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BRIEF COMMUNICATION

Altered Splenic Catecholamine Concentrations During Experimental Allergic Encephalomyelitis

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WESSELMANN, U., R. J. KONKOL, G. L. LEO, D. L. ROERIG AND D. R. HARDER. *Altered splenic catecholamine concentrations during experimental allergic encephalomyelitis.* PHARMACOL BIOCHEM BEHAV **26**(4) 851–854, 1987.—Catecholamine concentrations of the spleen were studied with neurochemical techniques in rats injected with myelin basic protein to produce an experimental allergic encephalomyelitis (EAE). Thirteen to 14 days postinoculation the affected rats showed peak clinical signs of weakness, especially in the lower extremities. Resolution of the disease then progressed rapidly with full clinical recovery at day 21. Splenic concentrations of norepinephrine (NE), 3,4-dihydroxyphenylalanine (DOPA), epinephrine (EPI) and 3,4-dihyxroxyphenylethylamine (DA) were determined by HPLC with electrochemical detection. DOPA concentrations were significantly increased (+62%) while DA concentrations were decreased (−29%) in the EAE rats on day 14 postinoculation. NE and EPI concentrations tended to be elevated in the EAE group, but this was not statistically significant. No differences in splenic catecholamines were detected on day 7 and 52 postinoculation between EAE and control animals. These results indicate that changes in the metabolic pathways of splenic catecholamines occur at the peak of the clinical symptoms of EAE; the increase in DOPA and the decrease in DA concentrations suggest that the activity of DOPA-decarboxylase or its co-factor is altered.

Catecholamines Dopaminergic system Spleen Experimental allergic encephalomyelitis Autoimmunity Autonomic nervous system

EXPERIMENTAL allergic encephalomyelitis (EAE) is a T-cell mediated [14,25], autoimmune, inflammatory disease of the CNS that has been widely studied as a model for demyelinating disorders [1, 10, 17].

EAE can be induced in experimental animals by footpad inoculation with myelin basic protein (MBP) emulsified in complete Freund's adjuvant (CFA) [15,16]. Clinically, the disease begins about 10 days postinoculation with weakness of the hind legs and progresses rapidly to the peak of the clinical manifestations (paralysis of the hind legs, fecal impaction, urinary retention) on day 14. About 21 days postinoculation there is virtually complete recovery of all clinical symptoms [3,15].

Recently it has been reported that the clinical course of EAE can be influenced by alpha- and beta-adrenergic receptor blockers [3]; however, it is not clear whether these drugs are acting through altering the immune response or vascular permeability or both. The present study was designed to further investigate the role of the adrenergic system in the course of EAE. It is well known that cells, which are involved in an immune response, perform in a milieu of hormones and neurotransmitters. In the last ten years evidence for a dynamic relationship between the nervous and the immune system has accumulated [2, 7, 13, 20, 21, 24]. Anatomical studies have shown that noradrenergic fibers of the sympathetic nervous system innervate lymphoid organs providing pathways by which the immune system and the autonomic nervous system can communicate with one another [4, 8, 9, 18, 19, 26]. Experimental evidence exists that the immune response can cause phasic changes in neurotransmitter levels in lymphoid organs [2,7]. So far neurotransmitter concentrations of lymphoid tissue during the clinical course of EAE have not been investigated.

The purpose of the present study was to determine

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whether changes occur in the catecholamine concentrations of the spleen during the development of EAE. The concentrations of norepinephrine (NE), 3,4-dihydroxyphenylalanine (DOPA), epinephrine (EPI) and 3,4-dihydroxyphenylethylamine (DA) in the spleen were investigated with neurochemical techniques before (7 days postinoculation), at the peak (14 days postinoculation) and about one month after recovery (52 days postinoculation) from the clinical manifestations of EAE.

METHOD

Animals

Male Lewis rats (3 months of age) weighing 270–350 g at the beginning of the experiment were obtained from Harlan Laboratories. They were kept under barrier-maintained conditions in a temperature controlled room with a 12/12 hr light-dark cycle. Purina Lab Chow pellets and tap water were available ad lib.

Sensitization

EAE was induced in the experimental group by sensitization with a mixture of CFA (containing myco-bacterium butyricum) and guinea pig MBP according to the method of Swanborg [23]. Fifty μl of the suspension was injected into the hind footpads of the rats (EAE group). Control animals received footpad injections of CFA alone (CFA group).

Clinical Assessment

The following grading system was used to assess the clinical state of the animals on a scale of 1–3: 0—normal, 1—loss of tail tone, 2—paresis of hind legs, 3—paralysis of hind legs.

Dissection:

Seven, 14 and 52 days postinoculation approximately equal numbers of experimental and control animals were sacrificed by decapitation. The spleens were rapidly removed and frozen on dry ice. All rats were killed at the same time of the day (between 10:00 and 12:00 a.m.) to avoid circadian variations in catecholamine metabolism [5].

Neurochemical Analysis

Spleens were weighed while frozen and then quickly homogenized in ice cold 0.1 M perchloric acid (50 mg tissue/ml 0.1 M perchloric acid). The homogenate was centrifuged at 1000/g for 20 minutes. The supernatant was prepared for HPLC injection by alumina extraction as described by Bioanalytical Systems [11,12]. One hundred μl of the resulting filtrate were used for analysis.

The concentrations of NE, DOPA, EPI and DA were determined by reverse phase HPLC with electrochemical detection. The HPLC system consisted of an LDC/Constametric III pump, an injection valve (Rheodyne No. 7125) with a 100 μl sample loop, an LC-4A electrochemical detector (Bioanalytical Systems) with a glassy carbon electrode and a Hewlett Packard (3390A) reporting integrator. The electrochemical detector was operated at an electrode potential of +0.7 V. Chromatographic separation was carried out on an IBM C-18 column (column size: 4.5×250 mm, spherical particle size: 5 μm). The mobile phase (pH: 3.05) consisted of 6.9 g $NaH_2PO_4 \cdot H_2O$, 0.75 g Na_2EDTA, 24 mg sodium octyl sulfate and 35 ml acetonitrile in one liter of deionized water, at a flow rate of 1.5 ml/min. At room temperature the absolute retention times of NE, DOPA, EPI, DA, and DHBA (3,4-dihydroxybenzylamine; internal standard) were 5.8, 8.1, 10.6, 24.1 and 12.9 minutes, respectively. The catecholamine/DHBA peak height ratio in comparison to an appropriate standard of known concentration was used to quantify the concentration of each catecholamine in the spleen. The lower limits of detectability for NE, DOPA, EPI and DA were 0.75, 1, 1, and 1 ng/g spleen, respectively.

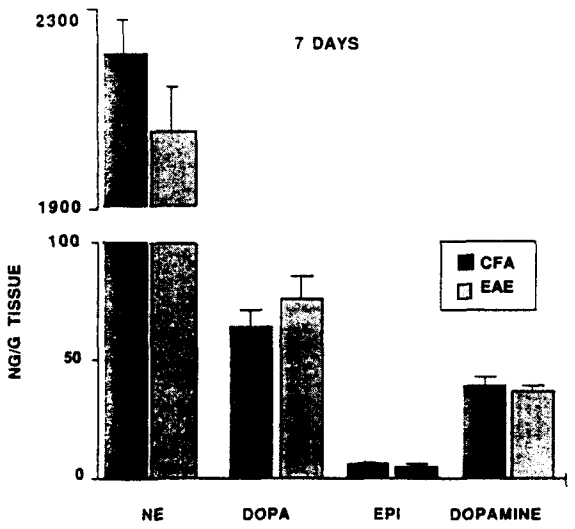

FIG. 1. Norepinephrine (NE), 3,4-dihydroxyphenylalanine (DOPA), epinephrine (EPI) and dopamine concentrations in the spleens of CFA-treated controls and EAE-treated rats, 7 days postinoculation. Each column represents the mean±SEM of tissue samples from 5–6 animals in duplicate determinations. No statistically significant differences were observed.

Statistical Analysis

Results are expressed as mean±SEM. The data were compared using Student's t-test (two-tailed). A level of $p<0.05$ was considered to be statistically significant.

RESULTS

Clinical Course of EAE

Immunization with MBP emulsified in CFA resulted in a time dependent development of clinical symptoms of EAE. On day 7 postinoculation no clinical signs were evident but by days 9–10 the first clinical manifestation of the disease observed was a loss of body weight. By day 14 all EAE rats were severely impaired (clinical scores: 2.7±0.2, mean±SEM) and had lost about 9% of preinoculation body weight, whereas the CFA group had gained about 10% of weight. Resolution of the disease then progressed very rapidly with complete recovery of all clinical symptoms on day 21 postinoculation. A group of 6 EAE rats was observed until day 52 postinoculation. These rats presented no residual clinical signs of the disease.

Neurochemistry

Figures 1, 2, and 3 show the concentrations of NE,

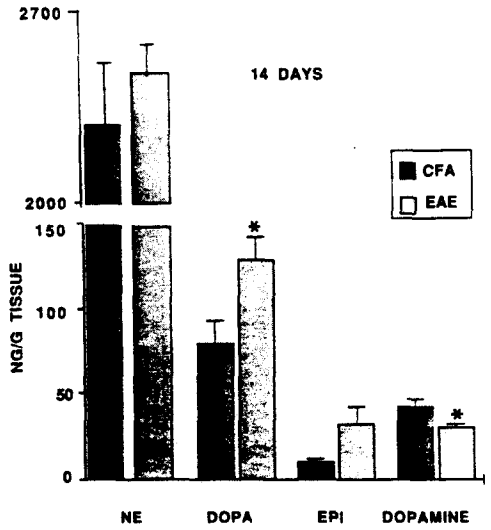

FIG. 2. Norepinephrine (NE), 3,4-dihydroxyphenylalanine (DOPA), epinephrine (EPI) and dopamine levels 14 days postinoculation (peak clinical incidence of EAE) in the spleens of CFA-treated (control) and EAE-treated animals. Results are given as mean±SEM of tissue samples from 5–8 animals in duplicate determinations. *$p<0.03$ as compared with control value by Student's t-test (two-tailed).

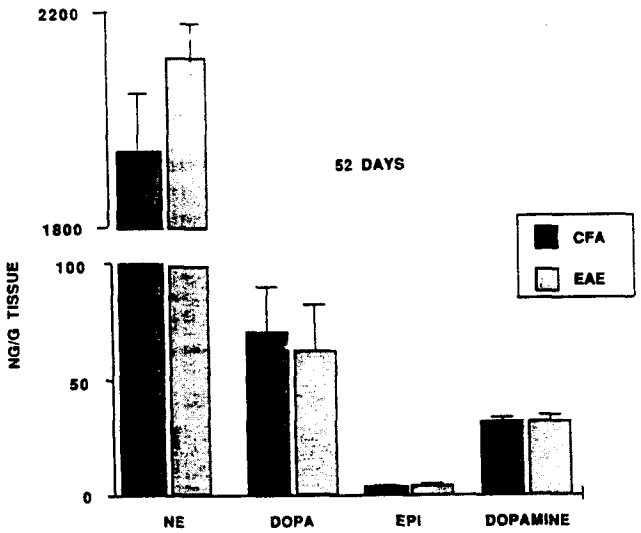

FIG. 3. Norepinephrine (NE), 3,4-dihydroxyphenylalanine (DOPA), epinephrine (EPI) and dopamine concentrations in the spleens of CFA-treated controls and EAE-treated rats, 52 days postinoculation. Each column represents the mean±SEM of tissue samples from 5–6 animals in duplicate determinations. No statistically significant differences were observed.

DOPA, EPI and DA in the spleens of EAE and control rats at 7, 14 and 52 days postinoculation. On day 7, before the onset of clinical symptoms, there was no statistically significant difference in splenic concentrations of NE, DOPA, EPI and DA between EAE rats and CFA-treated controls (Fig. 1). However, at the peak of the clinical manifestation of EAE (day 14 postinoculation) (Fig. 2), the DOPA concentrations were significantly increased (EAE, 128±13 vs. CFA, 79±13; mean±SEM, ng/g tissue; $p<0.03$), while the DA concentrations were significantly decreased (EAE, 30±2 vs. CFA, 42±5; mean±SEM, ng/g tissue; $p<0.03$). The NE and EPI concentrations tended to be elevated in the EAE group but did not achieve statistical significance. On day 52 postinoculation, about one month after the resolution of all clinical symptoms, no differences in splenic catecholamine concentrations between EAE and control animals could be observed (Fig. 3).

DISCUSSION

The present study demonstrates that EAE results in significant changes in the concentrations of certain catecholamines in the spleen which parallel the time course of the clinical symptoms. Neurochemical analysis revealed an increase in DOPA concentrations (+62%) in the spleens of the EAE group 14 days postinoculation, which was accompanied by a decrease in DA concentrations (−29%). One possible explanation of this finding is that DOPA-decarboxylase activity is impaired during the clinical course of EAE. DOPA-decarboxylase activity could be reduced either due to a direct effect of EAE on the enzyme or as a result of reduced availability of the enzyme cofactor, pyridoxal phosphate [6].

Although there was a significant decrease in DA concentrations, EPI and NE concentrations were not decreased in the spleens of EAE animals and actually tended to be slightly higher compared to controls. The reason why the splenic EPI and NE concentrations did not parallel the decrease in concentration of their precursor (DA) may result from several mechanisms: (1) Previous studies have shown that potent decarboxylase inhibitors have very little effect on endogenous NE levels in tissue [6]. (2) As the EAE rats were very sick on day 14 postinoculation, it is possible that the slightly elevated splenic EPI and NE levels were the result of stress induced increases in circulating catecholamines [22]. (3) Furthermore, it might be possible that the release of NE is inhibited during EAE.

The results demonstrate that the dopaminergic system is affected during the peak clinical manifestation of EAE. The increased splenic concentration of DOPA and the decrease in DA concentration at the peak of the clinical signs of EAE may be indicative of a dopaminergic role in the immune response in the spleen. However, it is not clear whether the observed events originate in the spleen and whether they are specific for lymphoid tissue.

The present data contrast with observations in another immunological model. Immunization with sheep red blood cells or continuous environmental antigen exposure results in a significant decrease in splenic NE concentrations [2,7], whereas, in the EAE model, no significant changes in splenic NE levels could be detected. These differences in neurochemical changes could be due to differences in the type of cells mediating the immune response in both models. Further studies have to show whether the changes observed in the splenic catecholamine concentrations of EAE rats also occur in other autoimmune diseases and other T-cell mediated immune responses.

In summary, a significant increase in DOPA and a decrease in DA concentrations have been shown to occur in the

spleen of rats at the peak of the clinical manifestation of EAE. Further work is in progress to elucidate the functional significance of this observation for the clinical course of EAE and the possible mechanism of the observed phenomenon.

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