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RAT HIPPOCAMPUS AND PRIMARY IMMUNE RESPONSE

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Abstract : During immune challenge hippocampal region shows time-dependent changes in neurotransmitter levels. Hence in the present study the effect of electrolytic lesion in the dorsolateral hippocampus (DLH) and ventral hippocampal formation (VHF) (to create a disturbance in neurotransmitter levels) on humoral immunity in albino rats has been studied along with appropriate controls. Haemagglutination titre, IgM and IgG levels were monitored on the 5th day after an immune challenge by sheep red blood cells (SRBC) suspension. Antigen challenged lesioned animals had low circulating antibody titre levels compared with the controls and their site-specific sham lesioned groups. The IgM levels were significantly lowered in both DLH and VHF lesioned and immunized animals compared to their immunized sham groups as well as immunized controls. However, only immunized VHF lesioned group showed a significant decrease in IgG level from their immunized sham group. It was concluded from the results that an intact hippocampal region is essential for the normal humoral immunity for the primary immune response in rats. Probably VHF region may be required for the secondary immune response as indicated by the alteration in IgG levels in these animals.

Key words : neuroimmunomodulation humoral immunity hippocampus IgM IgE

INTRODUCTION

The role of acquired immunity in the prevention and recovery from infectious diseases is of immense importance for survival. During an antigen entry one of the antigen specific immune response mounted is the humoral immunity. The humoral immunity is subjected to regulation by negative feed back mechanisms and also suppression at cellular level by cellular interactions, one of which being the neural modulation. Immunoregulatory roles of neurotransmitters are well documented and comprise of two aspects, modulation of immune function by neurotransmitters and

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effect of immune system on nervous system. Few reports on the role of neurotransmitters in this aspect reveal that the changes in immune competence following depletion of brain catecholamine leads to suppression of both IgG and IgM response (1). Further Cross et al (2) showed an enhancement in activity of splenic 'T' suppressor cell in central catecholamine depleted animals following immunization and that the central depletion of catecholamine inhibits the humoral antibody response only to 'T' cell-dependent antigens (3). An increased accumulation of norepinephrine metabolites in hippocampal region during the immune response in a time dependent manner was also reported by Zalcman et al (4). As hippocampal area is known to regulate the corticosteroid levels and hypersecretion of steroid occur in the absence of hippocampus, this study has been desinged to elucidate the role of hippocampus in primary immune response. Since rat hippocampal region is a large structure it is divided as dorso-lateral hippocampus (DLH) and ventro-lateral hippocampal formation (VHF) and lesioned separately and studied in individual groups of animals.

METHODS

Animals and groups

Male Wistar albino rats weighing 180–200 g were obtained from Indian Institute of Science, Bangalore, India and were housed under standard laboratory conditions (12 hr light and 12 hr dark) with food (Gold Mohor pellets, Hindustan Lever Ltd. Bangalore) and water ad libitum. The animals were divided into six groups as described below :

Group I comprised of normal rats to provide the basal values (n = 10) of immunoglobulins. Group II consisted of control rats which were immunized with sheep red blood cells (SRBC) to determine the immune response to this antigen (n = 10). Group III consisted of dorsolateral hippocampus (DLH) lesioned animals (n = 6). Group IV consisted of DLH lesioned animals immunized with SRBC (n = 6). Group V consisted of sham operated animals for DLH region (n = 6). Group VI animals were DLH sham lesioned and immunized with SRBC (n = 6). Group VII animals had lesion in ventral hippocampal formation (VHF) (n = 6). Group VIII consisted of VHF lesioned animals immunized with SRBC (n = 6). Group IX had sham operated animals for VHF lesion (n = 6). Group X consisted of VHF sham operated animals immunized with SRBC (n = 6).

Hippocampal lesion

Groups III, IV, VII and VIII were included to study the effect of lesion and its effect on immune response. Groups V, VI, IX and X were made to elucidate the effect of surgical injury influencing the immune mechanism. Bilateral lesions in DLH and VHF areas of hippocampus and sham operations were performed according to the rat brain atlas of Koing and Klippel (5) under pentathol sodium (Abbot laboratories, India Pvt. Ltd., Bombay) anaesthesia (40 mg/kg). The co-ordinates used were 2.58 mm anterior, 4.4 mm lateral and 5 mm depth from dura for DLH and 2.58 mm anterior, 4 mm lateral and 7.5 mm depth from dura for VHF lesion, respectively. Anode-electrolytic lesions were produced by coated stainless steel electrode (0.22 mm-

diameter) exposed 0.5 mm at the tip. Direct current at 2 mA for 45 second was used to produce lesion. The sham lesioned animals were subjected to the same procedure except for the passage of current.

Measurement of humoral immune response

For immunization, 5×10^9 sheep red blood cells (SRBC)/ml (obtained from the same animal source for all experiment) was injected intraperitoneally. The day of immunization was taken as day "0". In Sham immunized and lesion immunized animals the antigen challenge was carried out on the 10th day after surgery. The chosen parameters were studied on 5th day in group II and on 15th day in-groups III to X after immunization.

Light ether anaesthesia (Scientific Advance Company, Madras, India) was used for collecting blood samples. Blood sampling and sacrifice were done between 8.00–9.00 a.m. to avoid any circadian influence. The serum from the blood samples was separated and preserved at minus 20°C until use. Hippcampal lesioned and sham group animals were sacrificed using over dose of pentathol sodium. Brains of the lesioned animals were preserved in formalin and processed by routine paraffin technique to confirm the lesion site and using crysyl fast violet the neuronal damage in the area was assessed. If the lesion site was not accurate, the values from those animals were deleted and new animals were recruited to maintain the same number of animals in all groups.

The circulating antibody titre was determined by direct haemagglutination principle using titre plate and SRBC. For the quantification of IgM and IgG, antibodies to rat immunoglobulins were purchased from Pelfreeze Rogers (USA). The anti-IgG antibody was raised in rabbit (Code 11602) and anti-IgM antibodies were raised in Goat (Code 12606). The calibrated rat serum was used to quantify the immunoglobulin which was obtained from the Binding site Institute of Research, Birmingham (Code BP077). The technique used was the single radial immuno-diffusion (6) for the quantification of IgG and IgM. 40 mg of agarose was dissolved in 1.5 ml of barbitone buffer (pH 8.6) and boiled, cooled down to 45°C. Then mixed with 1.5 ml of pre-warmed phosphate buffered saline (PBS) with 100 μl of anti-IgG (12.6 μg/100 μl) and layered immediately over pre-coated double width glass slides and allowed to solidify at room temperature in a moist chamber. With the help of a template the wells were cut in the gel. A similar procedure was carried out for IgM with 100 μl of anti-IgM antibodies (210 μg/100 μL). The standard calibrated sera used at neat, one-half and one-fourth dilutions and a standard graph was plotted in a semi-log paper from which the unknown concentration were quantified. The migration of IgG was terminated after 24 hours and IgM after 48 hours by washing off the excess antibody with saline, slides were dried and the protein precipitin rings were stained with Amido Black. Their exact diameters were measured.

For statistical analysis One-way analysis of variance (ANOVA), followed by Tukey's multiple comparison was performed. The significance was fixed at $P<0.05$ level.

RESULTS

Circulating antibody titre levels

The anti-SRBC antibody titre levels are given in Table I. In unchallenged animals there was no significant change in the SRBC specific circulating antibody levels. After an immune challenge a marked decrease in the antibody titre levels was observed (F = 38.36, df = 4, 29) in both DLH and VHF lesioned and immunized animals when compared to their site specific sham operate as well as with the immunized control animals. However, DLH immunized sham animals showed a significant marked increase in circulating anti-SRBC antibody titre than the normal control immunized rats.

TABLE I : Rat serum anti-sleep red blood cells (SRBC) antibody titre levels (expressed as log 2 titre).

Groups	*Anti-SRBC antibody titre*	
	Non immunized animals	*Immunized animals*
Control	1.30±0.94	10.70±0.48
Sham DLH lesion	1.33±0.5	12.30±1.20‡
DLH lesion	0.83±0.4	8.00±1.20*
Sham VHF lesion	1.16±0.75	12.00±1.26
VHF lesion	0.50±0.54	7.90±0.81*

(imm. = immunized, Only significant effects are indicated)

* Titre decreased (P<0.05) in imm. VHF & DLH lesion groups compared to it's sham groups & also from the immunized controls.

‡ Titre increased (P<0.05) in imm. sham DLH animals compared to imm. controls.

IgM levels

The values are summarised in Table II. Unimmunized DLH sham operated animals alone showed a marked elevated levels of IgM compared to DLH lesioned and control animals (F = 4.4, df = 4, 31). IgM levels in all the other groups studied are similar to control animals.

After the antigen challenge immunized DLH and VHF lesioned animals showed an marked reduction in IgM levels, when compared to their site specific sham groups (f = 20, df = 4, 26) as well as from the immunized control rats. However, after the immune challenge DLH sham lesioned and immunized animals showed a significant

TABLE II : Rat serum IgM and IgG levels (mg%, Mean ± SD).

Groups	*Unimmunized groups*		*Groups*	*Immunized groups*	
	IgM	*IgG*		*IgM*	*IgG*
Control	63.1±2.9	1713±121.8	Imm. control	123.9± 6.0	2480±525
Sham DLH	66.3±2.0	1775±81.9	Sham DLH Imm.	139.5± 9.3‡	2810±212
DLH lesion	62.3±1.0†	1555±214.4†	Lesion DLH Imm.	109.0± 5.4*	2425±389
Sham VHF	63.1±1.7	1606±169.7	Sham VHF Imm.	128.0± 10.4	3065±321
VHF lesion	65.6±2.0	1637±79.1	Lesion VHF Imm.	106.0± 4.9*	2123±952**

(imm. = immunized)

* IgM level decreased (P<0.05) in imm. VHF & DLH lesion groups compared to the respective sham groups.

** Significant decreased (P<0.05) IgG levels in VHF Imm. lesion compared to imm. sham VHF and imm. controls.

‡ IgM level increased (P<0.05) in imm. sham DLH animals compared to imm. controls.

† Marked decreased IgM and IgG levels in DLH lesion compared to DLH sham unimmunized group

increase in IgM levels compared to controls.

IgG levels

The DLH lesioned animals showed a marked decrease (F = 2.76, df = 4, 33) in IgG levels from it's specific sham animals but not from the controls (Table II). Moreover the IgG levels in sham operated animals were similar to controls. All the other groups in the study showed similar levels to controls.

After an antigenic challenge IgG levels in both (DLH and VHF) sham groups were similar to the immunized control IgG levels. The immunized VHF lesioned animals showed a decrease in IgG levels compared to it's sham operated immunized group and immunized control group (F = 4.7, df = 4, 29).

DISCUSSION

An administered antigen elicits immune response, the magnitude of which is essentially depends on the endogenous factors and cellular interactions. The pattern of response in turn depends on the regulatory factors that regulate the effect. The present study was performed to analyse the level of IgM at one point, ie as the peak of IgM level occurs on the 5th day in such animals. The levels of both IgM and IgG in control group are well in agreement with the values of earlier reports in Wistar strain rats (7). Unimmunized DLH sham operated animals alone showed a marked elevated levels of IgM compared to DLH lesioned and control animals. IgM levels in all the other groups studied are similar to control animals. Hence this effect could be due to the site-specific cranio-cerebral insult that resulted from the surgical procedures. As this effect is not seen in the VHF sham groups and further site-specific sham groups alone are considered to evaluate the lesion effects, we can presume that the lesion in DLH area has a tendency to lower the circulating IgM levels even before an antigenic challenge.

In the present study, after an immune challenge the circulating anti-SRBC antibody titre level and IgM levels are significantly lowered in both DLH as well as in VHF lesioned and immunized animals, when compared with their site-specific sham operated and immunized controls. From these results, it can be concluded that the decreased antibody titre level observed could be due to the decreased IgM antibody synthesis. Since our study was confined to primary immune response, the altered IgM level is important. However after the immune challenge DLH sham lesioned and immunized animals showed a significant increase in IgM levels compared to controls. The elevated antibody titre seen in DLH sham operated and immunized animals could be due to the elevated IgM levels. This result indicates that, to have a normal humoral response in rat hippocampal region need to be intact.

The DLH lesioned animals showed a marked decrease in IgG levels as compared with it's specific sham operated animals, indicating that DLH region may have role in regulating circulating antibody levels in unchallenged animals. However, only immunized VHF lesioned animals showed a significant decrease in IgG level as compared to their immunized sham group. The analysis of IgG level indicates the need

for intact ventral hippocampal area to maintain normal circulating IgG levels, as IgG levels are considered more important during the secondary immune response. Further evidence is required to implicate the role of DLH and VHF in modulating IgG levels during the secondary immune response.

One of the possible reasons for the decrease in IgM after hippocampal lesion could be related to an alteration in the neurotransmitter levels in this area. Work of Qiu et al. (8) supports this suggestion, as they showed that the depletion of monoamine neurotransmitters or only of noradrenaline (NA) in CNS caused an impairment of anti-SRBC responses in rats. During phases of days 2–7 (peak periods of antibody response) post immunization, the metabolic alterations in NA, 5 hydroxytryptamine (5HT) and dopamine (DA) occurred in the CNS and in lymphoid organs of rats. Further, these authors reported that the metabolism of monoamine neurotransmitters in the hypothalamus and hippocampus was markedly increased, but NA content in spleen and thymus was significantly decreased. These time-dependent changes appear to be more important in the physiological perspective of the neural modulation of immunity. It appears that when these neurotransmitter changes were abolished by hippocampal lesion, the primary immune response was observed to be affected in the present study. Further corroborative evidence was provided by studies of Devoino et al. (9) who showed that within 20 min of inoculation with SRBC in rats DA levels were elevated in hypothalamus, amygdala and hippocampal areas suggesting nigrostiatal and mesolimbic dopaminergic structures are involved in the neural modulation of immune system.

Another possible cause for altered immune response could be the modified cellular level interactions. Though specific immune response are classified as humoral- and cell-mediated, macrophages are required for antigen presentation and the T-dependent antigen require T helper cell interaction for the transformation of B cell into plasma cell. It is important to note that the SRBC used in the present study is a T-dependent antigen. Lesion in the brain can induce the changes in the lymphoid organs at its cell distribution levels. Based on this, we in a previous study analysed the alteration of individual cell types in splenic population by separating them after lesioning DLH and also after an immune challenge (10). Our data indicated that there was a decrease in macrophages and ‘T’ cells in immunized lesioned animals, and an increase in macrophages in immunized sham animals. The recruitment of macrophages is essential for antigen processing and presentation, and at the same time T cells are essential for the transformation of B cells to plasma cells during T-dependent antigen processing. This concept is further supported by our earlier reports on DLH and VHF lesioned immunized animals in which there was a significant decrease in spleen plaque forming cells (11, 12). A decrease in immunoglobulin levels in the present study can be explained in the background of foregoing discussion.

An elevated antibody titre was seen in the present study in immunized sham DLH-lesioned animals as a result of an the

elevation of IgM level. The site specific sham effects have also been observed by us in an earlier study (13). This could be due to the resultant combination of neuro-surgical trauma and the release of regulatory factors from the inflammatory cells. Sham effects on immune regulation could be variable and this was also evidenced by the report of Nagy and Berczi (14) who have shown that in sham operated rats in which the only animal's skull was drilled showed significant higher antibody levels for the SRBC antigen than that of hypophysectomized and non operated groups. In the present study also sham operated animals of DLH differed from that of VHF sham operated animals.

It was reported that hypersecretion of ACTH occurs in the afternoon in the absence of hippocampus (15). However, such an increase in ACTH and consequently increased secretion of corticosteroid may not be the basis of the observed decreased anti-SRBC antibody and serum IgM response in DLH lesioned animals as in previous studies non lesioned immunized animals also showed a similar rise in corticosteroid levels, yet had a higher antibody response (11, 16–18). Thus an altered immunoglobulin levels observed in this study could not be attributed to changes in steroid levels. However, an altered receptor sensitivity for corticosteroid could be another possibility for such an effect, since a considerable heterogeneity in the activation of adrenal steroid subtypes in stressed rat, irrespective of peak level of corticosteroid has been reported by Miller et al. (19). It will be more fruitful if receptor sensitivity is also studied in future investigations on this subject.

On the basis of available results in the present study, it can be concluded that an alteration in the humoral immunity in hippocampal lesioned animals is not due to the circulating corticosteroid levels. More possible reason for these effects could be due to the modulation in the CNS neurotransmitter levels, or due to the resultant cellular changes observed in spleen (8–10). Observations of lesion induced histological changes and concurrent alteration in neurotransmitter profile in all these groups will throw more light on the exact cause for the observed immunological phenomenon.

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