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SPREAD OF SWINE HEMAGGLUTINATING ENCEPHALOMYELITIS VIRUS FROM PERIPHERAL NERVES TO THE CNS

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1. ABSTRACT

Swine hemagglutinating encephalomyelitis virus (HEV) strain 67N was inoculated into the sciatic nerve or the right leg crural muscle of rats. In both cases, the virus was isolated first from the caudal half of the spinal cord on day 2 after inoculation, and from the rostral half of the spinal cord and the brain on day 3. The virus titers in the brain reached a maximum when the infected rats developed CNS symptoms on day 5.

Using confocal laser scanning microscope, fluorescent positive cells were first found in the lumbar dorsal root ganglion (DRG) and spinal cord ipsilateral of the inoculated leg on day 3. Antigen positive neurons were found bilaterally in the lumbar DRG and spinal cord on day 4. On day 5 specific fluorescence was observed in the neurons of the cerebral cortex, hippocampus, brainstem and Purkinje cells in the cerebellum.

2. INTRODUCTION

The HEV strain 67N causes encephalomyelitis or vomiting and wasting disease in piglets (Andries et al., 1980; Mengeling et al., 1972; Roe et al., 1958). In experimental oronasal infection of piglets, the virus spreads to the CNS predominantly via the nerve pathways (Andries et al., 1980; Andries et al., 1981). In our experimental studies of HEV

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strain 67N, the virus caused encephalomyelitis in mice when inoculated by intracerebral (i.c.), intranasal (i.n.), intraperitoneal (i.p.) or subcutaneous (s.c.) routes, and was propagated mainly in the nerve cells. However, 20-day-old or older mice were resistant to the virus inoculated by i.n., i.p. or s.c. routes (Hirano et al., 1995). In experimental infections of rats, 4-week-old rats died of encephalitis after intravenous (i.v), i.p. and s.c. as well as i.c. and i.n. routes. However, the rats inoculated by s.c. route died a few days earlier than those i.p. and i.v. inoculated and the virus was isolated only from the spinal cord and the brain, but not from the spleen and liver. By immunohistochemistry, viral antigen positive neurons were found in the spinal cord and brain on day 4. These findings suggest that the virus might spread to the CNS by neural pathways rather than through the blood stream (Hirano et al., 1993).

To follow the virus spread from the peripheral nerve to the CNS, the virus was inoculated s.c. into the foot pad of the right leg. Rats were operated on to cut the sciatic nerve after virus inoculation. The fatal infection of HEV was aborted by cutting the sciatic nerve within 6 hours but not after 12 or more hours. When the virus was directly inoculated into the sciatic nerve, rats were protected from encephalitis by cutting the proximal segment to the inoculated site of the sciatic nerve within 1 hour following inoculation. These studies strongly suggest the crucial role of neural spread of the virus in the induction of fatal encephalitis with HEV in rats (Hirano et al., 1995).

In the present study, we attempted to analyze the virus spread to the CNS from the peripheral nerve after virus inoculation into the sciatic nerve or skeletal muscle using immunohistochemical techniques.

3. MATERIALS AND METHODS

Virus. Plaque-purified HEV 67N strain was propagated and assayed for infectivity in SK-K cells as described previously (Johnson et al., 1965). The infectivity titers were expressed in plaque-forming units (PFU).

Animals and inoculation: Four- to 8-week-old Wistar male rats were obtained from a commercial breeder colony, which was serologically negative for murine coronavirus infections. The virus (1×10^4 PFU/0.02 ml) was inoculated directly into the sciatic nerve under surgical operation, or into the crural muscle of right leg of rats anesthetisized with inhaled halothane.

Infectivity assay of the brain and spinal cord: Ten percent tissue homogenates were prepared from the brain and spinal cord and assayed for infectivity in SK-K cells (Johnson et al., 1965). The spinal cord was divided into 2 pieces, anterior and posterior half for each assay.

Immunohistochemistry: Rats were perfused with a fixative containing 4% paraformaldehyde in 0.1M phosphate buffer (pH 7.4) and the brain and spinal cord were cut out and processed for histopathological and immunohistochemical studies. For histopathological studies, serial sections of the CNS were stained by hematoxylin and eosin (HE). For immunostaining, coronal sections were obtained on a freezing microtome, treated with anti-HEV 67N mouse antibody (1:1000) at 4^0C overnight, and labeled with horse radish peroxidase (HRP)- or FITC-conjugated anti-mouse IgG goat serum at room temperature for 2 hours. HRP-labeled specimens were observed after visualization with the DAB reaction. FITC-labeled specimens were examined under a confocal laser scanning microscope.

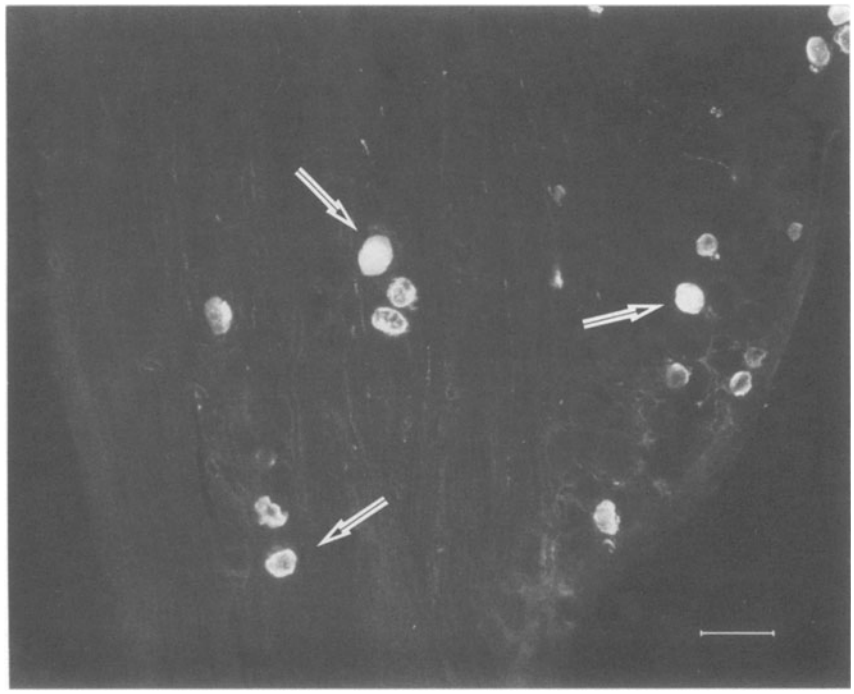

Figure 1. Ipsilateral L4 DRG on day 3 after viral inoculation. Many antigen positive neurons (arrows) are shown. FITC-labeling. Scale bar: 100 μm.

4. RESULTS

Infectivity in the spinal cord and brain: After inoculation with the virus (1×10^4 PFU) into the sciatic nerve or muscle of right leg, the virus was first isolated from the posterior half of the spinal cord at an infectivity titer of 10^5 to 10^6 PFU/0.2g. On day 3 the virus was detectable from the anterior half of the spinal cord and the brain. On day 5 the virus titer in the brain reached a maximum of 10^6 to 10^7 PFU/0.2g when the animals developed CNS signs consisting of ataxia, flapping ears and hypersensitivity. No virus was detected from the spleen, liver, and blood.

By HE staining a few neurons were pyknotic and the typical perivascular infiltration was not found in the spinal cord nor in the brain.

Immunofluorescence: On day 3 after inoculation, specific fluorescence was first found in the lumbar DRG neurons (Fig. 1) and lumbar spinal cord (Fig. 2) ipsilateral to the inoculated right leg. On day 4 antigen positive neurons were bilaterally distributed in the spinal cord, cerebral cortex, hippocampus, cerebellum and brainstem. In the spinal cord many neurons in the ventral and dorsal horn were antigen positive as were neurons in the contralateral DRG. At this time, antigen positive neurons were found in the thoracic and cervical spinal cord but the number of these cells in this area was smaller than in the lumbar spinal cord. In the cerebral cortex, pyramidal cells were infected in both supragranular

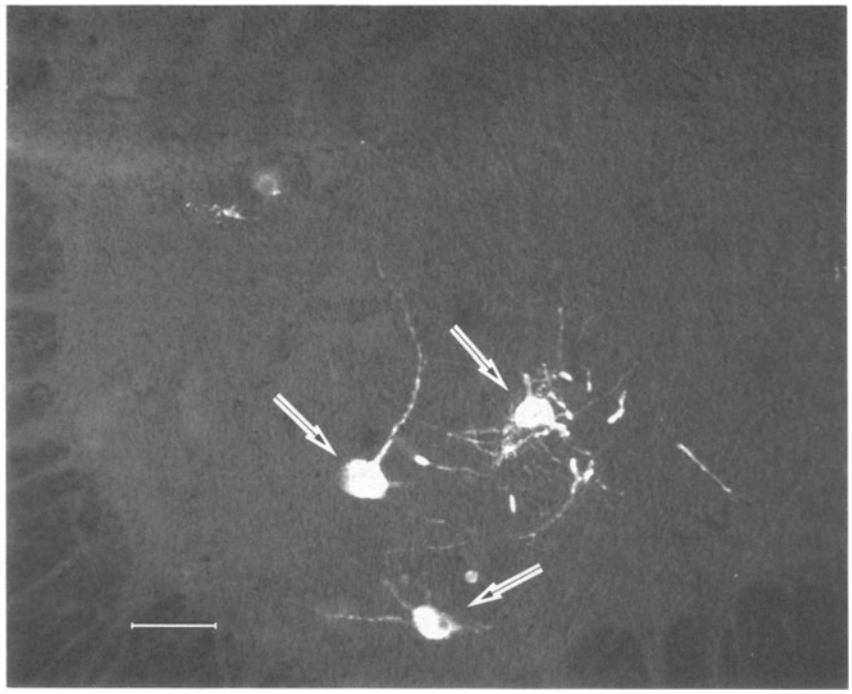

Figure 2. Ventral horn of ipsilateral lumbar spinal cord (L4) on day 3. A couple of HEV-positive motorneurons (arrows) are shown. FITC-labeling. Scale bar: 100 μm.

and infragranular layers, whereas neurons in the layer IV were generally not infected (Fig. 3). Clusters of antigen positive neurons were distributed in a patch-like manner. In the cerebellum, only a few Purkinje cells were antigen positive, but not neurons in the granular and molecular layers (Fig. 4). No fluorescence was detected in the choroid plexus and ependymal cells in the ventricle, and in extraneural tissues.

5. DISCUSSION

In the present study the experimental infection of rats with HEV was characterized by a selective vulnerability of neurons, apparently synchronous development of the virus growth within these neurons with a paucity of histopathological reactions, and a spread of virus from peripheral nerves or extraneural tissues to the CNS along neural pathways. These findings were different from those of HEV infection in rats by i.c. inoculation (data not shown). In rats infected by the i.c. route, virus antigen was widely found not only in neurons in the brain but also in the ependymal cells and in the choroid plexus in the ventricle. In the present study, antigen positive neurons were distributed in a patch-like manner in the cerebral cortex, but the ependymal cells and choroid plexus were negative. These findings suggest that virus infection in the CNS of i.c. inoculated rats might be established by cell to cell spread or via ventricles, and that HEV infection in this study might be established by virus spread through neural connections.

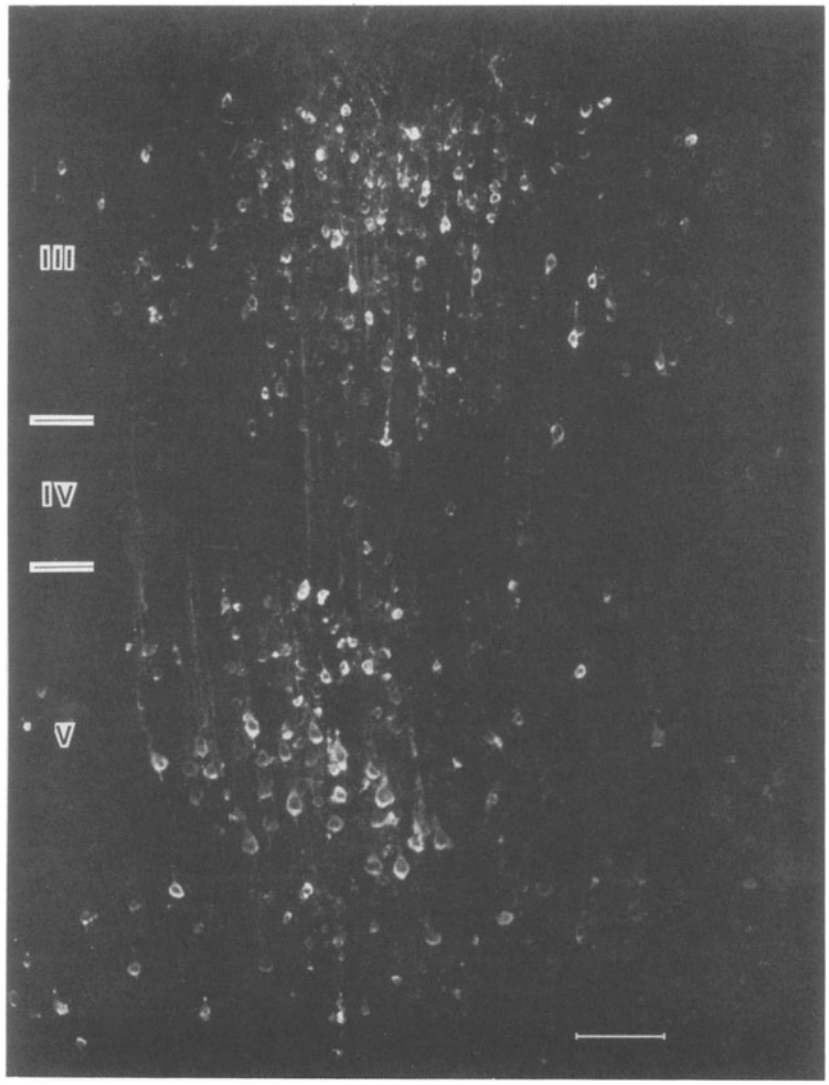

Figure 3. Cerebral cortex on day 4. Clusters of pyramidal neurons in the layer III and V are HEV-positive whereas neurons in layer IV were negative. FITC-labeling. Scale bar: 100 μm.

Our previous experiments showed that the infection of HEV was aborted by cutting the ipsilateral sciatic nerve within 6 hours after s.c. inoculation of the virus into the foot pad (Hirano et al., 1993). Furthermore, when the virus was directly inoculated into the sciatic nerve, only cutting of the proximal, but not the distal segment of the nerve within one hour post-infection prevented the rats from developing encephalitis (Hirano et al., 1995).

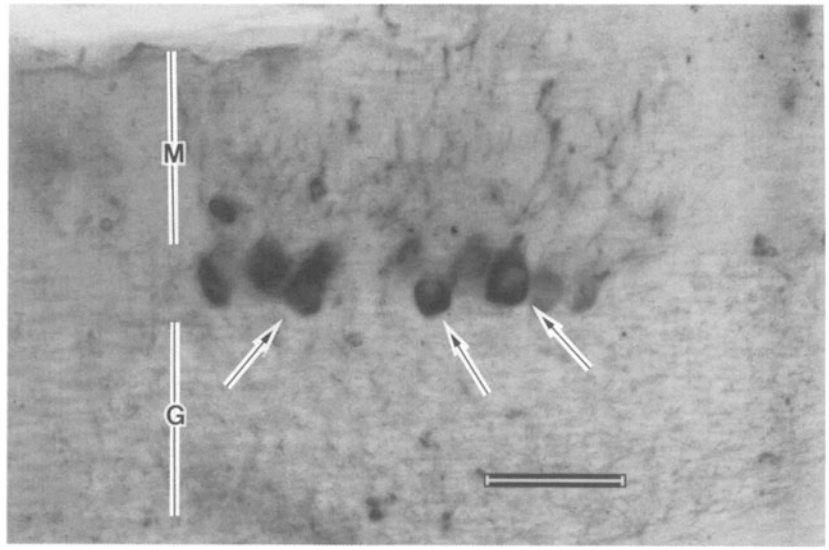

Figure 4. Cerebellar cortex on day 4. A cluster of HEV-positive Purkinje cells (arrows) are shown whereas no antigen positive neurons in the molecular (M) and granular (G) layers. DBA reaction. Scale bar: 50 μm.

In the present study, after virus inoculation into the sciatic nerve or the muscle, the virus was detectable from the lumbar spinal cord on day 2. By immunohistochemistry, specific antigen was found in the lumbar DRG neurons and in the ventral and dorsal horn neurons of the lumbar spinal cord ipsilateral to the inoculated leg on day 3. On day 4, specific fluorescence was also found in the contralateral DRG. On day 5, antigen positive cells were found bilaterally in the thoracic and cervical spinal cord, cerebral cortex and cerebellum. In the present study only neurons were found to be susceptible to HEV infection and, furthermore, only selective neuron populations. This was best demonstrated in the cerebellum where the Purkinje cells were infected, while there was no evidence of infection of any cells in the adjacent molecular or granular layers.

These findings are similar to those of experimental infection of mice with fixed rabies virus by the s.c. route (Roe et al., 1958).

This study clearly demonstrated that the virus spread via nerve pathways to the CNS from the sciatic nerve or extraneural tissue (gatrocnemius muscle) when inoculated directly with HEV, as demonstrated with rabies virus infections in mice (Johnson et al., 1965). From a viewpoint of neuroanatomy, HEV is useful as a transneural tracer for analysing neural connections in the CNS.

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