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

A. K. MOHARANA, S. K. BHATTACHARYA, P. K. MEDIRATTA AND K. K. SHARMA*

Department of Pharmacology, University College of Medical Sciences & GTB Hospital, Shahdara, Delhi – 110 095

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Abstract: The present study was designed to delineate the role of H₁ and H₂ histamine receptors in the neuro-immune regulation in rats. The effects of H₁- and H₂-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 H₁- (pheniramine and astemizole) and H₂-receptor antagonists (ranitidine and cimetidine) were observed to produce significant enhancement of anti-SRBC antibody response. However, only H₂-receptor blockers were observed to stimulate CMI response significantly. When administered by icv route, only H₂-receptor antagonists caused a statistically significant increase in both HI and CMI responses, while the H₁-receptor blockers failed to modify the same. Thus, H₂-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, neuro-immune interaction, humoral immune response, 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 H₁- and/or H₂-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,
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 x 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 x 10$^8$ SRBC ip on 1st
day, test groups of rats were treated with the H1-blockers (pheniramine and astemizole) and H2-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 $1 \times 10^8$ 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 H1- and H2-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:

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

**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 H1-blockers used were: pheniramine 10, 20 µg/rat, astemizole 5, 10 µg/rat, and for H2-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 administration of H1- and H2-blockers on the humoral and cell-mediated immune responses: Significantly higher values of anti-SRBC antibody titre (P<0.05)
Fig. 1: Effect of H₁ and H₂-receptor antagonists (ip) on anti-SRBC antibody titre in rats. Results are expressed as -log titre. Height of the bar represents mean ± SD (n=8). *P<0.05, **P<0.02 as compared to the control group.

Fig. 2: Effect of H₁ and H₂-receptor antagonists (ip) on anti-SRBC antibody titre in presence of 2-ME in rats. Results are expressed as -log 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 H₁ and H₂-receptor antagonists (ip) on cell-mediated immune response.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>% Increase in footpad thickness</th>
<th>% Leucocyte migration inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (Saline)</td>
<td>21.43±2.26</td>
<td>36.45±6.12</td>
</tr>
<tr>
<td>Astemizole (5)</td>
<td>20.37±1.75</td>
<td>44.26±9.20</td>
</tr>
<tr>
<td>Astemizole (10)</td>
<td>21.45±2.45</td>
<td>45.61±8.13</td>
</tr>
<tr>
<td>Pheniramine (10)</td>
<td>18.87±3.74</td>
<td>35.20±5.85</td>
</tr>
<tr>
<td>Pheniramine (20)</td>
<td>20.05±4.23</td>
<td>40.78±6.00</td>
</tr>
<tr>
<td>Ranitidine (10)</td>
<td>22.41±3.29</td>
<td>48.61±6.35</td>
</tr>
<tr>
<td>Ranitidine (20)</td>
<td>24.66±1.97</td>
<td>54.65±7.00</td>
</tr>
<tr>
<td>Cimetidine (10)</td>
<td>26.42±4.22</td>
<td>53.26±5.75</td>
</tr>
<tr>
<td>Cimetidine (20)</td>
<td>27.71±3.15</td>
<td>58.38±5.14</td>
</tr>
</tbody>
</table>

P<0.05; **P<0.005 as compared to the control group.

Values are mean ± SD (n=8). Ip: intraperitoneal

were observed with H₁-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 H₂-blockers were found to have raised
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,

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**TABLE II:** Effect of H₁- and H₂-receptor antagonists (iv) on cell-mediated immune response.

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

P<0.05; **P<0.005 as compared to the control group.
Values are mean ± SD (n=8)
Ipc: intraperitoneal
no significant alteration was observed with 
H₁-blockers on foot-pad thickness and % LMI, 
in the doses used. Only H₂-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 H₁-blockers 
on humoral and cell-mediated immune 
responses: Unlike their effect after ip 
administration, the icv administration of H₁- 
receptor blockers failed to produce a 
significant change in HI reponse. However, 
similar to their effect on peripheral 
administration, these H₁-receptor blockers 
did not show any effect on foot-pad thickness 
and % LMI. In contrast, rats exposed to H₂- 
receptor blockers exhibited marked 
engagement 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 

Both H₁- and H₂-receptors are distributed 
throughout the CNS and mediate various 
functions (9–10). However, the ineffectiveness of centrally administered H₁- 
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.

REFERENCES


