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HSV-1 Not Only in Human Vestibular Ganglia but Also in the Vestibular Labyrinth

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Key Words
Vestibular neuritis · Herpes simplex virus type 1 · Vestibular ganglia · Labyrinth · Benign paroxysmal positional vertigo

Abstract
Reactivation of herpes simplex virus type 1 (HSV-1) in the vestibular ganglion (VG) is the suspected cause of vestibular neuritis (VN). Recent studies reported the presence of HSV-1 DNA not only in human VGs but also in vestibular nuclei, a finding that indicates the possibility of viral migration to the human vestibular labyrinth. Distribution of HSV-1 DNA was determined in geniculate ganglia, VGs, semicircular canals, and macula organs of 21 randomly obtained human temporal bones by nested PCR. Viral DNA was detected in 48% of the labyrinths, 62% of the VGs, and 57% of the geniculate ganglia. The potential significance of this finding is twofold: (1) Inflammation in VN could also involve the labyrinth and thereby cause acute unilateral vestibular deafferentation. (2) As benign paroxysmal positional vertigo often occurs in patients who have had VN, it could also be a sequel of viral labyrinthitis.

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Introduction

The reactivation of latent herpes simplex virus type 1 (HSV-1) in human geniculate ganglia (GGs) and vestibular ganglia (VGs) is the assumed etiology of Bell's palsy and vestibular neuritis (VN) [Schuknecht and Kitamura, 1981; Furuta et al., 1993; Adour et al., 1996; Murakami et al., 1996]. It is widely accepted that after primary infection (stomatitis herpetica) HSV-1 ascends to associated sensory ganglia by retrograde axonal transport, e.g., via chorda tympani in the GG and via faciovestibular anastomosis to the VG [Bergström, 1973; Whitley, 1988]. A recent study reported the presence of HSV-1 not only in VGs but also in vestibular nuclei, thus indicating that viral migration can also occur via the vestibular nerve to the pontomedullary brainstem [Arbusow et al., 2000] (fig. 1). This finding raises the question of whether the virus could also migrate from the VGs to the peripheral receptors in the semicircular canals (SCCs) and the otolithic organs. Viral inflammation could also involve the peripheral labyrinth and thereby cause acute unilateral vestibular dysfunction typical of VN. To determine the distribution of HSV-1 DNA in VGs, GGs, SCCs, and macula organs (utricle and saccule), 21 randomly obtained temporal bones of 11 individuals were examined for HSV-1-specific DNA by nested polymerase chain reaction (PCR).

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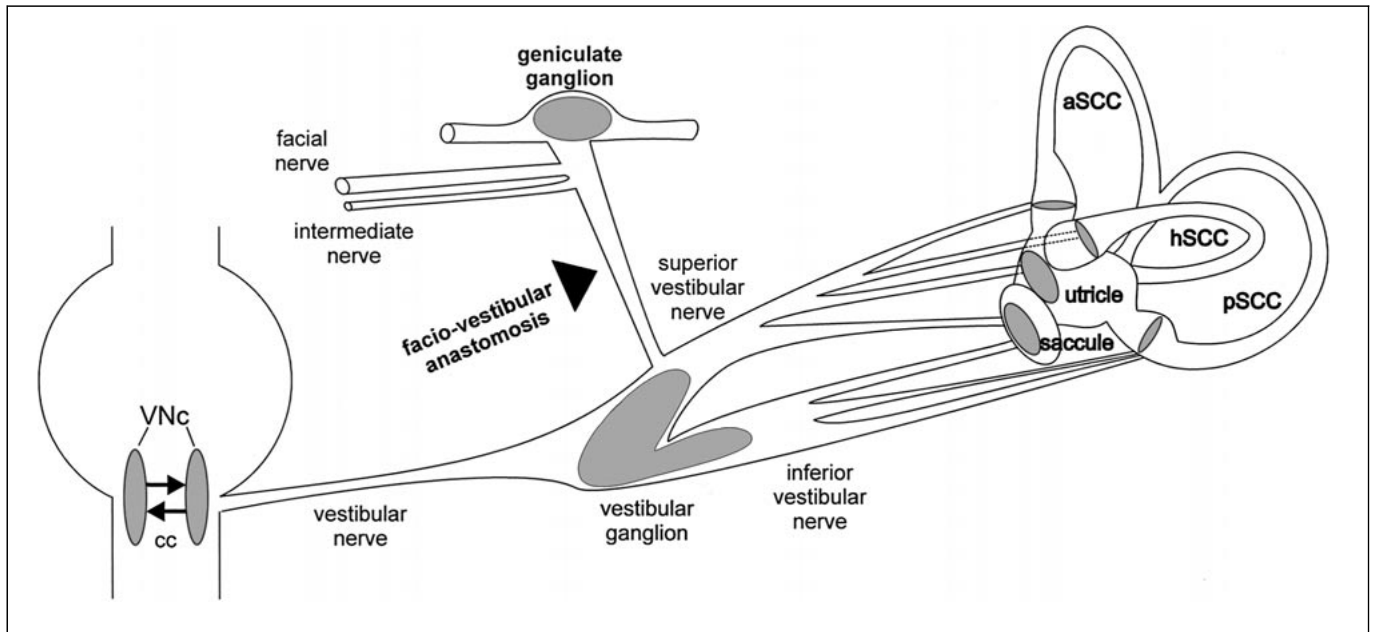

Fig. 1. After primary infection (stomatitis herpetica) HSV-1 ascends to the geniculate ganglion (GG) via chorda tympani and via the faciovestibular anastomosis to the vestibular ganglion (VG). Viral migration to the vestibular nuclei (VNc) and the human labyrinth is possible along the vestibular nerve. aSCC, hSCC, pSCC = Anterior, horizontal, and posterior semicircular canals; cc = commissural connections.

Table 1. Distribution of HSV-1-specific DNA in human VGs, GGs, and vestibular labyrinth (VL; semicircular canals, utricle, and saccule)

Temporal bone No.	Age	Sex	GG	VG	VL
1	42 years	f	+	–	–
2	4 months	f	–	–	–
3	4 months	f	–	–	–
4	53 years	f	+	+	–
5	53 years	f	+	+	–
6	37 years	m	–	+	+
7	37 years	m	–	+	+
8	27 years	m	–	–	–
9	27 years	m	+	–	–
10	58 years	f	–	–	–
11	58 years	f	+	–	–
12	43 years	m	+	+	+
13	43 years	m	+	–	–
14	35 years	m	+	+	+
15	35 years	m	+	+	+
16	35 years	f	–	+	+
17	35 years	f	–	+	+
18	35 years	m	–	+	–
19	35 years	m	+	+	+
20	50 years	m	+	+	+
21	50 years	m	+	+	+
Positives			12/21	13/21	10/21

Materials and Methods

The Ethics Committee of the Medical Faculty of the Ludwig Maximilians University of Munich approved the study. Twenty-one temporal bones of 11 individuals, obtained from the Institute of Forensic Medicine of the University of Munich, were dissected during autopsy and immediately stored at –20 °C. In all cases the cause of death was neither natural nor related to cranial nerve dysfunction. None of the individuals had had a history of central or peripheral vestibular vertigo, VN, benign paroxysmal positional vertigo, or Ménière's disease. Viral infections (human immunodeficiency virus; hepatitis C virus, hepatitis B virus, herpes simplex encephalitis) and immunosuppression were also excluded. As 80–90% of healthy adults have antibodies against HSV-1, the sera of the individuals were not checked for HSV-1-specific antibodies. Age distribution ranged from 4 months to 58 years (6 males and 5 females). The bony surface (from the entrance of the intrameatal duct to the GG on the opposite side of the temporal bone) was removed with a binocular and an electric drill until the dura that surrounds the vestibulocochlear and facial nerves was exposed. After the dural sac was opened, the VG and the facial and cochlear nerves were also removed. Then, the stapes was separated from the fenestra vestibuli, which was carefully opened and widened with a microdrill. Next the temporal bones were split into two halves along the internal auditory canal through the middle of the vestibulum with surgical scissors: one half contained the cochlear and the other the labyrinthine structures. Finally, a microforceps was used to carefully remove the SCCs, utricle, and saccule of each labyrinth from the bony structures (fig. 2a, b) and to pool them for DNA extraction (QUIAamp® DNA mini kit; Qiagen).

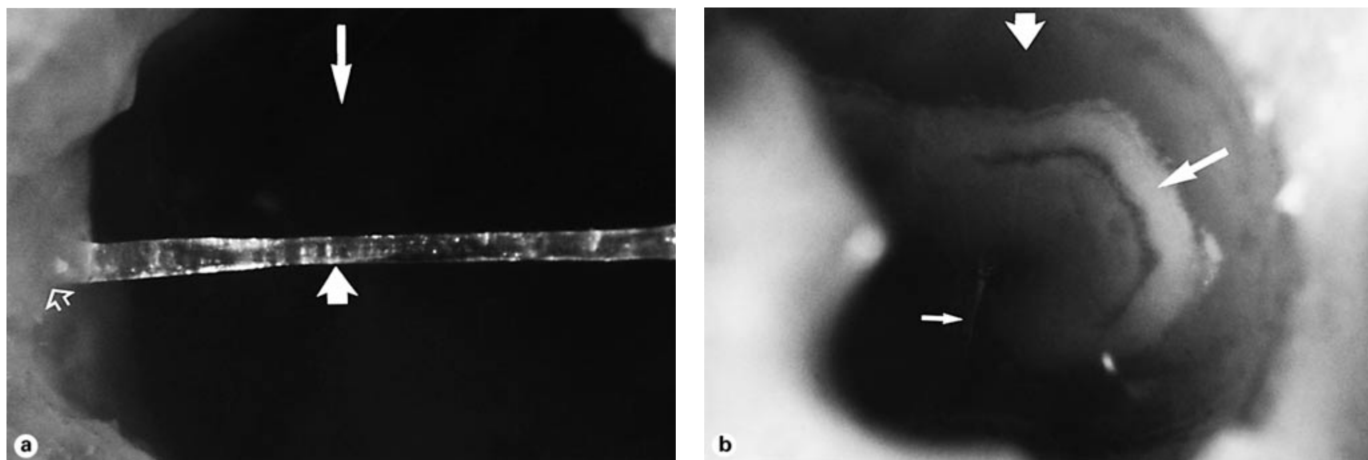

Fig. 2. a Human posterior SCC (short arrow) drawn out of its bony canal (open arrow); vestibulum (long arrow). **b** Macula utriculi (long arrow) in the human vestibulum (bold arrow); ductus utricularis (small arrow).

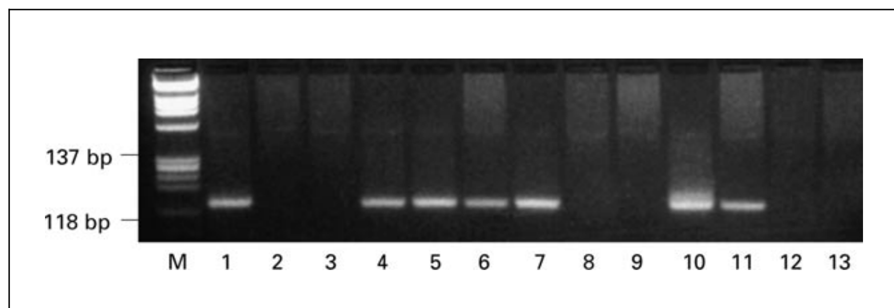

Fig. 3. HSV-1-specific PCR products (137 bp) from 10 human labyrinths. Lane M = Molecular weight marker; lane 1 = positive control; lane 12 = human blood DNA (negative control); lane 13 = water control; lanes 2 and 3 = labyrinths No. 2 and 3; lanes 4–7 = labyrinths No. 14–17; lanes 8–11 = labyrinths No. 14–17; lanes 8–11 = labyrinths No. 4–7.

Nested PCR for HSV-1 glycoprotein D gene was carried out according to the method described by Aurelius et al. [1991]. The sequences of the primers and their location in the glycoprotein D gene of HSV-1 were as follows: (1) outer 5′-primer (position 19–43): ATCACGGTAGCCCGGCCGTGTGACA; (2) outer 3′-primer (218–239): CATACCGGAACGCACCACACAA; (3) inner 5′-primer (51–71): CCATACCGACCACACCGACGA, and (4) inner 3′-primer (166–188): GGTAGTTGGTCGTTCGCGCTGAA.

All reagents and the equipment for PCR were purchased from Biometra, Göttingen, Germany. All DNA samples were amplified 25 times with the outer and then 25 times with the inner primers. The temperature profile was 30 s at 95 °C, 30 s at 60 °C, and 1 min at 72 °C. PCR products were analyzed on 3% agarose gels (fig. 3). To confirm the presence of amplifiable DNA in the nucleic acid preparations, a nonpolymorphic mitochondrial fragment of 530 base pairs was demonstrated in a parallel PCR [Schulz et al., 1998]. Human blood DNA from a healthy individual and a water control were included as negative controls for each PCR run. To exclude false positives, all positive reactions were repeated at least once.

Results

HSV-1 DNA was detected in 10 of the 21 vestibular labyrinths (48%). Four individuals showed bilateral HSV-1 infection of the labyrinth. HSV-1 infection of the labyrinth was always associated with the detection of HSV-1 DNA in the ipsilateral VG. In only 3 of 13 cases was HSV-1 DNA found in the VG but not in the ipsilateral vestibular labyrinth (table 1). Frequency and distribution of HSV-1 DNA among the VGs (13/21; 62%) and GGs (12/21; 57%) did not differ substantially from findings in earlier studies [Furuta et al., 1993; Schulz et al., 1998; Arbusow et al., 1999]. In 5 cases, HSV-1 infection of the VG was not associated with infection of the GG. As in other recent studies [Schulz et al., 1998; Arbusow et al., 1999, 2000], bilateral infection was a common finding; it was detected in the VGs of 6 and in the GGs of 4 individuals. As viral infections (human immunodeficiency virus; hepatitis C virus, hepatitis B virus, herpes simplex encephalitis) and immunosuppression were excluded in these indi-

viduals, bilateral involvement of the cranial nerve ganglia did not indicate a severe underlying disease.

Discussion

Several lines of evidence support a viral hypothesis of VN, for example temporal bone pathology in patients with VN [Schuknecht and Kitamura, 1981], its epidemic occurrence in certain periods of the year [Silvoniemi, 1988; Sekitani et al., 1993], and the common detection of HSV-1 DNA in human VGs and central vestibular nuclei [Furuta et al., 1993; Arbusow et al., 1999, 2000]. This is the first demonstration of HSV-1 DNA in the human SCCs and otolithic organs. It suggests that oculomotor signs in acute VN (horizontal rotatory spontaneous nystagmus to the nonaffected ear) may not be exclusively caused by viral inflammation of the superior vestibular nerve [Strupp and Arbusow, 2001] but also be a sequel of viral inflammation of the peripheral vestibular labyrinth.

Benign paroxysmal positional vertigo occurs relatively often in patients who have had VN (about 12%) [Büchele and Brandt, 1988]; it may also be induced by viral labyrinthitis. Inflammation of the labyrinth could lead to a loosening of otoconia, which enter the SCCs causing canalolithiasis and subsequently benign paroxysmal positional vertigo.

As there was no single case in which HSV-1 DNA was detected in the human labyrinth but not in the associated VG (table 1), a centrifugal viral migration from the VG to the peripheral vestibular labyrinth seems more likely than primary viral infection of the labyrinths (e.g., via fenestra vestibuli in the middle ear). Also the finding of 5 cases showing HSV-1 infection of the VG without involvement of the ipsilateral GG (table 1) does not necessarily exclude viral spreading from the GG to the VGs via faciovestibular anastomosis. The absence of viral DNA in the GG could be due to reactivation and subsequent clearance of the virus, as a result of virus- or immune-mediated destruction of the previously infected neurons [Whitley, 1988]. However, it is also possible that HSV-1 may first infect the VG, e.g., via infected T lymphocytes or via the vestibular labyrinth, and then secondarily migrate to the GG [Arbusow et al., 2000].

In conclusion, this study showed that HSV-1 DNA is a common finding not only in human VGs but also in the labyrinth. The pattern of viral distribution in this study does not allow us to determine a specific route of viral spreading. Several mechanisms are possible to explain the pattern of viral distribution: (1) viral migration along anastomoses, (2) hematogenic infection by HSV-1-infected T lymphocytes, and (3) direct infection of the vestibular labyrinth (e.g., via fenestra vestibuli in the middle ear). The next logical step is to determine the exact cellular location of HSV-1 in the human vestibular system by using in situ PCR methods that amplify viral DNA and RNA.

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