Activation of Latent Pseudorabies Virus Infection in Mice Treated with Acetylcholine

Seiichi TANAKA and Kazuaki MANNEN

Animal Laboratory Center, Oita Medical University, Hasama-machi, Oita 879-5593, Japan

Abstract: Pseudorabies virus (PrV) YS-81 strain latently infected in 6-week-old BALB/c mice was detectable by nasal swabbing, and serum antibody was shown to increase in titer after intraperitoneal injection for 3 days with acetylcholine or dexamethasone.

Key words: acetylcholine, latent infection, pseudorabies virus

Alphaherpesvirinae is known to persist inactive in infected animals and man mostly within ganglial neurons [1, 15], resulting in long-term infection with recurrent disease. Such latently infected viruses are often reactivated by some stresses [8, 9, 13]. Pseudorabies virus (PrV), a member of the alphaherpesvirinae, causes Aujezsky’s disease (AD) showing acute nervous symptoms in piglets while adult pigs are asymptomatic harboring the virus in the trigeminal ganglia [2, 5, 11, 12]. The latent viruses might be reactivated by stresses such as transportation, change of food, and/or any other disease [20, 21].

Several chemicals have been shown to activate latent viruses [3, 18], and glucocorticoids such as dexamethasone were reported to reactivate latent infection with equine Herpes virus 1 (EHV1) [14] or PrV [20]. Recently, neurotransmitters were proposed as activators of other latent herpes viruses [4, 8], and acetylcholine, a cholinergic neurotransmitter, was shown to reactivate latent PrV in swine [16] as well as in mice [17]. This study investigated the comparable effects of acetylcholine and dexamethasone in latently infected mice.

A PrV wild strain, YS-81, was grown on a porcine kidney cell (PK-15) culture, which was assayed for virus titers using cloned PK cells (CPK) [7]. Cells were grown in Eagle’s minimum essential medium (MEM) containing 5% fetal bovine serum, 1.5% NaHCO3, and 0.1% each of penicillin G potassium, streptomycin sulphate, and kanamycin sulphate. The CPK cells were also used for virus isolation in the acetylcholine reactivating test.

Thirty 6-week-old BALB/c mice purchased from Charles River Japan, Inc., received i.p. injections of 0.25 ml of anti-PrV swine serum showing a neutralizing antibody titer of 1:128, and 30 min later, they were infected i.p. with 100 LD50 of the YS-81 virus. Three of 30 mice died 1 week after challenge infection, and the remaining 27 survived for 2 months and were used as latently infected (LI) mice. The trigeminal ganglia (TG) of all the LI mice were examined for PrV as described in our previous report [17] after completing the experiment and latent infection was confirmed.

Daily for 3 days starting 8 weeks after virus inoculation, 6 LI mice were injected i.p. with 2.73 mg acetylcholine chloride (ACH). Another group of 6 mice received 0.5 mg dexamethasone (DEX) i.p. As controls, phosphate buffered saline (PBS) or sesame oil, the solvents of ACH and DEX, respectively were in-
Table 1. Detection of virus from nasal swabs of latently infected mice after treatment with acetylcholine or dexamethasone

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of mice tested</th>
<th>Days after treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Acetylcholine (2.73 mg)</td>
<td>6</td>
<td>0‡</td>
</tr>
<tr>
<td>PBS (Control)</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>Dexamethasone (0.5 mg)</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>Sesame oil (Control)</td>
<td>4</td>
<td>0</td>
</tr>
</tbody>
</table>

‡ Injected i.p. 2 months after antiserum and virus inoculation. Number of virus-positive mice.

Table 2. Neutralizing antibody titers at 14 days after starting of ACH or DEX treatment

<table>
<thead>
<tr>
<th>Treatment</th>
<th>NT antibody titer</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>Acetylcholine (2.73 mg)</td>
<td>1</td>
</tr>
<tr>
<td>Dexamethasone (0.5 mg)</td>
<td>3</td>
</tr>
</tbody>
</table>

† Injected i.p. 2 months after antiserum and virus inoculation. Number of virus-positive mice.

S. TANAKA AND K. MANNEN

... injected i.p. into 4 mice each. ACH and DEX were purchased from Wako Pure Chemical Industry. Sesame oil was purchased from SIGMA.

Daily after the first injection of the chemicals, nasal swabs were harvested as described previously [6]. Under anesthetization with Nembutal (Dainippon Pharmaceutical, Japan), 100 µL MEM was injected into one of the nasal cavities of the mice, and washing was harvested from the other cavity and mouth with a swab (MENTIP, Japan). The swabs were immersed in 100 µL MEM and stored at -80°C. For virus isolation, CPK cell cultures in 96-well microplates were inoculated with serial 2-fold dilutions of each swab sample, and the cultures were incubated in 5% CO2 at 37°C and checked daily for cytopathic effects (CPE). After incubation for 10 days, the cells were passaged onto another microplates, which were cultured for other 10 days. After three blind passages the viral presence or absence was determined by CPE.

At 14 days after the first injection of chemicals all the animals were sacrificed, and serum samples were examined for neutralizing antibody titers. Serial 2-fold dilutions of serum samples were mixed with the YS-81 virus (200 TCID50/mL). The mixtures were then incubated at 37°C for 1 h and were inoculated on the CPK cell cultures, which were incubated in 5% CO2 at 37°C. All culture wells were examined for CPE on days 7 and 11, and the reciprocal of the highest dilution that inhibited CPE was regarded as the neutralizing titer.

CPE was observed in some CPK cultures inoculated with the swab samples of days 1, 4, 5 and 6, more frequently from the DEX than from the ACH group. No virus was detectable in the control groups (Table 1).

As shown in Table 2, serum samples harvested 14 days after ACH treatment showed low antibody titers (1:2 to 1:16), while all of the DEX group showed higher antibody titers (maximum 1:64). No significant difference was seen in virus titers of the nasal swabs between the 2 chemical groups. No antibody was detectable in the control groups (data not shown).

The DEX [12, 19] and ACH [16] treatments were reported as reactivating latent infection of herpesvirus in swine. PrV latently infecting in the murine TG was shown to be reactivated after ACH treatment [17], and in this study, viral excretion and increased antibody titers were evident after ACH or DEX treatment, as observed in swine [12, 16, 19]. In mice 2.73 mg ACH is toxic, but pigs are resistant to 181.67 mg, though show some salivation, shivering and stomachache which subsides within an hour. In contrast, DEX causes severe toxic effects in swine but not in mice. There might be some species difference in sensitivity to these chemicals, which is of importance in studies on animal models for viral reactivation.

References