# Failure of Hypothalamic Lesions to Protect against Experimental Allergic Encephalomyelitis (EAE) in the rat (33551)

MARVIN E. ROSENTHALE AND D. L. MARGULES

*Pharmacological Evaluation and Psychopharmacology Sections, Wyeth Laboratories, Inc., Radnor, Pennsylvania 19087*

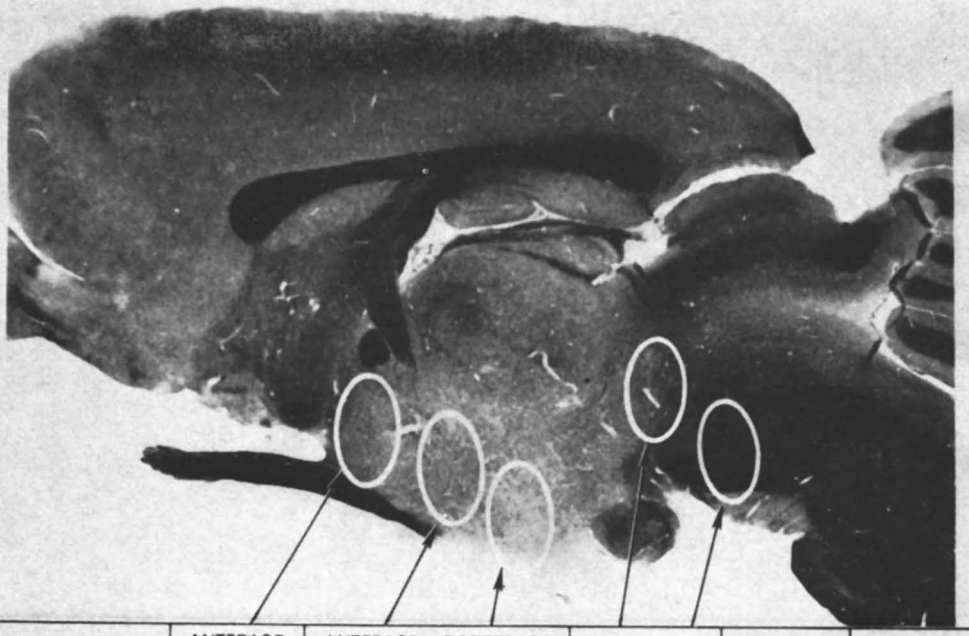
Immunogenesis can be profoundly affected by electrolytic lesions of the hypothalamus, as has been demonstrated in several species. Lethal anaphylactic shock is prevented by bilateral focal lesions in the tuberal region of the hypothalamus or midbrain recticulum of guinea pigs (1–4) or in the anterior hypothalamus of the rat (5). In the rabbit, electrolytic lesions of hypothalamic and thalamic structures suppress production of antibody and prolong retention of antigen in the blood (6). These hypersensitivities are of the immediate or humoral type, i.e., the responses are characterized by the production of circulating antibodies that interact with antigen, thereby releasing various humoral agents which immediately induce shock or affect target organs. This type of immune response may or may not be related to the tuberculin type of delayed or cellular hypersensitivity characterized by cell-mediated tissue damage. Whether the two are fundamentally distinct phenomena or only represent different stages of a single process is under intensive investigation (7, 8).

In the present study we attempted to determine whether electrolytic lesions of the hypothalamus can block a delayed type of immunologic response in the same fashion as they prevent the circulating antibody response (9). The test system chosen was experimental allergic encephalomyelitis (EAE), an autoimmune disease in which delayed hypensensitivity is thought to play the major etiologic role and which responds in a quantitative manner to various therapeutic agents (10–12).

*Methods.* Thirty-two male Lewis strain rats (Microbiological Associates, Bethesda, Md.) were anesthetized with pentobarbital on day 1, and mounted in a Kopf stereotaxic instrument. Electrolytic lesions were produced in various hypothalamic and tegmental areas by passing 2 mA of direct current for 10 sec through a glass-insulated platinum electrode with 1.5 mm of wire bared at the tip. The cathode was a rectally placed phone jack. Anterior preoptic lesions were produced by implanting anodal electrodes perpendicular to the skull at the level of the bregma, 1 mm lateral to the sagittal sinus, and 8 mm below the brain surface (i.e., at coordinates 0, 1, 8). Coordinates for the anterior hypothalamus were —1.5, 1, 8.5; for the posterior hypothalamus, —3, 1, 9; and for the midbrain tegmentum, —5, 1, 6 (in 3 rats) and —6, 1, 7 (in 8 rats).

EAE was initiated on day 2 by subplantar injection in the right hind footpad of 0.05 ml of an encephalitogenic emulsion of isologous spinal cord in complete Freund's adjuvant (12). Animals were weighed and observed during a 16-day period. On day 17 all animals were autopsied, blood was taken for hematologic studies, and stress organs were weighed. The brain and spinal cord were removed and fixed in formalin. Twelve to 14 cord sections and 4–5 brain sections were examined for each animal. Sections were stained with cresyl violet and scored for typical EAE inflammatory lesions on an arbitrary scale of 0 to 4, based on the type and degree of periovascular cuffing, granulomata, and neuronal degeneration (13). Electrolytic lesion sites were verified in every case.

*Results.* Fourteen days after administration of encephalitogenic antigen, symptoms typical of EAE (severe hind limb paralysis, abdominal wall flaccidity, fecal impaction, and urinary incontinence) developed in 11 of 12 (92%) of the control EAE rats. All control animals exhibited inflammatory lesions of the spinal cord and brain. The total leukocyte count was similar to that in normal rats, but the differential count showed a relative neutrophilia and lymphopenia. These responses



| AREA LESIONED | ANTERIOR PREOPTIC | ANTERIOR POSTERIOR HYPOTHALAMUS | | MIDBRAIN TEGMENTUM | | NORMALS | EAE CONTROLS |
|---|---|---|---|---|---|---|---|
| NO. RATS | 3 | 5 | 13 | 3 | 8 | 12 | 12 |
| CLINICAL SCORE: | | | | | | | |
| NO. PARAL. NO. DONE | 2/3 | 3/5 | 7/13 | 0/3 | 4/8 | 0/12 | 11/12 |
| PATHOLOGICAL SCORE: | | | | | | | |
| NO. POS. NO. DONE | 2/3 | 5/5 | 13/13 | 3/3 | 8/8 | 0/12 | 12/12 |
| MEAN CORD SCORE | 1.3 | 1.8 | 2.0 | 1.7 | 2.2 | 0 | 2.5 |
| MEAN BRAIN SCORE | 1.9 | 1.0 | 1.4 | 2.4 | 1.4 | 0 | 1.5 |
| HEMATOLOGY: | | | | | | | |
| WBC x $10^3/mm^3$ | 14.5 | 14.2 | 14.2 | 12.0 | 9.8 | 12.0 | 10.2 |
| POLYS % | 49 | 57 | 42 | 45 | 46 | 22 | 49 |
| LYMPHS % | 46 | 37 | 54 | 53 | 49 | 75 | 46 |
| STRESS ORGANS: | | | | | | | |
| THYMUS (mg) | 243 | 258 | 304 | 173 | 175 | 471 | 231 |
| SPLEEN (mg) | 381 | 332 | 467 | 388 | 351 | 426 | 400 |
| ADRENALS (mg) | 38 | 39 | 39 | 37 | 36 | 38 | 38 |

FIG. 1. Effect of electrolytic lesions of several brain areas on various parameters of EAE in Lewis rats. Lesions were unilateral in 2 of the 3 rats with anterior preoptic lesions, in 2 of the 13 with posterior hypothalamic lesions, and in 5 of the 8 with midbrain tegmental lesions. All other lesions were bilateral.

were accompanied by some involution of the thymus and spleen but not, as reported in a larger series (12), of the adrenals (Fig. 1). Control EAE rats gained weight similarly to normal animals until 9 days after antigen (day 11), when weight gain stopped and a precipitous drop in body weight ensued (Fig. 2).

Of the rats with electrolytic lesions, 16 of 32 (50%) exhibited paralytic signs of EAE. Histopathologic examination of the nervous system, however, revealed that 31 of the 32 (97%) had some degree of inflammatory lesion (Fig. 1). Neutrophilia, lymphopenia, and thymic involution occurred in all 32 animals. With the exception of the posterior hypothalamic group, the spleen weight was also reduced. These changes were similar to those in the EAE control rats. There was a severe drop in body weight for a 4-day period after the electrolytic lesions were induced. The magnitude of this drop varied, depending on the area of the lesion, the less severe losses occurring in animals with lesions in the anterior hypothalamic area. Recovery and

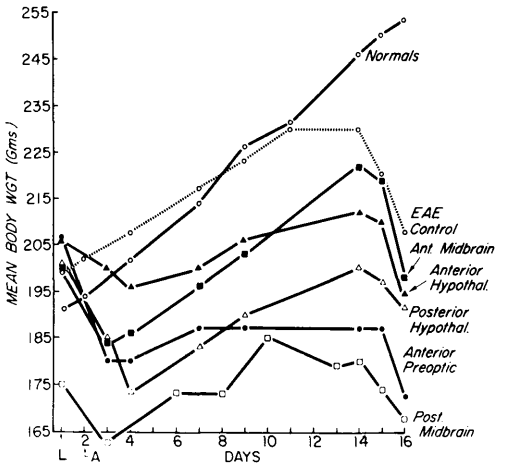



FIG. 2. Effect of electrolytic brain lesions on body weight of Lewis rats with EAE. Number of rats per group is shown in Fig. 1. Electrolytic lesions were produced at L and encephalitogenic antigen was administered at A.

some gain in body weight occurred after day 4 and continued until day 14, when body weight dropped as precipitously in all 32 rats as it had in the 12 EAE controls (Fig. 2).

*Discussion.* The results indicate that electrolytic ablation of hypothalamic areas can partially prevent the clinical but not the histological signs of EAE in the Lewis rat. Although these rats have definite histopathologic evidence of EAE, the lower clinical score (50% in rats with lesions against 92% in controls) suggests that the severity of the syndrome has been reduced.

The presence of EAE lesions in clinically "well" animals has been described by several other investigators (13–17). Paterson and Weiss (18), who studied the transfer of EAE by intracerebral lymphoid cells, postulated that the presence of microscopic lesions in the absence of clinical signs indicates a milder form of the disorder. Similarly, Levine *et al.* (19) demonstrated that the clinical but not the histologic lesions of EAE could be suppressed by immobilization stress. The initial stress, subsequent loss of body weight and possible release of ACTH (5) associated with electrolytic lesions thus may have attenuated the clinical signs of EAE. These animals probably also had fewer and less advanced microscopic lesions, indicating a less severe form of the disease.

The fact that electrolytic lesions did not prevent the changes in stress organ weights, body weights, and hematologic changes which occurred in the EAE control rats confirms the histopathologic pattern and indicates that the rats were indeed afflicted with EAE. The decrease in body weight, which began in all groups 9–12 days after sensitization (and before the appearance of maximum clinical paralysis) suggests that the onset of EAE occurred at this time. The initial appearance of histologic lesions in the neuraxis of various species 9 to 10 days after administration of antigen has been described (13, 20), and

closely correlates with our observed body weight changes. Body weight loss may therefore be a sensitive indicator of the onset of EAE.

Tuberal lesions of the hypothalamus, although effective in preventing anaphylactic shock in guinea pigs (21), fail to prevent the development of the Arthus and the Sanarelli–Shwartzman phenomena or turpentine-induced exzema in guinea pigs and rabbits (2). Thus, complete elimination of immunologic reactivity is not achieved by lesions of hypothalamic centers. Blaw *et al.* (10) demonstrated that humoral antibody is not necessary for the development of EAE in agammaglobulinemic chickens. Our inability to completely suppress EAE by electrolytic destruction of hypothalamic and tegmental areas in the rat is consistent with these results and may indicate a difference between delayed and humoral hypersensitivity responses, as represented by EAE and anaphylactic shock.

*Summary.* Thirty-two male Lewis rats were electrolytically lesioned in various hypothalamic and tegmental areas 1 day prior to the administration of an encephalitogenic antigen. Lesions of these areas have previously been shown to prevent anaphylactic shock. Ninty-two percent of the controls but only 50% of the rats with electrolytic lesions exhibited clinical signs of EAE 15 days after administration of antigen. Microscopic examination of nervous tissue, however, indicated that 31 of the 32 rats had inflammatory lesions of the central nervous system characteristic of EAE. Electrolytic lesions also did not prevent the changes in stress organ weights, body weights, and hematologic changes which occurred in control EAE rats. Hypothalamic lesions that would have blocked anaphylactic shock in the rat and guinea pig reduced the clinical signs of EAE but had no great effect on the histologic course of disease. Thus, delayed and humoral hypersensitivities, as represented by EAE and anaphylactic shock, may constitute different immunologic phenomena in the rat.

The authors thank Mr. L. J. Datko, Mr. J. Kassarich, Mr. F. Schneider, Mr. G. Keller, Mr. J. Kulak, and Mr. W. Carmint for their excellent assistance.

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Received Sept. 17, 1968. P.S.E.B.M., 1969, Vol. 130.