THE CENTRAL EFFECT OF BIOLOGICAL AMINES ON IMMUNOSUPPRESSIVE EFFECT OF RESTRAINT STRESS IN RAT

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Abstract - *The effects of some histaminergic agents were evaluated on stress-induced immunosuppression in immunized male rats. In rat immunized with sheep red blood cells (SRBCs), restraint stress (RS) prevented the booster-induced rise in anti-SRBC antibody titre and cell immunity response. Intracerebroventricular (I.C.V) injection of histamine (150 μg/rat) induced a similar effect with RS. Pretreatment with chlorpheniramine (50 μg/rat) reduced the inhibitory effect of RS on immune function . Also histamine could inhibit the effect of chlorpheniramine when injected simultaneously. Pretreatment with ranitidine (10 μg/rat) had not a significant effect. Serotonin (3 μg/rat) and dopamine (0.2 μg/rat) could reverse the effect of chlorpheniramine when injected with chlorpheniramine ($P<0.05$). Epinephrine (0.2 μg/rat) had not a significant effect. The results indicated that histamine mediates the immunosuppression of restraint stress by influencing the histamine H_1 receptor in the brain and this effects of histamine may be modulated by serotoninergic and dopaminergic system.*

Acta Medica Iranica 38 (3): 182-186; 2000

Key Words: Restraint stress, histamine, immunity

INTRODUCTION

Psychoneuroimmunology is a relatively new discipline which deals with CNS immune system interaction. Two pathways link the brain and the immune system: The autonomic nervous system and neuroendocrine outflow via the pituitary (1). Different studies have been conducted to understand the clinical implication and mechanisms of the effects of stress on the immune system.

The effect of stress on immune function is related to different factors such as type and duration of particular stressor being used, the specific animal tested, and the time of day that the stressor was applied (2,3,4). The restraint stress for 24h can reduce the humoral and cell mediated immunity (5,6). The role of dopaminergic (6,7,8), sympathetic nervous system (3,9) and different hormones such as growth hormone (2) , prolactin (10) , corticosteroides and endogenous opioids (11,12,13), and other agents such as substance P and adenosine (14,15) have been studied in immunosuppression related to stress. For example, the release of prolactin and growth hormone increases following restraint stress and induce immunopotentiator effect, but corticosteroids and opioids reduce immune system activity.

Histamine has direct effect on immune cells and reduces activity of this cells via H_2 receptors ((4, 16,17)). Also Histamine releases during stress in the brain and mediate the activity of different systems (18).

Histaminergic neurons mediate restraint stress-induced activation of 5-hydroxytryptaminergic and dopaminergic neurons (19,20) and the activity of noradrenergic neurons projecting to the hypothalamus (21) in the rats. Restraint stress-induced immunosuppression may be mediated by different mechanisms via some of hormones or nervous system (5,6,9,13,22). The role of histamine receptors in this effect of stress has not been studied.

MATERIALS AND METHODS

Subjects: 200 Male Wistar rats (200-250g) were used. They were housed in standard laboratory conditions of light (12h light- dark schedule) and temperature (22± 2 °C) and had free access to food and water.

Drugs: The drugs used were histamine, chlorpheniramine , ranitidine, serotonin, dopamine and epinephrine (all from Sigma) and ketamine for anesthesia (Park Davis) . Drugs were disolved / diluted in distilled water and were injected intracerebroventricularly (5 μl/rat) 15 minute prior to Restraint stress.

Stress procedure (6) restraint stress (RS) was applied in plexiglas restrainers (9 x 7 x 15 cm) . Control rats were left in their cages without food or water at the same time as the other rats were stressed, in order to avoid difference in food and water intake.

Stereotaxic method: The rats were anesthetized with ketamine (150 μg/rat ip) and according to Paxinos and Watson's procedure introduced in their Atlas (23) , the rats placed in an animal stereotaxic instrument and the incisor bar adjusted until the heights of lambda and bregma skull points were equal.

Drilling point on skull were AP= 0.8 mm and RL = 1.4 mm; the length of penetrating needle from the surface of skull was 3.7 mm.

A permanent cannula made by a 23-gauge needle was implanted to penetrate the ventricle and then it was fixed with dental cement . Rats were canulated before first and second injection of SRBCs for cellular and humoral immunity test respectively.

Immunological assay humoral immune response (5,6) : Rats were immunized with sheep red blood cells (SRBCs) (0.5 × 10^9 cells/ml/100g) on day 0. On day 7 they received a similar booster dose of the antigen and were exposed to Restraint stress (RS) for 24 h. Subsequently , the rats were lightly anesthetised with ether and then blood was collected from the supraorbital plexus using the micro capillary technique . A parallel (no RS) group was run and served as controls . The vehicle or drug treatments were made just prior to RS.

The serum was assayed for hemagglutination titre as follows: two fold dilutions (0.025 ml) of sera were made in the microtitre plates with saline. To each well, 0.025 ml of 1% (V/V) SRBCs was added. The plates were incubated at 37 °c for 1 h and then observed for hemagglutination . The highest dilution giving hemagglutination was taken as the antibody titre . The antibody titres were expressed in a graded manner , the minimum dilution (1/2) being ranked as 1 and the mean ranks of different groups were compared for statistical significances.

Cell - mediated response (6): Rats were immunized on day 0 with 0.5 ml SRBCs (5× 10^8 cells / ml) given on their back. They were then subjected to stress / vehicle / drug treatments from day 1 to day 5. On day 5 the rats were challenged by injecting 0.1 ml SRBCs into their left hind paw, whereas their right hind paw received saline (0.9%).

Twenty - four hours after challenging, the differences in paw volumes were measured by using the volume differential meter . The changes in foot pad thickness (as measured by differences in paw volumes) of different treatment groups were compared for statistical significance.

RESULTS

In rats immunized with SRBCs, a booster dose of antigen clearly augmented the humoral immune response to antigen . RS clearly attenuated the booster (SRBC) -induced rise in the secondary antibody titre (P<0.01 fig. 1) . Similar changes (suppression) were also seen when histamine (150 μg/rat) was given intracerebroventriculary (I.C.V) in nonstressed rats as well (Fig. 1).

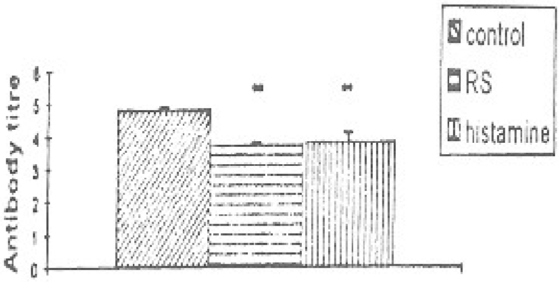

Fig. 1. The central effect of histamine on humoral immunity following restraint stress (RS) in rat. The effect of RS, histamine (150 μg/rat) and control (non stressed) groups were compared on antibody titre in immunized rat with SRBC. Each column shows mean ± SE of antibody titre related to six rats. ** P<0.01

Pretreatment with chlorphenıramine (50 μg/rat) reduced the immunosuppression effect of stress (P<0.01 Fig. 2).

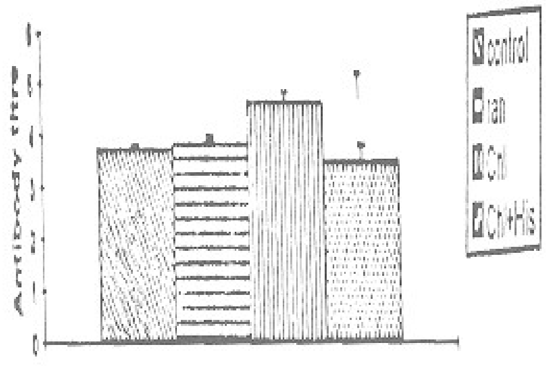

Fig. 2. The role of histamine receptors on the reduction of humoral immunity following restraint stress (RS) in rat. Centre The effect of pretreatment with ranitidine (ran. 10 μg/rat). chlorpheniramine (chl. 50 μg/rat), chl + histamine (his. 150 μg/rat) was compared on antibody titre. Each column shows mean ± SE of antibody titre related to six rats. ** P<0.01 (compared with control). T P<0.01 (compared with group received chlorpheniramine)

Histamine (150 μg/rat) when injected with chlorpheniramine (50 μg/rat) simultaneously inhibited the effect of chlorpheniramine (Fig. 2) . Ranitidine (10 μg/rat) did not reduce the inhibitory effect of RS on immune function (Fig. 2). Serotonin (3 μg/rat), dopamine (0.2 μg/rat) and epinephrine (0.2 μg/rat) were injected with chlorpheniramine in different groups of stressed rats.

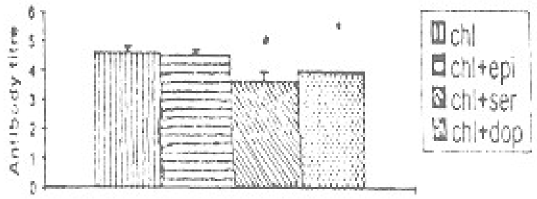

Fig. 3. The effect of serotonin, dopamine, and epinephrine on increment of antibody titre by chlorpheniramine following restraint strss (RS) in rat. histamine (chl, 50 μg/rat), chl + serotonin (ser, 3 μg/rat), chl + dopamine (dop, 0.2 μg/rat) chl + epinephrine (epi, .2 μg/rat) were compared on antibody titre in stressed rat. Each column shows mean ± SE of antibody titre related to six rats. * $P<0.05$

Serotonin and dopamine inhibited the chlorpheniramine effect on RS- induced immunosuppression ($P<0.05$) , but epinephrine had not a significant effect (Fig. 3). In rats immunized with SRBCs , and then challenged with SRBCs into the left paw (right paw received saline), there was a marked enhancement in the paw volume as measured by using the volume differential meter.

In the RS treated rats, there was significant reduction in paw volume difference compared to the control ($P<0.01$ Fig 4). Similar effects on paw volume changes were also seen after histamine (150 μg/rat) injection in non-stressed rats ($P<0.01$ Fig. 4).

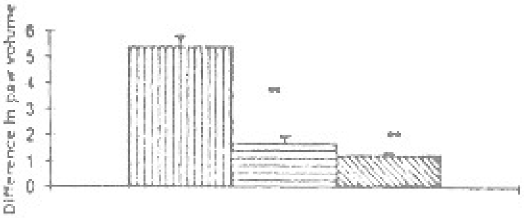

Fig. 4. The central effect of histamine on the cell immune response following restrains stress (RS) in rat. The effect of RS, histamine (his, 150 μg/rat) and control (non stressed) groups were compared on difference in paw volume (DIPV) in immunized rat. Each column shows mean ± SE of DIPV related to six rats. ** $P<0.01$

Pretreatment with chlorpheniramine (50 μg/rat) reduced the inhibitory effect of RS ($P<0.01$) . Ranitidine (10 μg/rat) did not show a significant response (Fig. 5). Also histamine (150 μg/rat) could inhibit the effect of chlorpheniramine when injected simultaneously (Fig.5). Chlorpheniramine when injected with serotonin (3 μg/rat) and dompamine (0.2 μg/rat) caused a significant reduction in inhibitory effect of chlorpheniramine on RS-induced immunosuppressoin ($P<0.05$ Fig. 6). But Epinephrine (0.2 μg/rat) had not a significant effect (Fig. 6).

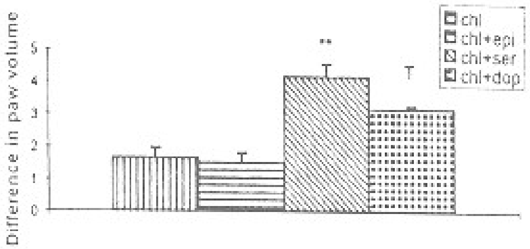

Fig. 5. The central role of histamine receptors on the reduction of cell immune response following restraint stress (RS) in rat. The effects of pretreatment with ranitidine (ran, 10 μg/rat), chlorpheniramine (chl, 50 μg/rat) and chl + histamine (his, 150 μg/rat) were compared on difference in paw volume (DIPV) in stressed rat. titre. Each column shows mean ± SE of DIPV related to six rats.
** $P<0.01$ (compared with control), T $P<0.01$ (compared with group received chlorpheniramine)

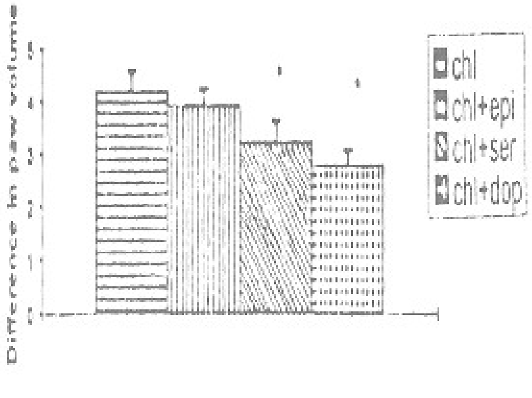

Fig. 6. The effect of serotonin, dopamine, and epinephrine on increment of immune response by chlorpheniramine following restraint strss (RS) in rat. The effects of chlorpheniramine (chl, 50 μg/rat), chl + serotonin (ser, 3 μg/rat), chl + dopamin (dop, 0.2 μg/rat) chl + epinephrine (epi, 0.2 μg/rat) were compared on difference in paw volume (DIPV) in stressed rat. Each column shows mean ± SE of DIVP related to six rats. * $P<0.05$

DISCUSSION

Complex neurochemical mechanisms are involved in the organism's biological response to noxious stimuli like stress. Stress can induce the immunosuppression effect in a specific condition.

It has been shown that the restraint stress (RS) for 24h reduces the humoral immune response (5,6) and RS for 1 h daily for five days reduces the cell-mediated immune response (5) . In this study RS induced a significant reduction in humoral and cellular immune function . The release of histamine and mediation of different nervous system activity following RS has been recognized (18,19,20,21). Similar immune response (immunosuppession) has been seen with histamine injection in the absence of RS. The similarity between histamine and RS effect on immune function can be a reason for the role of histamine in immunosuppression effect of RS . Pretreatment with ranitidine had not any effect on RS - induced immunosuppression but chlorpheniramine reduced the RS effect on immune function. This result shows that histamine affects the immune function via action on Hi receptor in the brain. Simultaneous injection of histamine and chlorpheniramine inhibited the effect of Hi receptor antagonist, so it can be concluded that the effect of chlorpheniramine is related to inhibition of Hi receptor and the other effects of drugs on different sites are not important.

Following RS, histamine increases the serotoninergic, dopaminergic and adrenergic nervous system via Hi receptor in the brain (19,20,21) . Serotonin and dopamine could reverse inhibitory effect of chlorpheniramine on RS- induced immunosuppression , but epinephrine could not induce significant effect . It can be concluded that the immunosupression effect of RS via histamine release can be modulated by serotoninergic and dopaminergic nervous system in the brain.

In summary, the RS induced immunosuprnssion by release of histamine and changing in dopaminergic and serotoninergic nervous system activity via Hi receptor affects stimulation by histamine.

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