# Effect of Hypothalamic Lesions on Experimental Autoimmune Diseases in Rats

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## INTRODUCTION

Experimental studies have indicated the possible involvement of the hypothalamus in immune modulation.[1] It has been shown that ablation of the anterior hypothalamus can inhibit antibody response to some antigens, delayed-type hypersensitivity to tuberculin, and anaphylaxis.[2,3] We examined the effect of electrolytic lesions of the anterior hypothalamus on the development of experimental autoimmune diseases, experimental autoimmune encephalomyelitis (EAE) and experimental autoimmune myasthenia gravis (EAMG). In addition, we examined the effect of depletion of hypothalamus-related neurotransmitters on EAE.

## MATERIALS AND METHODS

### *Anterior Hypothalamic Lesion (AHL)*

Female Lewis rats weighing 180 to 220 g were anesthetized and fixed in Kopf's stereotaxic apparatus, and nerve tissue (anterior hypothalamus, or hippocampus for control) was destroyed by passing 2.5 mA DC for 15 sec using an insulated stainless-steel electrode, as described previously.[4]

### *Administration of Neurotransmitter-Depleting Agents*

The lateral ventricle of anesthetized rats was reached with a fine Hamilton syringe according to the appropriate coordinates.[5] 6-Hydroxydopamine (6-OHDA) or 5,7-

dihydroxytryptamine (5,7-DHT) were administered in a single injection in a dose of 250 μg/1 μl saline solution either to the lateral ventricle or intraperitoneally. Reserpine, a nonspecific monoamine-depleting agent, was injected daily, 0.375 mg/kg subcutaneously, for up to 25 days.

### *Induction of EAE*

Animals were immunized with a 33% homogenate of rat spinal cord emulsified in complete Freund's adjuvant (CFA) as described previously.[6] AHL rats were immunized: (1) either two weeks or twelve weeks after the electrolytic procedure; (2) two weeks after administration of 6-OHDA or 5,7 DHT, or after the fifth day of reserpine injection. Clinical signs of EAE were assessed during the three weeks after immunization, and were scored from 1 to 4 as described previously.[6]

### *Induction of EAMG*

Rats were immunized with 40 mg of acetylcholine receptor (AChR) preparation emulsified in CFA containing 1 mg *M. tuberculosis* H37Ra, as described previously.[7] Immunization was performed three weeks after AHL and the rechallenge took place four weeks later. AChR was purified from the electric organ of *Torpedo californica* by solubilization of membrane fragments with 1% Triton X-100 followed by affinity chromatography on a *Naja naja siamensis* neurotoxin-Sepharose resin.[7] Animals were observed for clinical signs of disease up to 16 weeks after immunization; in animals with clinical signs, EAMG was proved by improvement after injection of edrophonium hydrochloride, as well as by decremental response in electromyography.[7] Serum antibody to AChR was determined four weeks after immunization.

### *Histologic and Biochemical Examinations in EAE*

Rats immunized for EAE were sacrificed after three weeks. Frozen sections of brain from AHL rats were examined, and rats with lesions not exactly located at the anterior hypothalamus were excluded from the study. Formalin-fixed sections of brain and spinal cord were processed for hematoxylin-eosin staining and histopathologic parameters of EAE were scored.[6] High-pressure liquid chromatography (HPLC) was used for determination of norepinephrine (NE), serotonin (5-HT), and dopamine (DA) in hypothalamus, hippocampus, and striatum.[8]

### *Lymphocytic Transformation* in Vitro

Spleen cells were suspended in enriched medium[6] and were cultivated in multiwell microplate ($2 \times 10^5$ cells/well) in the presence of concanavalin-A (Con-A) (1

$\mu$g/ml) or myelin basic protein (MBP) (10-200 $\mu$g/ml) for four days at 5% $CO_2$. One $\mu$Ci of [$^3$H]thymidine was added for the last 18 hours. Results were expressed as stimulation index (SI), namely, cpm ratio of cultures in the presence and absence of the stimulant (Con-A, MBP).

### *Antibody to Myelin Basic Protein*

Fifty-$\mu$l aliquots of diluted serum were reacted with MBP adhered to flexible microplates. After 90 min, $^{125}$I-labeled sheep anti-rat immunoglobulin (50,000 cpm) was added for 60 min. The wells were washed and counted for evaluation of percentage of $^{125}$I-binding.[9]

### *Antibody to Acetylcholine Receptor*

One-$\mu$l aliquots of test serum were incubated with 0.1 mg $^{125}$I-labeled alpha-bungarotoxin bound to AChR, as described.[10] Subsequently, 50 $\mu$l of 10% protein-A was added. The amount of radioactivity in the precipitate was determined in an autogamma scintillation counter.

## RESULTS AND DISCUSSION

The development of acute clinical EAE was inhibited by anterior hypothalamic lesions. Only 17% (5 of 29) of rats immunized two weeks after AHL developed clinical signs of EAE 10-15 days post immunization as compared with disease incidence of 90% (27 of 30) in control rats without AHL ($p < 0.001$). In addition, average duration of disease was shorter in sick animals with AHL: 1.3 $\pm$ 0.1 days as compared with 4.8 $\pm$ 0.6 days in control animals ($p < 0.01$); and severity of disease was milder in AHL rats: 2.2 $\pm$ 0.3 as compared with 3.2 $\pm$ 0.3 (maximal score of disease being 4) in control rats without AHL. However, the development of EAE was not affected when animals were immunized 12 weeks after AHL; in this case all animals (17 of 17) developed EAE 10-15 days after immunization. EAE was also not affected when animals were immunized two weeks after bilateral hippocampus lesions (BHL) were created as a control.

Histopathologic changes characteristic for EAE were found in all experimental groups of rats. Anti-MBP antibodies in sera of rats bearing AHL were lower than in the non-AHL control group as measured by percentage binding of protein-A to rat Ig: 13.0 $\pm$ 2.3 and 25.9 $\pm$ 3.9, respectively ($p < 0.01$). In addition, lymphocyte transformation *in vitro* in response to Con-A, was higher in cells obtained from AHL rats as compared with cells from the non-AHL control group: the stimulation index (SI) was 376.6 $\pm$ 75 and 198.2 $\pm$ 9.9, respectively. No stimulation to MBP *in vitro* was detected in both groups.

The development of acute EAE was also inhibited by the neurotransmitter-de-

pleting agents used.[11] As compared with a disease incidence of 98% (23 of 24) in control rats, only 32% (8 of 25) of reserpine-treated rats ($p < 0.01$), 52% (12 of 23) of rats receiving 6-OHDA intraventricular injections ($p < 0.01$), and 64% (9 of 14) of those injected intraventricularly with 5,7-DHT (not significant) developed EAE. No effect on EAE was found when 6-OHDA or 5,7-DHT were injected intraperitoneally. Content of CNS neurotransmitters, as determined by HPLC, was as follows: Striatal DA and hippocampal NE and 5-HT were similar in normal rat brains and in the brains of control rats with EAE (DA: 10.08 ± 1.26 μg/mg tissue; NE: 0.127 ± 0.014 ng/mg protein; 5-HT: 3.100 ± 0.345 ng/mg protein). Depletion of more than 90% ($p < 0.05$) of striatal DA content was found in both reserpine- and 6-OHDA-treated rats (0.788 ± 0.125 and 0.296 ± 132, respectively), and of 50% in 5,7-DHT-treated rats (5.48 ± 0.46). Hippocampal NE was almost totally depleted ($p < 0.05$) in both reserpine- and 6-OHDA treated rats (0.013 ± 0.003), but not in 5,7-DHT treated rats (0.105 ± 0.020). Depletion of about 50% of hippocampal 5-HT content was found in 5,7-DHT treated rats (1.682 ± 0.230), but also in reserpine- and 6-OHDA treated rats (2.05 ± 0.152 and 1.550 ± 0.104, respectively).

In contrast to the inhibitory effect of AHL on acute experimental autoimmune encephalomyelitis (EAE), AHL increases the incidence of chronic EAMG in AChR immunized rats. Three of 13 (23.0%) non-AHL control rats, and 12 of 18 (66.7%) AHL rats developed chronic EAMG 8-11 weeks post immunization ($p < 0.05$). The first phase of the disease was manifested in both groups by general muscle weakness, hunched back, and "fuzzy" fur, as well as by clinical improvement after injection of edrophonium hydrochloride. Average duration of the first phase was two days in non-AHL rats and seven days in AHL rats ($p < 0.02$); two AHL rats died of EAMG. Level of serum antibody to AChR was similar in the two groups: $1.0 \pm 0.1 \times 10^{-7}$ *M* in non-AHL and $1.65 \pm 0.15 \times 10^{-7}$ *M* in AHL rats.

Our results support previous observations[2,3] on the possible importance of the hypothalamus in modulation of immune functions. The results suggest that AHL has an inhibitory effect on different aspects of the immune response. Clinical signs of EAE, a cell-mediated autoimmune disease,[12] were suppressed if immunization took place at two weeks, but not 12 weeks, after the electrolytic damage to the anterior hypothalamus. This may indicate some compensation after several weeks of the unknown EAE inductive factors affected by AHL. The fact that cell infiltrates were present in the CNS of AHL rats may indicate that the affector limb of this specific immune response was not affected or was mildly inhibited in its intensity. Mononuclear cells reached the target organ, but in many AHL rats they were unable to propagate and cause clinical signs of EAE. Such a discordance between neurologic defecits and histopathologic evidence of EAE was described in many studies in which inhibitory regimens were assayed.[13] Suppression of cellular immune response *in vivo* may lead to inhibition of the development of EAE by decrease in T-helper or increase in T-suppressor activity. Increased Con-A response *in vitro* as demonstrated in the study may indicate an effect on the redistribution and possible entrapment of suppressor cells (specific T cells or nonspecific macrophages) within the spleen. Although antibodies are not likely to be important in disease induction, the decrease in anti-MBP antibodies following AHL may also be the result of decrease in T-helper activity or B-cell response.[14,15]

While acute EAE was suppressed by AHL, chronic EAMG, an antibody-mediated antoimmune disease,[16] was not suppressed and was even enhanced by AHL. This may indicate different hypothalamic influences on cellular and humoral immune responses. Although serum level of antibody to AChR did not change significantly by AHL, distribution and properties of antibodies may have been different. Moreover, the long period of time elapsed between AHL, AChR immunization, and EAMG appearance

might be crucial, as shown by the finding that AHL performed 12 weeks before encephalitogenic immunization did not affect the development of EAE.

The mechanisms by which anterior hypothalamic lesions influence autoimmune response and other immune reactions are not apparent. The neuroendocrine circuits via the hypothalamic-hypophyseal-adrenal axis may be involved. It is well established that lesions in the hypothalamus may change blood corticosterone and ACTH,[17] and that corticosteroids and different kinds of stress can modulate immune response.[18-20] Direct neuronal innervations from the hypothalamus to immune organs may also be involved; direct neuronal tracts between the hypothalamus and spinal sympathetic areas as well as sympathetic nerve endings in bone marrow and lymphatic organs have been demonstrated.[21,22] Furthermore, receptors to neurotransmitters have been demonstrated on lymphocyte membranes.[23] Humoral factors, such as neuropeptides and neurotransmitters, which can affect the single cell via specific receptors, may be the biological signals between the hypothalamus and the immune system. In the present study we tried to define the nature of some CNS neurotransmitters that may be involved in modulation of a classical T-cell-mediated response. We found that depletion from brain of the neurotransmitters dopamine and norepinephrine is associated with prevention of EAE. Thus, it is possible that dopaminergic and adrenergic pathways within the brain are necessary for the expression of the autoimmune disease.

## SUMMARY

The development of experimental autoimmune encephalomyelitis (EAE) was prevented in rats immunized with encephalitogenic antigen two weeks, but not twelve weeks, after stereotaxic electrolytic destruction of the anterior hypothalamus. Serum antibody level to the antigen myelin basic protein was decreased, and *in vitro* lymphocyte transformation response to a mitogen was increased. On the other hand, incidence and intensity of chronic experimental autoimmune myasthenia gravis (EAMG) induced by acetylcholine receptor immunization were higher in rats with anterior hypothalamic lesion. In addition, expression of EAE in rats was inhibited when dopamine and norepinephrine in brain were depleted due to intraventricular injection of 6-hydroxydopamine or subcutaneous injection of reserpine. The study indicates hypothalamic modulatory effects on autoimmune response as well as possible involvement of neurotransmitters in this kind of neuroimmunomodulation.

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