

***Prosopis juliflora* Pollen Allergen-Induced Changes in the Hypothalamic Electroencephalographic and Acetylcholinesterase Activity in the Rat**

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Electroencephalographic activity and acetylcholinesterase activity changes produced by sensitization of rats with *Prosopis juliflora* pollen allergen were studied in the hypothalamus. Acetylcholinesterase activity was also measured in the blood serum. At day 9 after the sensitization with allergen, the hypothalamic activity consisted of high-frequency, high-amplitude waves together with spiking. Challenge with an intravenous dose produced an increase in the electrical activity of the hypothalamus. Sensitization with allergen initially increased the acetylcholinesterase activity in hypothalamus, but later the enzyme activity showed a decrease. Challenge dose at day 9 after sensitization increased the enzymatic activity. Data of the present investigation appeared to show a correlation between changes in the electrical activity and enzyme activity with the sensitization of rat with allergen. The data thus indicate the possible involvement of hypothalamus in immune responses.

INTRODUCTION

There is growing evidence that the central nervous system modulates or influences the immediate hypersensitivity and also that it plays a significant role against lethal anaphylaxis sensitized with various allergens.^{1,2} The CNS modulatory effects on immune responses appears to be mediated by receptor-mediated interaction of neuropeptides released from the CNS with the immunocompetent cells.^{3,4} Immune responses produce brain functional changes which can be mediated by soluble factors released by activated immunologic cells.^{4,5} Central neurotransmitter signals so elicited may regulate the responsiveness of the cells of the immune system.^{3,6,7} There has been preliminary evidence to indicate the involvement of neurotransmitters such as serotonin and norepinephrine in the immune reactivity.⁸ Electroencephalographic activity (EEG) changes reflect the changes in brain functions.⁹ Since changes in brain activity produced by immune responses may be related to the process of neuroimmunomodulation, we studied both hypothalamic electric activity and acetylcholinesterase enzymatic activity (as a marker of cholinergic neurotransmitter system) in the immunosensitized rats.

Prosopis juliflora (Pj) pollen allergens have been isolated in this laboratory to analyze immunopathologic responses and desensitization.¹⁰ In the present paper we have determined the changes produced by this allergen in the hypothalamic activity to find out as to what extent the observed changes may be related to the modulation of immune responses. The data obtained showed that there was a correlation between development of immune response and the electrical activity and acetylcholinesterase activity changes.

MATERIALS AND METHODS

Extraction of Prosopis juliflora Pollen Allergen

Prosopis juliflora (Pj) pollen allergen extract was prepared in 0.15 M phosphate buffered saline solution, pH 7.2 (1.10 w/v). A homogeneous mixture was obtained after shaking for 48 hr at 4°C. The mixture was centrifuged at 15,000 rpm and supernatant was sterilized by a 0.45 μ m filter.

Estimation of Protein and Carbohydrate

Protein content of crude Pj pollen extract was estimated by the method of Lowry *et al.*¹¹ and verified by the method of Vernon and Roberts.¹²

The carbohydrate content of Pj pollen crude extract was estimated by the method of Koehler.¹³

Sensitization of Rats

Albino rats of Wistar strain of either sex and weighing 150–200 g. were used in this study.

Rats were sensitized by 0.5 ml of Pj pollen extract (100 µg/ml, protein content) with equal volume of *B. pertussis* vaccine (BPV) administered intraperitoneally.

Animals were challenged by a dose of 0.250 µg/ml Pj allergen injected intravenously.

In control animals saline solution with BPV was administered intraperitoneally.

Testing of Allergenic Activity

The allergenic activity in the rats was tested by skin prick test as wheal formation. The animals that showed positive responses were taken for further study in this investigation.

Implantation of Electrode in Rat Hypothalamus

Animals were anesthetized by intraperitoneal injection of Nembutal (36 mg/kg) for stereotaxic implantation of the electrodes in the hypothalamus according to the method given by Klemm.¹⁴ The electroencephalogram (EEG) of the hypothalamus was recorded through a suitable connector and cables on Grass Polygraph (79 D EEG Polygraph). Recordings were obtained from conscious, unrestrained sensitized animals and were compared with those of control animals.

Determination of Enzymatic Activity

Rat Brain Homogenate

Rats were killed by cervical decapitation. Hypothalamus and cerebral cortex were rapidly excised, weighed, and homogenized in ice-cold 0.25 M sucrose (1:20 w/v).

Separation of Blood Serum

Blood was collected and kept at 4°C for clotting. After one-half hour the clot was detached from the side of glass wall and cut into pieces. After 4 hr serum was taken out and centrifuged at 3500 rpm.

Estimation of Acetylcholinesterase

Whole homogenate and serum were treated with 1% triton-X for 2 min to achieve the activation of the enzyme.

Acetylcholinesterase (AChE) activity was determined by method of Ellman *et al.*¹⁵ The assay mixture contained 0.1 M phosphate buffer (pH 8.0), 0.075 M acetylcholine thioiodide, 0.01 M dithionitrobenzoic acid, and the enzyme extract in a final volume of 3.0 ml. Absorbance was recorded against reagent blank at 412 nm in a Shimadzu UV spectrophotometer. Enzyme activity was calculated as $\mu\text{mol/gm tissue/min}$ and expressed as percent of activity. Data were analyzed using Student's *t* test.

RESULTS

The protein content of crude Pj pollen extract was found to be 7.9 mg/ml by the method of Lowry and 8.0 mg/ml by the method of Vernon and Roberts. The carbohydrate content was found to be 2.0 mg/ml, which was one-fourth of the total protein content of crude Pj pollen allergens.

Allergen-Induced Electrophysiological Changes in Hypothalamus of Rat Brain

Electroencephalographic activity of the hypothalamus was recorded on the 3rd, 9th, 15th and 25th day after sensitization of the rat (FIG. 1A). The allergen-induced effects on EEG activity began to appear on the 3rd day after sensitization. At day 9 after sensitization the EEG of hypothalamus showed high-amplitude and high-frequency waves with spiking. After that the electrical activity began to decrease and reached near-control level by about 25th day after the sensitization.

FIGURE 1B shows the EEG recordings obtained at different intervals after the administration of a challenging dose in the rats that had been sensitized by allergen 9 days before. It was observed that the challenging dose stimulated the hypothalamic EEG activity and the maximum activation seemed to occur at 10 minutes after the administration of the challenging dose.

Allergen-Induced Changes in the Activity of Acetylcholinesterase (AChE)

FIGURE 2A shows the changes in AChE activity at 3,9,15, and 25 days after sensitization in the hypothalamus, cerebral cortex, and blood serum. The data show that the enzymatic activity increased in hypothalamus, cerebral cortex and serum on the 3rd day after sensitization. But at the 9th, 15th and 25th day, the AChE activity was found to decrease in the hypothalamus, cerebral cortex and serum. No significant change was found at day 25 after sensitization.

A challenging dose was also found to produce activation of enzymatic activity in the hypothalamus of rats at day 3 and 9 after sensitization (FIG. 2B). A challenging dose, however, did not produce the enzymatic activity changes in the cerebral cortex and the serum. At day 15 and 25 the challenging dose did not produce alterations in the enzymatic activity in hypothalamus, cerebral cortex and serum. At day 25 after sensitization AChE activity returned to the control value.

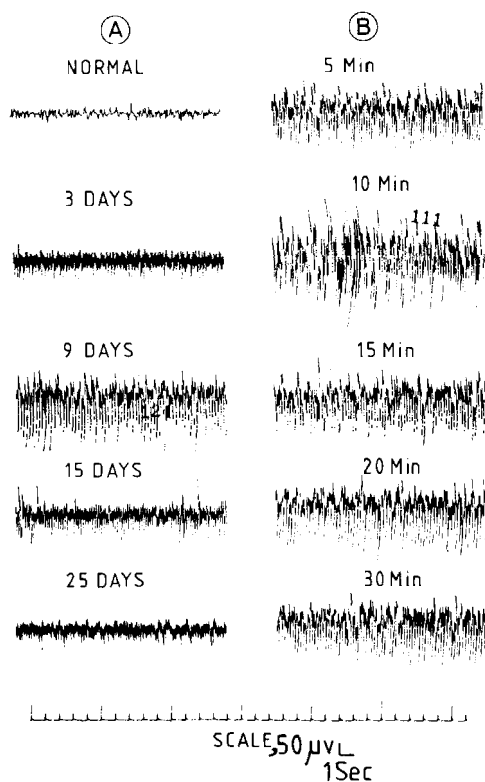


FIGURE 1. Electroencephalographic recording of hypothalamus from (A) normal sensitized rat at 3, 9, 15 and 25 days after sensitization by *Prosopis juliflora* pollen allergens with adjuvant and (B) 9-day-sensitized rat in which a challenging dose (0.250 mg/ml) was given for 1 to 30 minutes.

DISCUSSION

The data presented in this study clearly show that allergen-induced immune reactions in the rat are associated with changes in hypothalamic electric activity as well as in the cholinergic activity. Further, there is an activation of hypothalamic electric

activity even after the animal is challenged with a administrative dose of Pj pollen allergens. Very conspicuous activation of EEG activity is observed 9 days after sensitization. This shows similarity with the kinetics of reaginic antibody formation in the rat after primary immunization with antigen and adjuvant.¹⁶ This elevation of hypothalamic electrical activity is concomitant with the antibody formation. When a sensitized animal was challenged with allergen, the electric activity was increased in 10 minutes. This type of stimulation in sensitized rat is similar to that produced by stress or shocks given to an animal. Thus, results of this study show that allergen elicited a distinct response in the hypothalamus linked with the immune response, which in turn was evoked by a challenging dose of Pj pollen allergens.

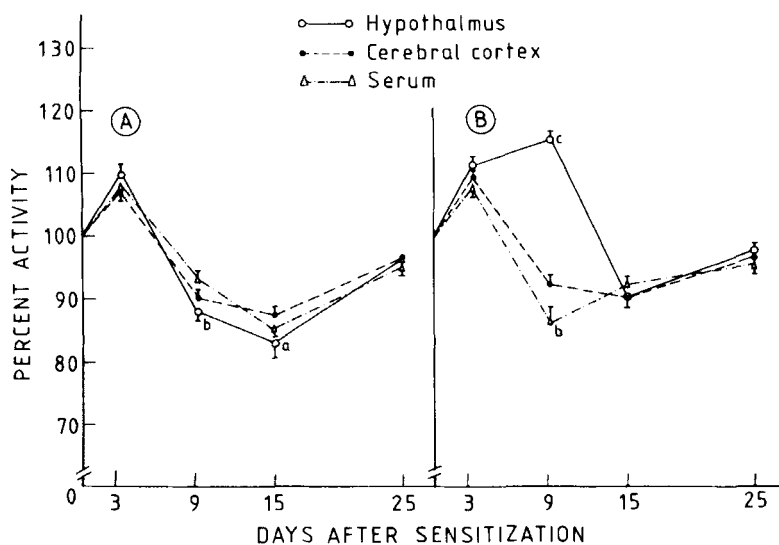


FIGURE 2. Percentage change in AchE activity in (A) sensitized rat at 3, 9, 15 and 25 days after sensitization and (B) sensitized rats in which a challenging dose (0.50 mg/ml) was given. Control was taken as 100%. Each point represents the mean of not less than six values, and the SEM is shown by vertical bars. a, $p < 0.04$; b, $p < 0.02$; c, $p < 0.03$.

Another significant finding of the present study is the stimulation and inhibition of the hypothalamic cholinergic neurotransmitter systems. So far, there have been no reports concerning the involvement of the cholinergic neurotransmitter in neuroimmunomodulation, although the role of serotonin and noradrenalin neurotransmitter has been indicated.^{8,17} Our results show an increase in AchE activity in 3-day-sensitized rats, but it decreased thereafter. The increase may be due to excess release of Ach in the 3-day-sensitized rats, but afterwards depletion in Ach might be associated with the decrease in enzymatic activity. The enzymatic activity increased in the hypothalamus of the 9-day-sensitized animal after challenge. The elevation of enzymatic activity observed after sudden shock could be due to release of bound acetylcholine when animal was completely sensitized. The observations from the present study thus suggest

the involvement of a cholinergic neurotransmitter in the neuroimmunomodulation responses.

In summary the elevation and depletion of electric activity and enzymatic activity seemed to correlate with the degree of sensitization of the rat. This is indicative of the neuroimmunomodulatory role of the brain region.

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