Inhibition of Efferent Sympathetic Activity in Experimental Allergic Encephalomyelitis (36637)

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Experimental allergic encephalomyelitis (EAE) represents a delayed (cellular) hypersensitivity reaction to nervous tissue and is considered a useful model of human demyelinating diseases (1). Clinical symptoms consisting of irregular gait, flaccid paralysis of hindquarters, urinary incontinence and fecal impaction result from characteristic lesions in the central nervous system.

Previous studies have demonstrated that systemic blood pressure and heart rate are diminished and cardiovascular reflexes and vascular responses to electrical stimulation of the hypothalamus and midbrain are inhibited in rats with EAE (2, 3). On the other hand, peripheral blood vessels respond normally to activation of sympathetic nerves. In view of these findings it was considered of interest to determine if the lesions also influence spontaneous sympathetic outflow and thus affect cardiovascular function.

Methods. Male Lewis strain rats (Microbiological Associates, Bethesda, MD) weighing 150–200 g were utilized. EAE was induced as previously described (2, 3). Briefly, a subplantar injection of 0.05 ml of an encephalitogenic emulsion consisting of syngeneic spinal cord (10 mg) in complete Freund's adjuvant (0.1 mg of *Mycobacterium tuberculosis*) produced maximal symptoms on days 13–15. Experiments were performed at this time.

Animals were anesthetized with Dial–urethane solution [0.6 ml/kg, ip; each milliliter contained allobarbital (100 mg), urethane (400 mg) and monoethylurea (400 mg)] and supplemented with ether during the operative procedure. Artificial respiration with room air was utilized and decamethonium bromide injected (0.75 mg/kg, iv) to eliminate movement. Additional doses were administered during the course of the experiment.

The lumbar sympathetic chains were exposed by a midline abdominal incision. One chain was freed, placed on bipolar platinum electrodes (0.008 in. diam) and cut distally and the area bathed in mineral oil. Nerve activity was amplified by a Tektronix model 122 preamplifier and visualized on a model 502A oscilloscope. Polaroid photographs of tracings were taken. The output of the preamplifier was integrated by a Beckman 9873B and/or 9852 coupler and recorded on an oscillograph (4). "Nerve activity" (expressed in arbitrary units) represents the prevailing signal minus the level observed following crushing of the nerve proximal to the electrode at the end of the experiment. Blood pressure was taken from a carotid artery and norepinephrine (NE) and acetylcholine (ACh) were injected into a jugular vein. Data are presented as means ± standard errors. Fifteen control and 16 EAE animals were used in this study.

Results. Animals afflicted with EAE weighed less than controls (Table I) as in previous studies. Similarly, systemic blood pressure and heart rate were significantly reduced in the EAE group (2, 3).

Spontaneous electrical activity of the lumbar sympathetic chain of representative control and EAE rats is illustrated in Figs. 1 and 2. The signal was also integrated and recorded on an oscillograph. Table I shows that mean spontaneous (base line) nerve activity of EAE rats was approximately one-half of that prevailing in the control group. Two doses of norepinephrine (NE; usually 2 and 4 μg/kg) and two doses of acetylcholine

TABLE I. Body Weight, Heart Rate, Blood Pressure and Sympathetic Nerve Activity.[a]

	Control	EAE
Body weight (g)	237 ± 5	183 ± 6[b]
Heart rate (beats/min)	345 ± 7	278 ±11[b]
Systemic blood pressure (mm Hg)		
Baseline control	105 ± 3	90 ± 3[b]
Response to:		
Norepinephrine		
low dose	+40 ± 3	+38 ± 2
high dose	+63 ± 4	+56 ± 3
Acetylcholine		
low dose	−35 ± 2	−34 ± 1
high dose	−43 ± 2	−46 ± 4
Integrated nerve activity		
Spontaneous baseline	8.9 ± 1.1	4.4 ± 0.8[b]
Response to:		
Asphyxia	+7.6 ± 1.5	+1.1 ± 0.6[b]
Norepinephrine		
low dose	−4.4 ± 0.7	−1.9 ± 0.4[b]
high dose	−5.5 ± 0.8	−2.5 ± 0.5[b]
Acetylcholine		
low dose	+0.3 ± 0.1	+0.6 ± 0.2
high dose	+0.6 ± 0.2	+0.9 ± 0.3

[a] Fifteen control and 16 EAE rats were used. Norepinephrine was usually injected in doses of 2 and 4 μg/kg and acetylcholine at 0.25 and 1 μg/kg.

[b] $p < .05$, group comparison t test.

(ACh; usually 0.25 and 1 μg/kg) were injected iv. These agents produced approximately equivalent changes in pressure in the two groups. As pressure rose following NE injection, nerve activity diminished (Fig. 1). Effects on pressure and nerve activity were dose related. NE also reduced firing in EAE rats but the magnitude of the effect was much smaller (Table I). However, when the reduction is considered relative to the degree of base line activity it is evident that NE produced roughly the same effects in both groups. ACh lowered blood pressure and caused a small augmentation of nerve activity. The magnitude of the latter effect in controls was considerably less in the present series of experiments than previously observed in hypertensive rats (Sprague-Dawley females) (4). Nerve activity also increased slightly during the hypotensive response to ACh in EAE rats. Asphyxia, *i.e.*, cessation of artificial respiration for 60 sec, enhanced nerve activity markedly in control animals. Blood pressure and heart rate fell toward the end of the 60-sec period. Firing also increased in the EAE group but to a much lesser extent.

Discussion. As in previous series of experiments (2, 3) blood pressure and heart rate of EAE rats were reduced relative to controls. The purpose of the present series was to study efferent sympathetic nerve activity. The lumbar sympathetic chain is involved in cardiovascular control since its stimulation causes marked vasoconstriction (2, 3). Further, as seen above, it participates in reflexes. Spontaneous electrical activity of the lumbar chain was considerably reduced in EAE. Levels in afflicted rats were only about one-half of those prevailing in controls indicating that the lesions also affect spontaneous sympathetic outflow. Normal vascular responses to sympathetic chain stimulation occur in EAE animals attesting to the functional integrity of the sympathetic chain and the peripheral nerve-arterial and arteriolar muscle system (2, 3).

Asphyxia for a period of 60 sec resulted in a large increase in nerve firing in controls. However, the augmentation in EAE rats was only very slight showing that the response to this stimulus is also attenuated by the lesions.

The iv administration of NE elevated pressure and reduced sympathetic outflow in both groups. The inhibition was smaller in absolute terms in EAE but, of course, the base line level was also lower. Intravenous ACh reduced blood pressure but resulted in only a very slight increase in nerve activity. The response was considerably less than in previous series (4). The effects in controls and EAE were not significantly different, in marked contrast to the response to asphyxia.

These experiments, therefore, demonstrate that spontaneous sympathetic outflow is reduced in EAE. This inhibiton is at least partially responsible for the reduced blood pressure in the afflicted group.

Summary. Prior experiments indicated that systemic blood pressure and heart rate were

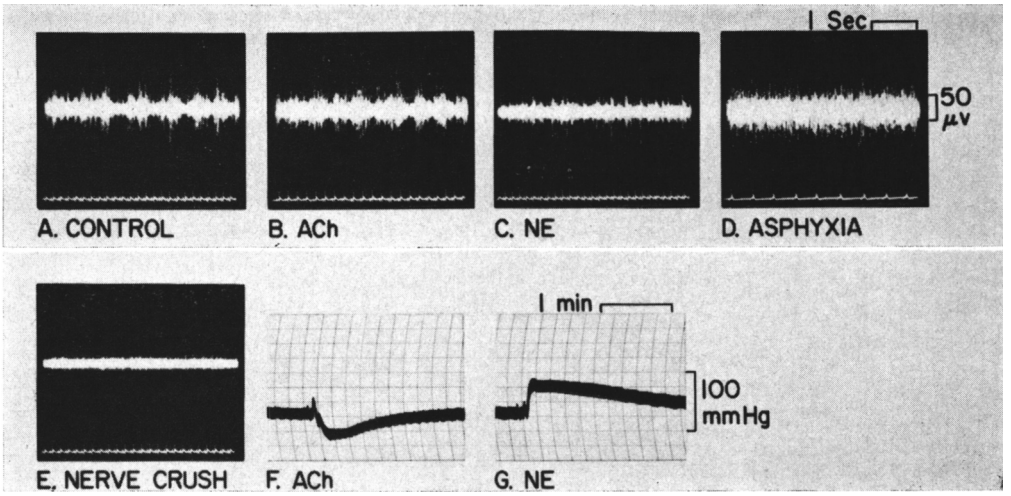

FIG. 1. Sympathetic nerve activity in a control animal. (top) lumbar sympathetic chain activity; (bottom) lead II electrocardiogram; (A) spontaneous nerve activity; (B) increase in firing following the iv injection of 1 μg/kg of acetylcholine [blood pressure trace appears in (F)]. The trace of sympathetic firing was taken as the pressure response approached its maximum. (C) Inhibition of nerve activity during the rapid rise in blood pressure caused by the iv injection of 4 μg/kg of norepinephrine [blood pressure trace appears in (G)]. (D) Nerve activity toward the end of a 60-sec period of asphyxia; (E) tracing after crushing the nerve proximal to the recording site at the end of the experiment.

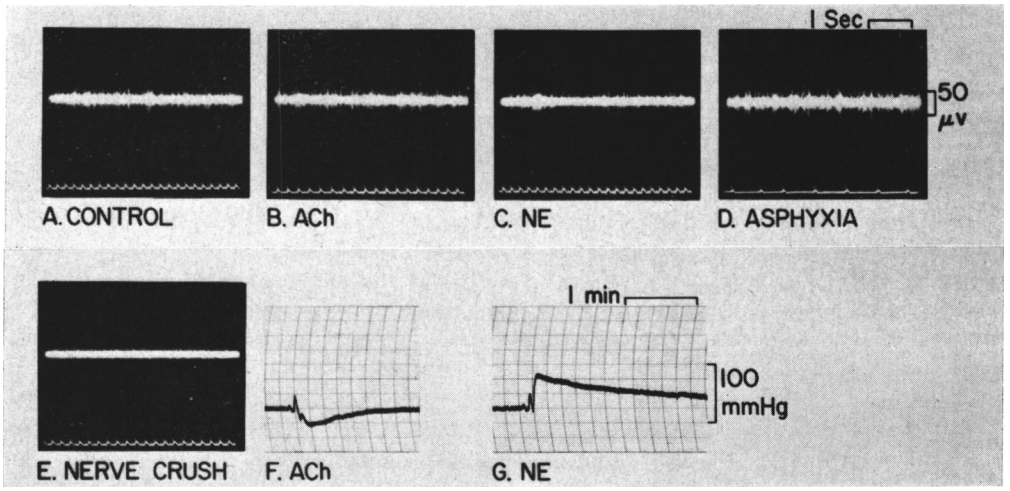

FIG. 2. Sympathetic activity in an EAE rat.

diminished and cardiovascular reflexes attenuated in rats with EAE. The present series demonstrated that spontaneous sympathetic outflow is reduced in afflicted animals and that this most probably contributes to the lower blood pressure.

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