip administratin of H_1- and H_2-blockers on the humoral and cell-mediated immune responses: Significantly higher values of anti-SRBC antibody titre ($P<0.05$)

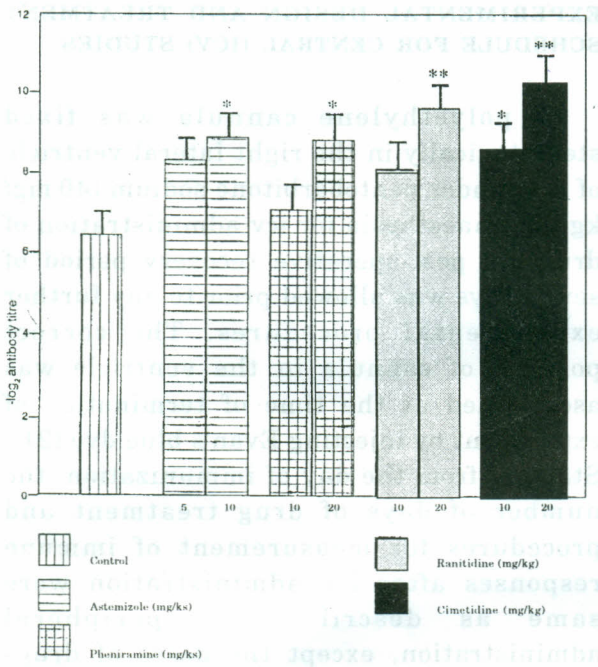

Fig. 1: Effect of H_1- and H_2-receptor antagonists (ip) on anti-SRBC antibody titre in rats. Results are expressed as $-\log_2$ titre. Height of the bar represents mean ± SD (n=8).
*P<0.05,**P<0.02 as compared to the control group.

were observed with H_1-blockers, albeit at higher doses (astemizole 10 mg/kg and pheniramine 20 mg/kg) used, in comparison to the control (6.50 ± 1.41). Rats exposed to H_2-blockers were found to have raised

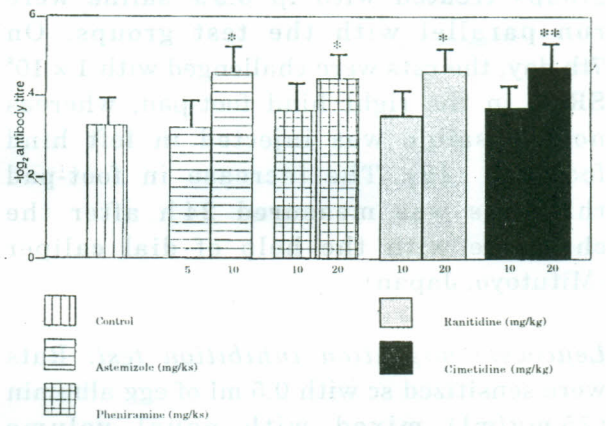

Fig. 2: Effect of H_1- and H_2-receptor antagonists (ip) on anti-SRBC antibody titre in presence of 2–ME in rats. Results are expressed as $-\log_2$ titre. Height of the bar represents mean ± SD (n=8).
*P<0.05,**P<0.02 as compared to the control group.

TABLE I: Effect of H_1- and H_2-receptor antagonists (ip) on cell-mediated immune response.

Treatment (mg/kg, ip)	*% Increase in footpad thickness*	*% Leucocyte migration inhibition*
Control (Saline)	21.43±2.26	36.45±6.12
Astemizole (5)	20.37±1.75	44.26±9.20
Astemizole (10)	21.45±2.45	45.61±8.13
Pheniramine (10)	18.87±3.74	35.20±5.85
Pheniramine (20)	20.05±4.23	40.78±6.00
Ranitidine (10)	22.41±3.29	48.61±6.85
Ranitidine (20)	24.66±1.97*	54.65±7.00*
Cimetidine (10)	26.42±4.22*	53.26±5.75*
Cimetidine (20)	27.71±3.15**	58.38±5.14**

P<0.05; **<0.005 as compared to the control group.
Values are mean ± SD (n=8)
Ip: intraperitoneal

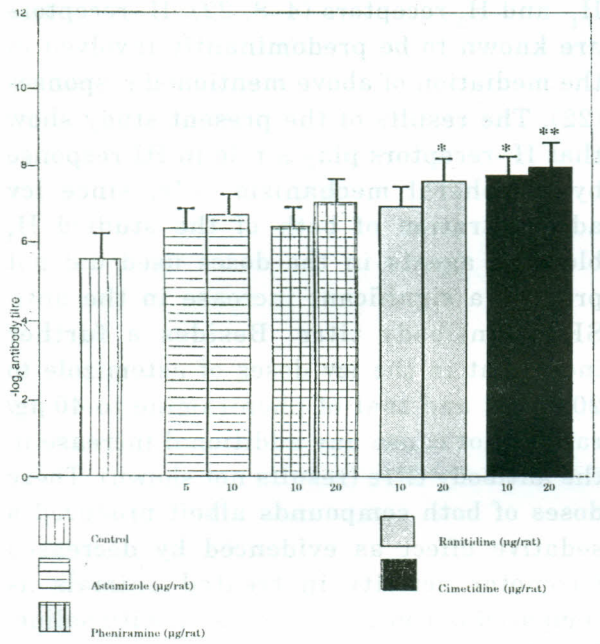

Fig. 3: Effect of H_1- and H_2-receptor antagonists (icv) on anti-SRBC antibody titre in rats. Results are expressed as $-\log_2$ titre. Height of the bar represents mean ± SD (n=8).
*P<0.05,**P<0.005 as compared to the control group.

antibody titre with higher dose of ranitidine, i.e. 20 mg/kg (P<0.02) and both doses (10, 20 mg/kg) of cimetidine (Fig. 1). The titre of 2-ME resistant antibody showed similar trend (Fig. 2). In contrast to HI responsiveness,

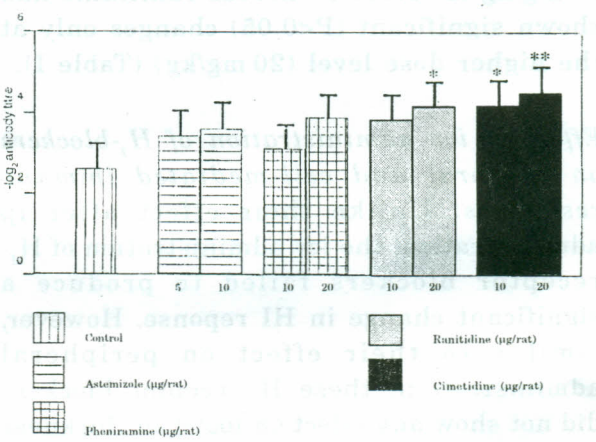

Fig. 4: Effect of H_1- and H_2-receptor antagonists (icv) on anti-SRBC antibody titre in presence of 2–ME in rats. Results are expressed as $-\log_2$ titre. Height of the bar represents mean ± SD (n=8).
*P<0.05,**P<0.005 as compared to the control group.

TABLE II: Effect of H_1- and H_2-receptor antagonists (icv) on cell-mediated immune response.

Treatment (µg/rat, icv) thickness	*% Increase in footpad inhibition*	*% Leucocyte migration*
Control (Saline)	20.21±2.28	34.15±6.81
Astemizole (5)	21.27±4.31	41.89±5.08
Astemizole (10)	21.02±3.50	42.21±9.14
Pheniramine (10)	18.86±2.03	36.23±7.82
Pheniramine (20)	20.91±1.84	40.53±8.53
Ranitidine (10)	21.92±4.89	46.37±7.00
Ranitidine (20)	23.67±2.01*	52.91±5.87*
Cimetidine (10)	25.88±3.65*	50.28±6.21*
Cimetidine (20)	26.81±3.16**	55.67±5.00**

P<0.05; **<0.005 as compared to the control group.
Values are mean ± SD (n=8)
Ip: intraperitoneal

no significant alteration was observed with H_1-blockers on foot-pad thickness and % LMI, in the doses used. Only H_2-blockers produced a marked increase in CMI with both doses of cimetidine, i.e. 10 mg/kg ($P<0.05$) and 20 mg/kg ($P<0.005$), whereas ranitidine had shown significant ($P<0.05$) changes only at the higher dose level (20 mg/kg) (Table I).

Effect of icv administration of H_1-blockers on humoral and cell-mediated immune responses: Unlike their effect after ip administration, the icv administration of H_1-receptor blockers failed to produce a significant change in HI reponse. However, similar to their effect on peripheral administration, these H_1-receptor blockers did not show any effect on foot-pad thickness and % LMI. In contrast, rats exposed to H_2-receptor blockers exhibited marked enhancement in both HI and CMI responses, i.e. with ranitidine in a dose of 20 µg/rat ($P<0.05$) and with cimetidine 10 µg/rat ($P<0.05$) and 20 µg/rat ($P<0.005$) a significant rise in anti-SRBC antibody titre (with and without 2-ME) was observed as compared to the control animals (Fig. 3 and 4). Ranitidine in the higher dose was found to increase foot-pad thickness and % LMI significantly ($P<0.05$), whereas in case of cimetidine treated groups, such a change was observed with both 10 µg/rat ($P<0.05$) and 20 µg/rat ($P<0.005$) doses (Table II).

DISCUSSION

Histamine has been shown to inhibit several diverse peripheral immune functions, including the production of lymphokines and antibodies, leucocyte- and macrophage-migration inhibition and modulation of a variety of T-cell and B-cell functions through H_1- and H_2-receptors (4–8, 22). H_2-receptors are known to be predominantly involved in the mediation of above mentioned responses (22). The results of the present study show that H_1-receptors play a role in HI response by peripheral mechanism only, since icv administration of both of the studied H_1 blocking agents in the doses used did not produce a significant increase in the anti-SRBC antibody titre. Besides a further increment in the icv doses of astemizole to 20 µg/rat and that of pheniramine to 40 µg/rat did not cause any additional increase in the antibody titre (results not shown). These doses of both compounds albeit produced a sedative effect as evidenced by decreased locomotor activity in treated animals as compared to control rats treated with saline. In addition the higher dose of astemizole (20 µg/rat) produced significant mortality (9/16, i.e. approximately 56% mortality). This study also shows that CMI response was significantly increased with H_2-receptor blockers when administered by peripheral route, whereas H_1-antihistamines had no such effect. This indicates that there may be separate histaminergic receptor-mediated mechanisms involved in the regulation of HI and CMI responses in peripheral immune system of rats. Further, icv administration of only H_2-blockers caused a significant enhancement of both HI and CMI responses, whereas H_1-blockers were effective only on ip administration and failed to show a significant response on icv dosing.

Both H_1- and H_2-receptors are distributed throughout the CNS and mediate various functions (9–10). However, the ineffectiveness of centrally administered H_1-blockers to produce significant change in both

HI and CMI functions in the present study emphasizes that H_2-receptors probably play a dominant role in the integrative areas of the brain which are responsible for maintenance of the neuro-immune axis. Barring few exceptions, i.e. release of vasopressin via H_1 receptors, a generalized inhibitory effect for H_2-receptor antagonists on stress-induced release of neurohormones has also been suggested (9, 14, 16–17, 23). The present results emphasize that H_2-histamine receptors may have a major contribution towards the histaminergic mechanisms involved in neuro-immune-endocrine axis. Such an assumption that both in the periphery and via central nervous system H_2-histamine receptor mechanism plays a major role as compared to histamine-H_1 receptors in producing an immunomodulatory response has been corroborated by a number of *in vitro* studies in which mediators of immune responses were measured (24–30) as well as *in vivo* studies, where both HI and CMI responses were investigated by employing different techniques (31–34).

In conclusion the results of this study suggest an immunomodulatory role of H_1-histamine antagonist drugs only in HI response that is mediated by peripheral and not by central nervous system effects. On the contrary H_2-histamine receptor active drugs modulate both HI and CMI responses by acting on peripherally active immune cells as well as through the neuronal structures involved in the central integration of immune system control.

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