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REGIONAL SPECIFICITY SEEN WITHIN HYPOTHALAMUS IN NEUROIMMUNOMODULATION

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Abstract : Wistar strain male albino rats lesioned and sham lesioned at Ventromedial hypothalamus (VMH) were used to study the neuroimmunomodulation by this brain region. Except the decrease in thymus weight/body weight ratio ($P<0.01$), and its cellularity ($P<0.02$) in immunized VMH lesion animals, the rest of the parameters like plaque forming cells (PFC), antibody titre, leukocyte migration inhibition index, foot pad thickness in the lesioned as well as the lesion immunized animals never deviated from their respective sham and immunized sham animals. Sham operated belongs to VMH when compared to control rats, showed marked decrease in spleen weight ($P<0.001$), thymus weight ($P<0.02$) and decrease in popliteal lymph node weight ($P<0.001$) ratios. After immunization, the immunized sham animals showed a marked decrease in antibody titre ($P<0.05$), PFC ($P<0.05$), spleen ($P<0.05$) and thymus ($P<0.001$) weight ratios with the significant increase in splenic cell count ($P<0.01$) compared to immunized control rats. VMH may be one of the information receiving center. However, from these results, it is infered that VMH could not be a modulating center for the many of the parameters studied as far as neuroimmunomodulation is concerned.

INTRODUCTION

The various biological functions that are encompassed by the term homeostasis include interactions between the various endocrine and immune systems. The hypothalamus is situated anatomically to function both as neuroendocrine transducer and as an integrating center for a variety of behavioural and autonomic responses. Stein et al (1) identified in a number of experimental systems the importance of anterior hypothalamus in immune regulation. Lesions located in the anterior hypothalamus area, which included damage to the posterior preoptic area, the suprachiasmatic nuclei and portions of the ventromedial nucleus, reduce the occurrence of the lethal anaphylaxis in the sensitized guineapigs (2). Rats with electrolytic anterior hypothalamic lesions showed changes in lymphoid tissue cellularity and a decrease in response to concavallin A (Con A) and these changes were not mediated by corticosteroids (3). Bilateral lesions in the preoptic anterior hypothalamic area resulted with the decreased splenic natural killer cell activity and mediated via pituitary factors (4). Additional supporting evidences for involvement of hypothalamus in immune regulation was given by a decrease in noradrenaline turnover in the hypothalamus of rats during the peak of immune response to sheep red blood cells (SRBC) and this neuronal activity was mimicked by injection of soluble mediators released by activated immunological cells (5). Changes in electrical activity of ventromedial hypothalamic neurons in the anesthetised rats have been recorded after induction of an immune response with SRBC or with trinitrophenylated haemocyanin. Animals

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failed to respond to antigen manifested no change in their firing rate (6). Though this evidence indicates that flow of information from activated immune system of VMH, this area was not modulating many aspects of the natural immune response (7). However, perusal of literature have shown no information regarding the involvement of VMH area in immunomodulation of acquired immunity and hence forms the basis of this work.

METHODS

Experimental animals were adult male Wistar strain albino rats (180-200 g) housed under standard laboratory conditions fed with food and water *ad libitum*. The animals were divided into six groups as shown below :

Group 1: Control rats (n=15) were included to reveal the basal immune states for the immune parameter studied and to exclude errors induced by body weight differences or differences between individual rats.

Group 2: Immunized control rats (n=15). These rats were immunized with SRBCs. This group gave the normal responses of animals for this particular dose of antigen.

Group 3: VMH sham operated animals (n=7). This group was used to elucidate the effect of non-specific stress and surgical injury influencing the immune mechanism.

Group 4: VMH lesioned rats (n=7). This group was used to detect the change in basal immune states due to the lesion.

Group 5 : VMH sham animals immunized with SRBC (n=7) to elucidate the effect of non-specific stress and surgical injury influencing the immune mechanism during an immune response.

Group 6 : VMH lesioned animals immunized with SRBC (n=7). This group was studied to detect the effect of lesion during an immune response.

Surgical procedure : Bilateral lesions and sham operations were performed according to Konig and Klippal atlas (8). The coordinates were 4.38 mm anterior, 0.5 mm lateral and 8.9 mm depth from dura using pentothal sodium (40 mg/kg) anesthesia. Anodal electrolytic lesion was produced by coated stainless steel electrode with 0.22 mm dia., which was exposed only at the tip. Direct current of 2 mA for 10 sec was used. The sham lesioned animals were subjected to the same procedure omitting the passage of current.

Immunization : To immunize, 5x10°9 SRBC/ml in saline was injected intraperitoneally. The day of immunization was considered as "0" day. In sham and lesion animals immunization was carried out on the 10th day after surgery. The parameters were studied on 15th day in groups 3,4,5,6 and on 5th day in control immunized animals (group 2).

In each animal the following parameters were studied :

Humoral immunity :

1. Plaque-forming cells (PFC) (9).
2. Antibody titre (Direct hemagglutination).

Cell mediated immunity :

3. Leukocyte migration inhibition (LMI) (10).
4. Foot pad thickness (10).

Organ weight/body weight ratio of Spleen, thymus and popliteal lymphnode.

Cell count of spleen and thymus :

The blood samples for serum were collected from jugular vein using ether anesthesia within 2 min (accepted procedure for stress free sample), between 8-9 AM to avoid circadian rhythm induced changes and preserved at –20°C until used. The thymes, spleen and popliteal lymphnode were collected, aseptically weighed and kept in minimum essential medium (AT 045 by Hi Media Lab., India).

At the same time of sacrifice the brain of each lesioned animal was removed and the site and extent of lesion was confirmed histologically using Cresyl fast violet staining. The data from

animals in which lesions located in sites other than VMH were discarded and this number was duly replaced from the stock.

Preparation of cell suspension of spleen and thymus, PFC assay and LMI were performed as described else where (11). Finally the PFC/ million of splenocytes were calculated. The thymocytes were used as a migrating population to study the effect of migration inhibition factor released by the splenocytes. The splenocytes and thymocytes were mixed in a ratio of approximately 3:1 so as to provide a migrating cell density of $80x10^6$ cell/ml.

$$\text{LMI index} = \frac{\text{Area of migration of cells in presence of antigen}}{\text{Area of migration of cells in absence of antigen}}$$

The PFC and FPT assays were performed only in the immunized groups of animals.

Statistical analysis : All data are expressed as Mean ± SD in Table I and II. To determine whether sham and immunized sham animals differ from controls and immunized controls, they were compared by Student 't' test with their respective controls. To evaluate the overall differences between the groups, one way analysis of variance (ANOVA) was performed. When there is a significant F test ratio in these comparisons the Tukey's multiple comparison was performed to test the null hypothesis among various groups.

RESULTS

The fact that surgical trauma and stress could modulate the immune functions studied, the sham and immunized sham groups were utilized to draw the meaningful specific lesion effects.

Compared to control rats, VMH sham animals showed marked decrease in spleen weight ($P<0.001$), thymus weight ($P<0.02$) and increase in popliteal lymphnode ($P<0.001$) ratios. After immunization the immunized sham animals showed marked decrease in antibody titre ($P<0.05$), PFC ($P<0.05$), spleen ($P<0.05$) and thymus ($P<0.001$) weight ratio with a significant increase ($P<0.01$) in splenic cell count.

Except the decrease in thymus weight/body weight ratio ($P<0.01$), and its cellularity ($P<0.02$) in immunized VMH lesion animals, in the rest of the parameters the lesion as well as the lesion immunized animals never deviated from their respective sham and immunized sham animals.

DISCUSSION

Though the observation of behavioural changes were not the primary objective, these changes help us to confirm the site of lesion. Immediately after the recovery from anesthesia the lesioned animals but not sham animals started to run continuously in the cage as if they were searching for something and had a tendency to escape from the cages. This hyper running activity originating after VMH lesion has also been reported by Yokawa et al (12).

TABLE I : The results of cell mediated and humoral immunity.

Group	*PFC*	*Antibody*	*LMI Index*	*FPT %*
Control (n=15)	—	1.5 ± 0.9	0.85 ± 0.04	—
Imm. Control (n=10)	563 ± 62.0	10.3 ± 0.7	0.2 ± 0.07	29 ± 3.2
VMH Sham (n=7)	—	0.4 ± 0.8	0.87 ± 0.07	—
VMH lesion	—	0.3 ± 0.8	0.86 ± 0.04	—
VMH Imm. Sham	503 ± 93	9.4 ± 0.79	0.26 ± 0.05	33 ± 6
VMH Imm. lesion (n=7)	408 ± 52	9.7 ± 0.49	0.24 ± 0.06	29 ± 7

Imm = immunized. The values expressed as Mean ± SD.

Due to this continuous locomotive behaviour some animals died due to exhaustion and hence more stock animals were utilized to replace the loss. However, these changes disappeared within 12 hours. After 12 hours all the animals slowly became hyperphagic, which lead to appreciable increase in body weight within the period of observation (15 days).

In the present study immunized sham animals showed significant decrease in PFC and antibody titre compared to controls but not from immunized lesion animals. This is also well in agreement with the earlier reports. Goldstein (13) pointed out that localized injuries to both hypothalamus and thalamus have no significant effect on production of antibody forming cells in spleen. A small decrease observed in antibody titre in all the groups of animals undergoing operation was also noticed. This effect was not connected with the injury to the components of a hypothetical midbrain system regulating immunogenesis, but occurred as a consequence of cranio cerebral trauma.

In this study humoral and cell mediated immune parameter did not alter due to destruction of VMH region. According to Jankovic et al (14) chronic electrical stimulation of hypothalamic dorsomedial nucleus and sensory motor cortex increased performance of both humoral and cell mediated immune response in rats immunized with bovine serum albumin. Less pronounced potentiation of immune reactivity was observed in rats with stimulation of posterior hypothalamic area. On the other hand stimulation of Ventromedial area failed to increase the immune capacity. Neither stimulation or destruction of VMH causes modulation in immunity studied, this reveals that VMH may be one of the information receiving center from immune system rather than modulating center as far as neuroimmunomodulation is concerned. Saphier et al (15) who recorded a peak electrical response in preoptic area/anterior hypothalamus area in conscious rats, during the time of antibody appearance in blood also suggest that this is not the only site involved in neuroendocrine responses to immunoregulatory factors.

It has been reported that the placement of lesions within the reticular formation resulted in thymic involution (16). Cross et al (3) reported that anterior hypothalamic lesion causes a decrease in splenocyte and thymocyte number which return to normal by day fourteen and seven respectively after surgery. This is well in agreement with our VMH lesion animals. He concluded this as a short lived effect and this could be mediated via neurohumoral secretions. He further hypothesized that irritation of hypothalamus resulted with prolonged immunosuppression by abnormal continued release of these neuro secretions. The transient nature of most immune alterations following lesion at central nervous system suggest that brain may have alternate routes or neuronal plasticity that can mediate these responses and restore homeostasis.

However, in immunized lesion animals of VMH in the present work showed marked decrease in thymic weight ratio as well as its cellularity even after 15th day. As this decrease was observed after an immune challenge, it could be due to the sequence of events following an immune response, which include the changes seen in mediators, neurotransmitters, hormones etc. Besedovsky et al (5) reported a decrease in norepinephrine content of hypothalamus following immunization as a result of decrease in norepinephrine turnover. Moreover, the animals responding poorly to antigen fail to show such changes. The NE level and its turnover rate is not known in VMH lesioned rats after an antigenic challenge. At present we could not delineate the exact cause such as neural, neuro humoral factors, hormonal or neurotransmitters and it needs further in-depth study.

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