Cardiovascular and Somatic Reflexes in Experimental Allergic Encephalomyelitis

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Experimental allergic encephalomyelitis (EAE) has been induced in a number of species by the injection of heterologous, homologous or isologous nervous tissue incorporated in complete or incomplete Freund's adjuvant.1-3 In rats clinical symptoms occur 11 to 15 days after inoculation and consist of ataxia or paresis, i.e., grossly irregular gait and weakness of one or both hind legs, followed by flaccid paralysis of the hindquarters, urinary incontinence, fecal impaction and weight loss.2,3 Generally, remission of neurological signs and clinical improvement are observed during the third week after inoculation. The central lesions are characterized by the infiltration of lymphocytes and other mononuclear cells into the adventitial sheath (Virchow-Robin space) of small veins and subpial and perivascular parenchyma and by perivenous breakdown of myelin.4-8

Immunologic and pathologic evidence indicates that EAE represents a delayed (cellular or tuberculin) type of reaction to tissue autoantigen and can thus be classified as an experimental autoallergic or autoimmune disease.7-8 EAE is thought to occur as a result of mechanisms by which animals sensitized to a component of nervous tissue tend to reject their own nervous tissue in a manner similar to the rejection of a tumor transplant or skin graft.9 Human demyelinating diseases as a group are characterized by destruction of the myelin sheaths of nerve fibers, the relative sparing of the axis cylinders and the presence of perivenous lesions throughout the brain and spinal cord. Like EAE, the human diseases are thought to have an allergic etiology. EAE has, therefore, been considered a useful model of the human condition.4,10,11

The purpose of the present investigation is to determine whether the paralysis involves somatic reflexes and to ascertain whether the dysfunction extends to the cardiovascular system.

Methods

Male Lewis strain rats (Microbiological Associates, Bethesda, Md.) were used in these experiments. Isologous spinal cord, free from blood and meninges, was homogenized in distilled water to give a 40% weight/volume preparation. Phenol was added to a concentration of 0.5%. An emulsion was prepared from equal volumes of the homogenate and complete Freund's adjuvant* (Difco) in a Virtis homogenizer. Single doses of 0.05 ml of the emulsion were injected into the right hind foot pad in 150 to 200 g rats. Signs of the disease appeared within 11 days and severe paralysis became evident on the thirteenth to fifteenth day after inoculation. Approximately 90 to 95% of the animals developed symptoms of EAE but only severely paralyzed animals (about 65% of the total injected) were used for subsequent experiments.

Animals were anesthetized either with Dialurethane solution† (0.65 ml/kg) or with chloralose (52 mg/kg) and urethane (520 mg/kg) administered intraperitoneally. Isometric contractions of the gastrocnemius-soleus-plantaris group of muscles were recorded from their severed tendons by a Grass model FT03 force transducer and an Offner oscillograph. A steel pin was driven into the head of the femur in order to stabilize the leg. Muscle contractions were induced by single supramaximal pulses (4 to 6 v in normal and 3 to 7 v in paralyzed rats) of 1 msec duration applied to the distal end of the ligated sci-

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* Complete Freund's adjuvant contains Arlacel A (mannide mono-oleate), Bayol F (paraffin oil) and killed dried tubercle bacilli (4 mg/ml).
† Ciba Pharmaceutical Products Company. Each ml contains allobarbital 100 mg, urethane 400 mg, and monoethyurea 400 mg.
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The hindquarters of rats were perfused by a method described by Brody et al.12 The abdominal aorta was exposed by a midline incision and cannulated in central and peripheral directions below the renal arteries. Blood was pumped from the central cannula to the hindquarters by a Sigmamotor pump (model T8). Blood obtained from donor animals was used to fill the tubing. Systemic and perfusion pressures were recorded from T-tubes located proximally and distally to the pump, respectively. The output of the Sigmamotor pump was set at a level at which perfusion pressure approximated systemic blood pressure. Flow remained constant for the remainder of the experiment. Over the range of perfusion pressures encountered, pump output was independent of perfusion pressure; therefore, changes in per-

![Graph](https://example.com/graph.png)

**Response of the gastrocnemius-soleus-plantaris group of muscles to sciatic nerve stimulation in normal and paralyzed rats. Ordinate: isometric tension. Abscissa: initial stretch of muscles. Left: resting and total tension. Lower portion of each bar signifies resting tension and the upper portion indicates active tension. Right: active tension. Top bars were transposed from the left portion of the figure to a common base line on the right. Height of each bar indicates the mean response and the line above it, the standard error.**

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fusion pressure were proportional to changes in vascular resistance. Heparin (10 mg/kg) was used as the anticoagulant. The central ends of the severed cervical vagus nerves were stimulated bilaterally using bipolar electrodes, pulses of 0.5 msec duration, 1 v intensity at a frequency of 50/sec for periods of 10 seconds. The lumbar sympathetic chains were stimulated at low voltages (1.5 or 2.5 v, divided equally in both groups) and high voltage (6 v) with pulses of 1 msec duration at frequencies of 5 to 20/second for periods of 5 seconds. Drugs were injected intra-arterially into the tubing between the Sig- mamotor pump and the hindquarters in volumes of 2.5 and 5 µliters. Base line resistance values were calculated by dividing perfusion pressure (mm Hg) by flow (ml/min). Ten normal and 10 paralyzed rats were used.

Data are presented as means ± standard errors. The Student t-test was used to indicate statistically significant differences. Each rat was used for only one type of experiment.

Results

NERVE-MUSCLE STUDIES

As the gastrocnemius-soleus-plantaris muscles were extended as a group from their "initial lengths" they exerted progressively greater resting tension (fig. 1). The force of muscular contraction resulting from sciatic nerve stimulation (active tension) also increased up to a maximum as muscles were elongated. Maximal active tension was exerted at an increase in length of approximately 12 mm. The resting and active tensions developed by the paralyzed rats were similar to the values obtained for the normal group and no statistically significant differences between groups could be detected at any of the muscle lengths examined (P > 0.05).

Anterior tibial muscles were activated reflexly at elongations of 2 and 3 mm. At these lengths resting tension was 5 ± 1 and 9 ± 1 g, respectively, for normal rats and 3 ± 1 and 6 ± 2 g for the paralyzed group. The differences were not statistically significant (P > 0.05). In normal animals reflex contraction of the muscle was elicited by stimulation of the ipsilateral posterior tibial nerve (flexor reflex, fig. 2). The active tension developed could be increased by stretching the muscle from 2 to 3 mm. On the other hand, reflex contraction of the muscle could not be induced in

![Figure 2](http://circres.ahajournals.org/)

**Figure 2**

Response of anterior tibial muscles to reflex activation and to sciatic nerve stimulation. Ordinate: active isometric tension. Abscissa: initial stretch of muscles. Left: reflexly induced active tension due to ipsilateral posterior tibial nerve stimulation. Reflex stimulation yielded no response in paralyzed rats (indicated by zeros just above base line). Right: active tension induced by sciatic nerve stimulation.

<table>
<thead>
<tr>
<th>TABLE 1</th>
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<p>| Hemodynamic Data from Normal and Paralyzed Rats (means ± SE) |
|---|---|---|---|---|</p>
<table>
<thead>
<tr>
<th></th>
<th>Normal rats</th>
<th>Paralyzed rats</th>
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<tbody>
<tr>
<td><strong>A. Intact rats, sciatic nerve stim. (8)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systemic blood pressure, mm Hg</td>
<td>149 ± 5</td>
<td>119 ± 5 ( ^* )</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>403 ± 9</td>
<td>305 ± 12 ( ^* )</td>
</tr>
<tr>
<td><strong>B. Rats with perfused hindquarters (10)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systemic blood pressure, mm Hg</td>
<td>90 ± 3</td>
<td>83 ± 6</td>
</tr>
<tr>
<td>Perfusion pressure, mm Hg</td>
<td>100 ± 6</td>
<td>95 ± 5</td>
</tr>
<tr>
<td>Flow, ml/min</td>
<td>3.8 ± 0.6</td>
<td>3.3 ± 0.3</td>
</tr>
<tr>
<td>Resistance, pressure/flow</td>
<td>35 ± 6</td>
<td>31 ± 4</td>
</tr>
</tbody>
</table>

\( ^* \)Numbers in parentheses indicate number of rats in each group.

\( ^+ \)P < 0.05, paired comparison t-test.
any of the paralyzed rats. However, stimulation of the motor nerve, i.e., the sciatic, caused the anterior tibial muscles of both groups (compared at 3 mm stretch) to contract with approximately equal vigor ($P > 0.05$, fig. 2). In normal rats sciatic nerve stimulation produced considerably greater active tension than could be induced by reflex activation.

**BLOOD PRESSURE AND HEART RATE**

Blood pressure and heart rate were recorded in eight normal and eight paralyzed rats. These animals were used subsequently for determining the blood pressure response to sciatic nerve stimulation. Pressure and heart rate were found to be significantly lower in paralyzed rats than in controls ($P < 0.05$, table 1, part A).

**CARDIOVASCULAR REFLEXES**

In preliminary experiments it was observed that sciatic nerve stimulation at various frequencies, voltages, and pulse durations consistently reduced blood pressure. At submaximal and maximal voltages a progressively greater depressor response was observed as the frequency of sciatic nerve stimulation was increased from 10 to 20 to 40 pulses/sec (figs. 3, 4). At each point the magnitude of the response was significantly less in paralyzed rats than in the normal group ($P < 0.05$). The intravenous injection of methacholine chloride (0.25 and 1 $\mu$g/kg) also caused a smaller decrease in blood pressure at each dose in paralyzed than in normal rats ($P < 0.05$). The intravenous injection of norepinephrine (1 and 4 $\mu$g/kg) caused an approximately equal increase in blood pressure in both groups.

Bilateral central vagal stimulation increased perfusion pressure in animals whose hindquarters were perfused at constant flow (figs. 5-10).
This response was significantly reduced in paralyzed rats ($P < 0.05$). However, vasoconstriction resulting from stimulation of the lumbar sympathetic chains at various parameters or from the intra-arterial administration of norepinephrine was similar in both groups.

Systemic blood pressure was slightly lower in the perfused paralyzed rats than in controls but not to a statistically significant ex-

![Comparison of the magnitude of systemic blood pressure responses to sciatic nerve stimulation, methacholine and norepinephrine in normal and paralyzed rats.](image)

**FIGURE 4**

Comparison of the magnitude of systemic blood pressure responses to sciatic nerve stimulation, methacholine and norepinephrine in normal and paralyzed rats. Sciatic nerve stimulation at low and high voltage and methacholine reduced blood pressure while norepinephrine increased it. Ordinate: changes of blood pressure in mm Hg. Abscissa: frequencies or intravenous doses. Points represent mean values and lines above or below them, the standard error.

![Vasoconstrictor response to central vagal stimulation, lumbar sympathetic chain stimulation and norepinephrine in the perfused hindquarters of a normal rat.](image)

**FIGURE 5**

Vasoconstrictor response to central vagal stimulation, lumbar sympathetic chain stimulation and norepinephrine in the perfused hindquarters of a normal rat. Top trace in each panel: systemic blood pressure (mm Hg). Bottom trace: perfusion pressure (mm Hg). Left panel: bilateral central vagal stimulation for 10 seconds. Middle and right panels: lumbar sympathetic chain stimulation for five seconds at voltages and frequencies indicated. Right panel also illustrates effects of intra-arterially administered norepinephrine in total doses (µg) indicated.
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Comparison of vasoconstrictor responses to central vagal and lumbar sympathetic chain stimulation and norepinephrine in the perfused hindquarters of normal and paralyzed rats. Ordinate: increase of perfusion pressure in mm Hg. Left panel: response to central vagal stimulation. Middle and right panels: response to sympathetic chain stimulation and to intra-arterial norepinephrine at frequencies or total doses indicated. Mean values and their standard errors are shown.

FIGURE 6

Comparison of vasoconstrictor responses to central vagal and lumbar sympathetic chain stimulation and norepinephrine in the perfused hindquarters of normal and paralyzed rats. Ordinate: increase of perfusion pressure in mm Hg. Left panel: response to central vagal stimulation. Middle and right panels: response to sympathetic chain stimulation and to intra-arterial norepinephrine at frequencies or total doses indicated. Mean values and their standard errors are shown.

Although not studied as extensively analogous responses were obtained with the anterior tibial muscle. On the other hand, reflex contraction of the anterior tibial muscle, a response which involves the participation of the spinal cord, was completely blocked in EAE rats. Thus, it appears that the functions of peripheral motor nerves, the neuromuscular junction, and skeletal muscle are not deficient in EAE but that spinal cord reflexes are inhibited.

The reflex effects of sciatic nerve stimulation on blood pressure were examined at several frequencies and voltages. In normal rats increasing either the frequency or intensity of stimulation caused a progressively greater reduction in blood pressure although the slope of the response curve was not particularly steep. The magnitudes of these effects were significantly less in paralyzed than in normal animals. However, the reduction of blood pressure caused by an intravenously administered depressor agent (methacholine) was also diminished. The reduced responsiveness to methacholine can be attributed, at
least partly, to the lower base line level of blood pressure since it has been demonstrated in other systems that the magnitude of depressor or dilator effects depends upon control pressure.\textsuperscript{13,14} Therefore, in order to obtain a more meaningful contrast, the depressor response to each parameter of sciatic nerve stimulation in each rat was expressed as a percentage of the magnitude of the response to the lower dose of methacholine. The lower dose of methacholine was selected because the magnitude of its effect was more nearly in the range of the depressor response to sciatic nerve stimulation. Although the mechanisms involved in the effects of methacholine and sciatic nerve stimulation are not necessarily the same, we calculated the responses to nerve stimulation as percentages of responses to an internal standard (methacholine) in order to adjust the effects of nerve stimulation to the apparent decreased reactivity of the cardiovascular system in EAE. In normal animals the responses to nerve stimulation at low voltage and frequencies of 10, 20, and 40 pulses/second averaged 59, 65, and 90% and at high voltage 72, 87, and 100% of those caused by methacholine. On the other hand, the percentages obtained in paralyzed rats for the same stimulation parameters were 18, 28, 41, and 29, 32, 48, respectively. In each case the difference between normal and paralyzed animals was significantly different ($P < 0.05$).

Thus, the responses to nerve stimulation were reduced out of proportion to the effects of methacholine in paralyzed rats.

Reflex activation of the sympathetic nervous system was induced by bilateral afferent vagal stimulation. The magnitude of the vasoconstriction in the perfused hindquarters was reduced significantly in paralyzed rats. Only one set of stimulation parameters was used in comparing the results in both groups because qualitatively different responses can be obtained when varying frequencies and voltages are employed. However, additional stimulation parameters were tested in paralyzed rats to make sure that the parameters used for comparison did produce optimal results. Stimulation of the lumbar sympathetic chains caused comparable vasoconstrictor responses in the hindquarters in both groups as did the intra-arterial injection of norepinephrine. These results indicate that the peripheral sympathetic nerves, the release of transmitter material at the nerve endings and the responsiveness of the vascular smooth muscle in the hindquarters function normally in EAE but that reflex responses requiring the participation of the spinal cord and higher structures are inhibited.

The blood pressure and heart rate of animals tested subsequently for response to sciatic nerve stimulation were reduced significantly in paralyzed compared to normal rats. In perfusion experiments basal blood pressure tended toward lower levels in paralyzed rats but in the number of animals studied the reduction was not statistically significant. The vascular resistance in the hindquarters of the two groups was very similar.

It is concluded from these experiments that peripheral nerve-muscle elements in blood vessels and skeletal muscle respond normally in EAE but that spinal cord and possibly higher reflexes are depressed.

**Summary**

Experimental allergic encephalomyelitis (EAE) in rats is characterized by flaccid hindlimb paralysis, urinary incontinence and fecal impaction. Somatic and cardiovascular reflexes were studied in these animals. At varying degrees of stretch of the gastrocnemius-soleus-plantaris group of muscles or of the anterior tibial muscle, stimulation of the sciatic nerve increased the isometric tension of these muscles similarly in normal and paralyzed rats. Reflex contraction of the anterior tibial muscle was produced in normal rats by posterior tibial nerve stimulation. This response was completely blocked in paralyzed rats. Stimulation of the central end of the ligated sciatic nerve at various parameters reduced blood pressure. The magnitude of this effect was less in the paralyzed group. Although vasoconstriction in the perfused hindquarters resulting from bilateral stimulation of the cervical vagus was also reduced in para-
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lyzed rats, the response to lumbar sympathetic chain stimulation was the same in both groups. Thus, in EAE the peripheral nerve-muscle elements in blood vessels and the skeletal muscle responded normally but effects requiring the participation of the central nervous system were depressed.

References


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