The effect of trigeminal nerve and ganglion manipulation on recurrence of ocular herpes simplex in rabbits

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Latent herpes simplex virus (HSV) has been demonstrated in the trigeminal ganglia of experimentally infected rabbits between episodes of spontaneous ocular recurrence. In three experiments reported here, the normal pattern of recurrence was modified by manipulation of the trigeminal nerve and ganglion. Temporary retrobulbar disruption of trigeminal nerve function in chronically infected animals significantly decreased the number of ocular HSV isolations obtained during the 20 weeks immediately following surgery. Stereotaxic interruption of intracranial trigeminal nerve function prior to initial HSV infection dramatically reduced the incidence of peripheral recurrence of HSV. In chronically infected animals, stereotaxic stimulation of the trigeminal ganglion caused a marked increase in positive cultures within 2 days. These studies provide additional evidence for the theory that the reservoir for latent ocular HSV in rabbits is the trigeminal ganglion. Moreover, the studies suggest that the transmission of latent HSV from the trigeminal ganglion to its infectious form in the peripheral tissues involves the trigeminal nerve. We have shown that mechanical and stereotaxic stimulation of the trigeminal ganglion is a reliable and rapid means of precipitating peripheral ocular shedding of HSV on command, a finding which should prove most productive in future research.

Key words: latent herpes simplex virus, ocular herpes simplex, reactivation of herpes infection, rabbit, trigeminal ganglion, stereotaxis, trigeminal nerve.

One of the most problematic aspects of herpes simplex keratitis is the continuing recurrence of disease. In studying this problem, most researchers have used the rabbit as their experimental model. Among other advantages, rabbits exhibit spontaneous recurrences of laboratory-induced ocular herpes simplex infection. Like man, they display periodic shedding of virus in the tear film, whether or not there is clinically recognizable disease.1, 2

The source of recurrent virus shedding remained unknown for many years. Goodpasture,8 in 1929, was the first to suggest that the central nervous system, particularly sensory ganglia, might be the reservoir of latent herpes virus infection. His theory had little experimental support until 1971, when Stevens and Cook4 utilized organ culture techniques to demonstrate...
latent herpes simplex virus in the spinal ganglia of experimentally infected mice. Working with Stevens and Cook, we applied the organ culture technique to the rabbit model of recurrent ocular herpes infection. This was done not only to test the ocular system, but because the mouse does not show the spontaneous recurrence pattern found in human beings. We tested ocular, adnexal, and neural tissues for the presence of latent and nonlatent McKrae strain HSV between recurrences. We never found infectious virus in the trigeminal ganglion or in ocular or adnexal tissue, including the conjunctiva, nictitating membrane, cornea, iris, or lacrimal gland. However, we did isolate latent herpes virus in 73 per cent of trigeminal ganglia, using organ culture methods. Knots and associates confirmed this work with a different strain of HSV. They also detected virus in the fifth nerve nucleus of the brain of 36 per cent of animals. Recently, latent herpetic infection of human sensory ganglia (sacral and trigeminal) has been documented.

On the basis of these data, it seems reasonable to hypothesize that in the rabbit, the trigeminal ganglion acts as the reservoir for latent herpes simplex infection, with its axons functioning as conduits or in some other way to transmit the infection to the peripheral ocular tissues. In this paper, we report the results of our experiments, which further support the role of the trigeminal nerve and ganglion in recurrent ocular herpes simplex in rabbits. The experiments document the patterns of HSV shedding after the following manipulations: (1) temporary retrobulbar disruption of trigeminal nerve function in chronically infected animals, (2) stereotaxic interruption of intracranial trigeminal nerve function prior to initial HSV infection, and (3) stereotaxic stimulation of the trigeminal ganglion in chronically infected animals.

The last-named maneuver is a reliable means of producing ocular shedding of HSV on command. Such a model is needed in order to study the process of herpes reactivation more effectively.

Materials and methods

Male New Zealand albino rabbits (4 to 6 kilograms) were inoculated with McKrae strain HSV (10⁷ primary rabbit kidney plaque-forming units per milliliter) without scarification. The animals were bilaterally infected by applying 0.1 ml. of virus suspension to the eye, then rubbing the closed lids for 30 seconds.

 Conjunctival swab cultures for HSV were inoculated onto primary rabbit kidney cell monolayers as outlined. Procedures for isolation, passage, and neutralization have been previously described.

Production of retrobulbar trigeminal nerve dysfunction (experiment 1). Being careful to avoid widespread damage to other ocular tissues, we produced trigeminal denervation of the eye by retrobulbar electrocautery. An insulated Hy-frecator probe, ½ inch in diameter, was inserted through a 5 mm. long incision at the rostral end of the lower conjunctival cul-de-sac. The probe was guided to engage the foramen, through which pass the orbital branches of the trigeminal nerve. Then high energy diathermy was applied. The success of the procedure was determined by testing corneal and lid sensation. The procedure was carried out in the opposite sham-operated eyes, without the use of electric current. To prevent neurotrophic keratitis in the operated eyes, a lateral one third tarsorrhaphy was performed on all eyes.

Intracranial procedures (experiments 2, 3, and 4). Intracranial manipulations of the trigeminal nerve and ganglion were executed with a standard small animal stereotaxic apparatus with rabbit adaptor (David Kopf Instruments, Tujunga, Calif.). Using the skull sutures as landmarks for alignment, we developed stereotaxic coordinates for the intracranial portion of the trigeminal nerve and ganglion. Anesthesia for these procedures consisted of ketamine hydrochloride 50 mg., azepromazine 15 mg., and scopolamine 0.25 mg. given intramuscularly and pentobarbital sodium (Nembutal) 20 mg. given intravenously. Lesioning or stimulation was accomplished by means of the LM-4 radio-frequency lesion maker (Grass Instrument Co., Quincy, Mass.). In experiment 2, intracranial destruction of the nerve was produced with an Epoxyllo-insulated 22-gauge platinum 10 per cent iridium wire, which had approximately 1 mm. of its tip exposed. Lesions were made by generating 100 to 125 volts of initial current at 6 milliamperes for a duration of 30 seconds. The success of the procedure was assessed by the loss of lid, conjunctival, and corneal sensation. Lateral tarsorrhaphies were performed on all eyes. In experiment 3, the ganglion was thermally stimu-
Table I. Experiment 1: Retrobulbar trigeminal nerve disruption of recurrent ocular shedding of HSV

<table>
<thead>
<tr>
<th>No. of positive HSV cultures/No. of eyes shedding</th>
<th>Operated group (11 eyes)*</th>
<th>Sham-operated group (11 eyes)*</th>
<th>Probability</th>
</tr>
</thead>
<tbody>
<tr>
<td>20 weeks prior to operation</td>
<td>101/9†</td>
<td>93/11</td>
<td>&gt;0.4</td>
</tr>
<tr>
<td>20 weeks after surgery</td>
<td>17/9</td>
<td>62/10</td>
<td>0.001</td>
</tr>
<tr>
<td>20-40 weeks after surgery</td>
<td>29/9</td>
<td>30/9</td>
<td>0.5</td>
</tr>
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</table>

*Eleven animals were used. One eye was operated and the other sham operated.
†Each entry represents the number of positive isolations from 1,320 cultures taken (11 eyes, 6 days per week for 20 weeks) over the number of eyes shedding virus out of the 11 eyes being cultured.

lated by stereotaxic application of low-voltage current through the 22-gauge electrode, by direct injection of 50 ng. of 1:1000 aqueous adrenaline with a No. 22 microsyringe, or most frequently, by blunt mechanical trauma with a 14-gauge solid trochar. The last-named procedure was chosen for trigeminal stimulations in experiment 4. In sham-operated animals, the stereotaxic probe was brought within 2 mm. of the trigeminal ganglion and no other stimulus was given.

Results

Experiment 1: Retrobulbar destruction of the trigeminal nerve. In 18 animals displaying chronic bilateral shedding of herpes virus, ocular trigeminal nerve function was interrupted on one side by retrobulbar electrocautery. The other eye was sham operated. Eleven animals survived for the 60 weeks of the experiment. Only four eyes manifested complete loss of corneal, conjunctival, and lid sensation, despite our aggressive attempts to destroy nerve function. Although the other seven lost corneal sensation, they retained variable lid and conjunctival sensitivity. Sensation in all animals had returned to normal 20 weeks after surgery.

In Table I, results of daily (6 days per week) conjunctival swab cultures for HSV are shown. During the control period, 20 weeks prior to operation, the number of positive cultures was essentially the same in all eyes. In the 20 weeks immediately following surgery, decreased sensation on the lesioned side was accompanied by a strikingly significant decrease in positive cultures. On the lesioned side, there were 17 positive cultures, as compared to 62 positives on the sham-operated side (p = 0.001). When trigeminal nerve function returned, 20 to 40 weeks after surgery, the number of cultures was equal in both groups.

Those eyes in which temporary trigeminal dysfunction was complete showed a more striking decrease in the number of isolations of HSV than those in which dysfunction was partial. These findings lend support to the hypothesis that nerve integrity is necessary for virus shedding.

Experiment 2: Effect of interruption of trigeminal nerve prior to infection on HSV recurrence rate. In this stereotaxic experiment on previously uninfected animals, we made unilateral intracranial destructive lesions in the trigeminal nerve just anterior to the ganglion. The opposite side was sham operated. Two weeks later, survivors were bilaterally infected with McKrae strain HSV. Acute infection, as monitored by culture and slit-lamp biomicroscopic examination, was identical both in the eyes with and without trigeminal innervation.

Beginning the third week after infection, conjunctival swab cultures to document HSV shedding were taken 6 days per week. Results are shown in Table II. The 13 rabbits with unilateral loss of trigeminal sensation at the start of culturing showed only 10 positive HSV cultures, compared to 67 positive cultures from the opposite, sham-operated eyes. Statistically, this result is highly significant (p = 0.001). Interestingly, in the two operated eyes in which we failed to produce any peripheral loss of ocular sensation (i.e., trigeminal remained intact), nine of 65 cultures (13.8 per cent) were positive. These results are essentially indistinguishable from those in the sham-operated eyes in which nerve function was not altered.
Experiment 3: Results of stereotaxic trigeminal stimulation on HSV shedding.
This was a pilot experiment in which stereotaxic procedures were carried out bilaterally on infected animals, between episodes of spontaneous ocular shedding of HSV. Eighteen ganglia were stimulated in one of three ways. Nine were mechanically traumatized with a 14-gauge blunt trochar, six were thermally stimulated with low-voltage radio-frequency current, and the remaining three were chemically stimulated with locally delivered aqueous epinephrine. Twelve sham-operated ganglia served as controls. Just prior to stimulation, all eyes were negative for HSV for at least 3 weeks and were clinically negative. After operation, eyes were cultured daily for 12 days.

The most effective means of eliciting HSV shedding, mechanical trauma, resulted in 35 per cent positive cultures during the 12-day test period. Adrenaline caused 28 per cent positive cultures, and thermal stimulation produced 20 per cent. Sham operation elicited only 12 per cent positive cultures.

In Table III, the combined results of all three methods of stimulation are compared with those from the sham-operated animals. In eyes in which the ganglion was stereotaxically stimulated, there was a statistically significant (p = 0.01) increase in positive cultures. The response to the stimulus was surprisingly rapid. Within 2 days, the first positive cultures appeared in six of 18 eyes of the stimulated group, but in none of the 12 shams. In addition, stimulated ganglia produced a longer sequence of shedding. This was a pilot experiment using bilateral stimulation of the various types mentioned.

Experiments 4A, 4B, and 4C: Effect of unilateral mechanical stereotaxic trigeminal ganglion stimulation on HSV shedding. In experiment 4A, a more rigorous protocol was followed. Between recurrences, bilaterally infected animals were stimulated on one side only, with the mechanical stimulus exclusively. This experi-

Table II. Experiment 2: HSV isolation in eyes infected after stereotaxic lesion of the intracranial portion of the trigeminal nerve

<table>
<thead>
<tr>
<th></th>
<th>Operated</th>
<th>Sham</th>
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<tbody>
<tr>
<td>No. of eyes yielding virus/No. of eyes cultured</td>
<td>6/13</td>
<td>11/13</td>
</tr>
<tr>
<td>No. (and percentage) of positive cultures of 585 taken in each group</td>
<td>10* (1.7%)</td>
<td>67* (11.5%)</td>
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</table>

*The difference is chi-square significant at the p = 0.001 level.

Table III. Experiment 3: Stereotaxic trigeminal ganglion stimulation in latently infected rabbits

<table>
<thead>
<tr>
<th>HSV isolations/total cultures taken in 12 days after surgery</th>
<th>Stimulated operated</th>
<th>Sham operated</th>
<th>Chi-square probability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eyes showing positive cultures within 2 days of operation</td>
<td>6/18</td>
<td>0/12</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Eyes showing more than 5 positive cultures in 12 day test period</td>
<td>7/18</td>
<td>1/12</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

mental design was used to test for a possible "cross-over effect" which might occur if stimulus on one side induced shedding in the opposite eye.

Preoperatively, the animals were divided into matched groups by analysis of virus shedding during the 8 months prior to manipulation. One trigeminal ganglion was mechanically stimulated in each of six animals. Five animals were unilaterally sham operated. Another five were not operated on at all. After the operations, the animals were cultured each day for 12 days. Statistical analyses during the 12 day test period showed that the sham-operated and unoperated animals were equivalent, both on the operated and unoperated sides. However, as Table IV illustrates, both eyes of unilaterally, mechanically stimulated animals showed a statistically significant (p = 0.01) increase in ocular HSV shedding. Thus, this manipulation produced both a direct and a cross-over effect, although the cross-over effect was less intense.
Table IV. Experiment 4A: Unilateral stereotaxic trigeminal ganglion stimulation in latently infected rabbits

<table>
<thead>
<tr>
<th></th>
<th>Stimulated (6 animals)</th>
<th>Sham (5 animals)</th>
<th>Control (5 animals)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of positive HSV cultures within 12 days/No. of eyes yielding virus</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Experimental eyes</td>
<td>25*/(4/6)†</td>
<td>7/(2/5)</td>
<td>10/(3/5)</td>
</tr>
<tr>
<td>Opposite eyes</td>
<td>16*/(3/6)</td>
<td>2/(1/5)</td>
<td>2/(1/5)</td>
</tr>
<tr>
<td>Total of both groups</td>
<td>41*/(7/12)</td>
<td>9/(2/10)</td>
<td>12/(4/5)</td>
</tr>
</tbody>
</table>

*Chi-square significant at p = 0.01 level comparing stimulated to sham and/or control groups.
†Numbers in parentheses are eyes yielding positive HSV cultures/number of eyes in the group.

Table V. HSV isolations from both eyes of unilaterally stimulated animals during 12 day test period

<table>
<thead>
<tr>
<th></th>
<th>Experiment 4B*</th>
<th>Experiment 4C†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Original stimulated group (6 animals)</td>
<td>0/113</td>
<td>0/136</td>
</tr>
<tr>
<td>Original sham-operated group (5 animals)</td>
<td>9+/110‡</td>
<td>1+/115</td>
</tr>
<tr>
<td>Original control group (5 animals)</td>
<td>0/92</td>
<td>6+/112‡</td>
</tr>
</tbody>
</table>

*Experiment 4B was carried out 7 weeks after experiment 4A. All three “original” groups were cultured. Only the five animals in the original sham group were stimulated. Experiment 4C was carried out 5 weeks after experiment 4B. All three “original” groups were cultured. Only the five animals in the original control group were stimulated.
‡Difference between these groups and the combined results of the other groups in each column is Chi-square significant at p = 0.01 level.

In the stimulated animals, the rapidity of response to stimulus was good in both eyes. In mechanically traumatized animals, 33 per cent (4/12) of eyes yielded HSV within 40 hours, as compared to 10 per cent (2/20) of the eyes in the sham and control animals. A long sequence of positive cultures (five or more out of 12 cultures) was more frequently encountered in mechanically stimulated animals (42 per cent), compared to the sham and control groups (10 per cent).

After the experimental period ended, to demonstrate that mechanical manipulation really was associated with HSV shedding, we subsequently stimulated the sham and then later the control animals of experiment 4A. The original stimulated group served as controls. These results are shown in Table V. After a period of 7 weeks, the original sham-operated animals were subjected to unilateral stereotaxic mechanical stimulation in experiment 4B. Five weeks after this, in experiment 4C, the original control animals were similarly treated. In each case, mechanical stimulation elicited a small, but statistically significant number of HSV sheddings, compared to the animals which were not manipulated.

Discussion

The results of all these experiments are consistent with the neuronal hypothesis, which proposes that the trigeminal ganglion and nerve are somehow involved in the recurrent shedding of infectious virus at the eye. In the retrobulbar trigeminal disruption experiments, viral recurrences at the eye decreased when nerve function (sensation) was impaired, returning to normal when the nerve function was restored. When trigeminal function was damaged before HSV infection, recurrences on that side were only 10 per cent of normal. It is possible that recurrences were not completely eliminated, because some branches of the nerve remained, allowing virus to reach and parasitize the ganglion. Or, perhaps other mechanisms or other ganglia such as the superior cervical or ciliary ganglia may account for some HSV recurrences. Worthy of note is the fact that acute HSV infection (dendritic keratitis) in denervated eyes was indistinguishable from that in the normal eyes. This observation substantiates the proposition that nerve supply is not important in the morphogenesis of dendritic figures.

If the neuronal hypothesis is correct, manipulation of the trigeminal ganglion ought to effect the shedding of HSV at the eye. In fact, these experiments utilizing stereotaxic trigeminal stimulation do precipitate a rapid shedding of virus. In 48 hours, 33 per cent of eyes shed HSV (experiments 3 and 4A). Experiments 4B and 4C gave further proof that mechanical
stimulation was associated with shedding of ocular HSV.

Based on these findings, a direct neurosurgical approach for the stimulation of the trigeminal ganglion was then developed. It has improved the percentage of eyes responding to mechanical stimulation. It will facilitate more effective study of peripheral HSV shedding following central nervous system stimulation.\(^1\)

Further work is underway to explain two unanswered questions—how mechanical stimulation results in enough virus shed at the eye to produce positive cultures within 48 hours, and what is the source of the cross-over effect.

We thank Dr. Earl Bogdanoff for statistical analyses.

REFERENCES