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Transsynaptic neuronal loss induced in hippocampal slice cultures by a herpes simplex virus vector expressing the GluR6 subunit of the kainate receptor

PETER J. BERGOLD*†, PATRIZIA CASACCIA-BONNEFIL‡, ZENG XIU-LIU*, AND HOWARD J. FEDEROFF§

*Department of Pharmacology, ‡Program of Anatomy and Cell Biology, State University of New York–Health Science Center at Brooklyn, New York, NY 11203; and §Departments of Medicine and Neuroscience, Albert Einstein School of Medicine, Bronx, NY 10461

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ABSTRACT **Patients with severe temporal lobe epilepsy lose neurons within the CA3 and hilar regions of the hippocampus. Loss of CA3 and hilar neurons was also induced by transducing organotypic hippocampal slice cultures with a replication-defective herpes simplex virus (HSV) vector expressing the GluR6 kainate subtype of the glutamate receptor (HSVGluR6). In transduced fibroblasts, HSVGluR6 expressed a M_r 115,000 protein that reacted with anti-GluR6 serum. After exposure of fibroblasts to HSVGluR6, a kainate-dependent toxicity appeared in cells that were previously resistant to kainate. Microapplication of nanoliter amounts of recombinant HSV stocks into organotypic hippocampal slice cultures resulted in localized transduction and gene transfer at the site of microapplication. Microapplication of 100 HSVGluR6 virions into CA3 stratum pyramidale induced a large loss of CA3 pyramidal cells and hilar neurons, despite the small number of transduced neurons. This effect was not seen when 100 virions of HSVGluR6 were microapplied to CA1 stratum pyramidale. Tetrodotoxin or *N*-methyl-D-aspartate receptor antagonists inhibited the large loss of CA3 and hilar neurons, suggesting that the small cluster of HSVGluR6-transduced cells induced an *N*-methyl-D-aspartate-dependent transsynaptic loss of non-transduced neurons.**

In the hippocampus of patients with temporal-lobe epilepsy, CA3 and hilar neurons are often damaged (1–4), most likely due to excessive synaptic release of glutamate (5, 6). Seizure activity and cell loss can be induced experimentally by stereotaxic application of kainate, a glutamate agonist (7). Neuronal loss is observed both at the site of kainate injection and at distant sites, suggesting that kainate depolarization of neurons at the injection site induces a transsynaptic excitotoxicity (8). A similar excitotoxic mechanism may be responsible for CA3 neuronal loss in patients with temporal-lobe epilepsy. During seizures in such patients, the recurrent excitatory synapses and intrinsic burst firing of a cluster of CA3 neurons may result in excitotoxic cell loss throughout the CA3 and hilar regions (5). Burst firing of CA3 neurons may result from enhanced expression of non-*N*-methyl-D-aspartate (non-NMDA) receptors in the hippocampus of epileptic patients (9, 10).

Recently five cDNAs (GluR5, GluR6, GluR7, KA-1, and KA-2) have been cloned which define a family of kainate receptor subunits (11–15). Introduction and expression of a GluR6 cDNA into *Xenopus* oocytes or human 293 embryonic kidney cells induces a rapidly desensitizing, kainate-gated ion channel (11). This channel is activated by kainate, but not by the glutamate agonists (±)-α-amino-3-hydroxy-5-methylisoxazole-4-propionate (AMPA) and NMDA (11). Coapplication of kainate with the lectin Con A reduces the desensitization of the GluR6-encoded channel. This channel may be more permeable to Na^+ and K^+ than to Ca^{2+} due to the presence of an arginine at residue 690 (16). This arginine residue is also found in other non-NMDA receptors displaying a similar ionic permeability (17). The arginine residue is not encoded by the gene for GluR6; instead a glutamine codon in the GluR6 mRNA is converted to an arginine codon by RNA editing (16).

We have constructed a herpes simplex virus (HSV) vector, HSVGluR6, that directs the expression of a cDNA encoding the edited form of the GluR6 kainate receptor subunit under the control of the HSV immediate early (IE) 4/5 promoter. This viral vector was applied by a microapplication method that permits localized infections of organotypic hippocampal slice cultures. HSV vectors transduce genes with very high efficiency, so that small numbers of virions are needed to produce a biological effect (18, 19). The microapplication method permits transfer of genes into identified hippocampal loci, since slice cultures retain much of the laminar organization, cellular heterogeneity, and synaptic connections of transverse hippocampal slices (20–22). Microapplication of HSVGluR6 to CA3 stratum pyramidale, but not CA1 stratum pyramidale, resulted in a transsynaptic neuronal loss that spread throughout the CA3, hilar, and dentate gyrus regions of the slice culture. The cell loss seen in the HSVGluR6-transduced slice cultures was similar to the hippocampal damage resulting from kainate injection *in vivo* or in patients with temporal lobe epilepsy.

MATERIALS AND METHODS

Cell Lines and Organotypic Slice Cultures. NIH 3T3 mouse fibroblasts were grown in Dulbecco's modified Eagle's medium plus 10% calf serum, 2 mM glutamine, and streptomycin (100 units/ml) and penicillin (100 units/ml) (all medium components were from GIBCO). Vero monkey kidney cells were grown in an identical medium except that 10% fetal bovine serum was used. Both cell lines were maintained at 37°C in a 5% CO_2 atmosphere. Organotypic slice cultures were established from 400-μm hippocampal slices from Sprague–Dawley rat pups 10 days after birth (23). These cultures were maintained at 35°C in a 5% CO_2 atmosphere for 3 weeks prior to use. All reagents were obtained from Sigma unless otherwise specified.

Construction and Titer of HSVGluR6. A GluR6 cDNA containing the complete coding sequence of GluR6 (a gift of S. Heineman, Salk Institute, La Jolla, CA) was digested with

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Abbreviations: NMDA, *N*-methyl-D-aspartate; AMPA, (±)-α-amino-3-hydroxy-5-methylisoxazole-4-propionate; CNQX, 6-cyano-7-nitroquinoxaline-2,3-dione; 7-CK, 7-chlorokynurenate; CPP, 3-[(*R*)-2-carboxypiperazine-4-yl]propyl-1-phosphonate; HSV, herpes simplex virus; IE, immediate early; X-Gal, 5-bromo-4-chloro-3-indolyl β-D-galactopyranoside.

†To whom reprint requests should be addressed.

*Eco*RI and *Xba* I (all restriction enzymes were from New England Biolabs), and the resulting 4.5-kb fragment was cloned into the replication-defective HSV vector HSVPrpuc and packaged into HSV virions (18, 19). The titer of HSV-GluR6 virions was determined by extraction of DNA from HSVGluR6 viral stock, followed by serial dilution, binding to nylon membranes, and hybridization to a ^{32}P-labeled probe corresponding to the β-lactamase sequence present in the HSV vectors. The amount of hybridization was compared with a similar hybridization to HSVlac, which was titered independently by staining β-galactosidase-positive cells with the chromogenic substrate 5-bromo-4-chloro-3-indolyl β-D-galactopyranoside (X-Gal) (24).

Preparation of Antisera and Immunoblot Analysis. The GluR6 cDNA was digested with *Eco*RV and *Xba* I and cloned into the *trpE* fusion vector pATH2. A M_r 77,000 fusion protein was induced in *Escherichia coli* RR1 (pATH-GluR6) and purified (25, 26). Purified TrpE–GluR6 fusion protein (200 μg) was emulsified with TiterMax adjuvant (CytRx, Norcross, GA) and injected into New Zealand White rabbits. After 1 month, the rabbits were boosted three times at 2-week intervals with 200 μg of fusion protein. Two of the three rabbits gave similar antisera (anti-GluR6) as measured by immunoblot assay. Vero cells (2×10^5) were infected with 4×10^5 HSVGluR6 virions in 1 ml buffered with 25 mM Hepes, pH 7.4. After 1 hr, the medium was exchanged with Vero culture medium and the cells were incubated overnight. The cultures were washed with 137 mM NaCl/2.6 mM KCl/9.5 mM NaH_2PO_4/1.4 mM KH_2PO_4, and the cells were scraped into 50 mM Tris, pH 7.5/1 mM EDTA/1 mM EGTA/5 mM benzamidine/0.1 mM leupeptin/0.1 mM phenymethylsulfonyl fluoride with aprotinin at 50 kallikrein inhibitory units/ml. An equal volume of 10 mM Tris-HCl, pH 6.8/1% SDS preheated to 100°C was added and incubated at 100°C for an additional 5 min. An equal volume of Laemmli sample buffer was then added and the sample was electrophoresed and immunoblotted with a 1:250 dilution of anti-GluR6 serum (27). The immunocomplexes were visualized with the ECL kit (Amersham) according to the manufacturer's instructions.

Toxicity Assay of NIH 3T3 Fibroblasts. NIH 3T3 cells (2×10^4) were transduced with 4×10^4 HSVGluR6 virions for 1 hr. The culture was washed and 24 hr later incubated with culture medium containing various combinations of 10 μM kainate, 1 μM Con A, and 50 μM 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) (Tocris Neuramin, Bristol, England; CNQX was added to the cultures 30 min before the addition of kainate plus Con A). The number of adherent cells was determined in a 0.5-cm^2 region of the culture dish before and after the 24-hr incubation period.

Microapplication of Recombinant HSV Vectors. After 14 days *in vitro*, slice cultures were examined and those similar in appearance to the culture shown in Fig. 3*A* were included in this study. At 21–28 days *in vitro*, recombinant HSV stocks were microapplied to slice cultures with pulled glass micropipettes positioned with a manual micromanipulator (Narishige, Greenvale, NY) placed on a vibrationless table (Kinetic Systems, Spring Valley, NY). Fifty to 250 nl of viral stock was microapplied extracellularly at a flow rate of 50 nl/min. In some experiments, 50 μM 3-[(*R*)-2-carboxypiperazin-4-yl]propyl-1-phosphonate (CPP), 200 μM 7-chlorokynurenate (7-CK) (Tocris Neuramin), or 0.2 μg of tetrodotoxin per ml was added to the medium 1 hr prior to microapplication. Transduced slice cultures were maintained for an additional 24 hr and stained with either cresyl violet or X-Gal (24). The cultures were scored by two independent observers with the criterion that a positive result was a complete loss of pyramidal cells between the microapplication site and the hilus.

PCR Assay of HSVGluR6 in Organotypic Slice Cultures. At 30 min, 6 hr, or 16 hr after microapplication of 250 nl of HSVGluR6 virus, the tissue at the microapplication site and at a distant site (0.4 mm away) in stratum pyramidale was dissected and frozen. As a control for the amount of virus stock delivered by the micropipette, 31, 63, 125, 250, and 500 nl of HSVGluR6 virus stock were microapplied into separate tubes and frozen. The virus stock or the tissue punches were thawed and incubated in 10 mM Tris·HCl, pH 7.5/100 mM NaCl/25 mM EDTA/0.5% SDS with proteinase K (0.1 mg/ml) and salmon sperm DNA (20 μg/ml) for 10 hr at 50°C. The DNA was extracted with phenol/chloroform, precipitated with ethanol, and suspended in 30 μl of 10 mM Tris·HCl, pH 8.0/0.1 mM EDTA. Ten microliters of each sample was added to a mixture containing 10 mM Tris·HCl (pH 8.3), 1.5 mM $MgCl_2$, 50 mM KCl, 0.01% gelatin, 0.2 mM each dNTP, 0.2 μM oligonucleotide primers (sense, nt 2159–2188 of the GluR6 sequence, 5′-GCTCTCATGCAGCAAGGTTCT-GAACTCATG-3′; antisense, nt 2812–2783, 5′-AAACT-GAGAGCACCAAGCCGGCTGCCAGGA-3′), and 0.5 unit of *Taq* DNA polymerase (Boehringer Mannheim). The DNA was amplified for 30 cycles consisting of annealing at 65°C for 1 min, extension at 72°C for 1.5 min, and denaturation at 92°C for 1 min.

RESULTS

HSVGluR6 Directs the Expression of GluR6 Protein and Renders Fibroblasts Sensitive to Kainate. A replication-defective HSV vector containing a cDNA encoding GluR6 (HSVGluR6) was constructed and packaged into virions (Fig. 1*A*). The titer of the resulting HSVGluR6 stock was 4×10^5 virions per ml. HSVGluR6 transduced and expressed GluR6 cDNA into both neuronal and nonneuronal cells. An anti-GluR6 serum detected a prominent 115-kDa protein from HSVGluR6-transduced Vero cultures, but not from non-transduced cultures (Fig. 1*B*). In the Vero cultures, weakly immunoreactive proteins of 115 and 75-kDa were seen. The prominent 115-kDa protein was not detected on immunoblots of HSVGluR6-transduced cultures with preimmune serum (data not shown). The apparent molecular mass of this protein is similar to the predicted size of the GluR6 protein.

HSVGluR6 transduction of NIH 3T3 fibroblast cultures also made the cells sensitive to kainate toxicity. HSVGluR6-transduced NIH 3T3 cultures were exposed to kainate (10 μM) and Con A (1 μM), which resulted in both activation and reduced desensitization of the GluR6-encoded ion channel (12). A large and significant loss of NIH 3T3 cells was seen in kainate plus Con A-treated cultures after HSVGluR6

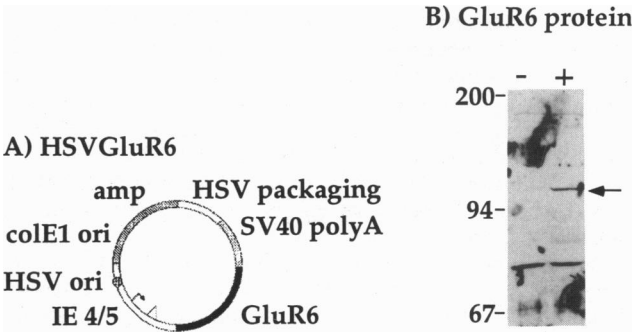

FIG. 1. The recombinant HSV vector HSVGluR6 directs the expression of GluR6 protein. (*A*) Genome of HSVGluR6. ori, Origin; amp, ampicillin-resistance gene; SV40 polyA, simian virus 40 polyadenylylation signal. (*B*) Immunoblot analysis of HSVGluR6-transduced Vero cells. Protein extracts from mock-infected (−) or HSVGluR6-transduced (+) Vero cultures were electrophoresed, blotted onto nitrocellulose, and reacted with anti-GluR6 serum. Arrow shows the 115-kDa GluR6 polypeptide present in the HSV-GluR6-transduced cultures. At left, the migration of molecular size (kDa) markers is shown.

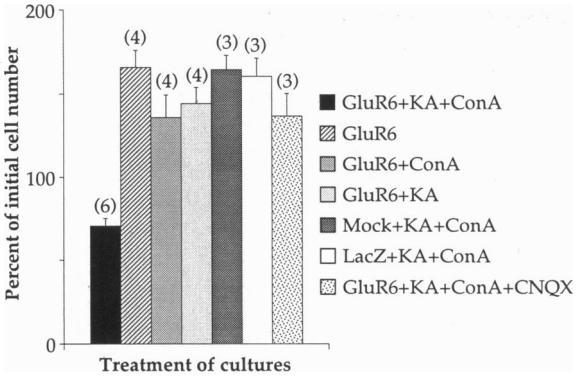

FIG. 2. Kainate-dependent loss of HSVGluR6-infected NIH 3T3 fibroblasts. Fibroblast cultures were plated and 24 hr later were transduced with virus [HSVGluR6 (GluR6) or HSVlac (LacZ)] or mock-transduced (Mock). After an additional 24 hr cultures were either left untreated or treated with Con A, kainate (KA), and CNQX in various combinations. Fibroblasts contained within a 0.5-cm^2 region of a tissue culture dish were counted at the time of treatment and again after a 24-hr incubation period. The number of cells remaining after the 24-hr incubation period is expressed as the percent of initial cell number + SEM. The cell number in HSVGluR6-transduced cultures treated with kainate plus Con A was significantly ($P < 0.05$, by analysis of variance using the Bonferroni inequality) less than that for all other groups. Number of times each microapplication was performed is indicated in parentheses.

transduction (Fig. 2). The kainate toxicity was reduced by pretreatment with the competitive non-NMDA channel antagonist CNQX (50 μM). No sensitivity to kainate and Con A was observed in nontransduced cultures or in cultures transduced with a HSV vector which expresses *E. coli* β-galactosidase (HSVlac). Insignificant cell loss was seen in HSVGluR6-transduced cultures that were untreated or treated alone with either kainate or Con A. These data suggest that activation of the GluR6-encoded ion channel with kainate and Con A resulted in the cell loss in HSVGluR6-infected fibroblast cultures.

Microapplication of HSV Vectors to Hippocampal Slice Cultures Results in Localized Gene Transfer. In unstained hippocampal slice cultures, the CA1 and CA3 strata pyramidale were easily identified. This permitted delivery of 50–250 nl of recombinant HSV stock into specific hippocampal regions via a glass micropipette placed within the explant by a manual micromanipulator. With this method, 125 HSVlac virions (250 nl) were microapplied to CA3 or CA1 stratum pyramidale and the cultures were maintained for an additional 24 hr. Staining of these slice cultures with X-Gal showed a cluster of β-galactosidase-containing cells centered at the microapplication site (Fig. 3 *A* and *B*). In some microapplications a portion of the virus stock spread down the explant surface and transduced cells at the edge. No cell loss, other than the damage induced by insertion of the micropipette, was observed with HSVlac (Fig. 3 *A* and *B*; Fig. 4).

Microapplication of HSVGluR6 to CA3 Stratum Pyramidale Results in Transsynaptic, NMDA-Dependent Excitotoxicity. HSVGluR6, in contrast to HSVlac, induced cell loss in slice cultures 24 hr after microapplication. When 50 HSVGluR6 virions (125 nl) were microapplied to CA3 stratum pyramidale, a small cell loss was observed only at the microapplication site (Fig. 4). In contrast, when 100 HSVGluR6 virions (250 nl) were microapplied to CA3 stratum pyramidale, darkening cells, an early sign of neurodegeneration, were

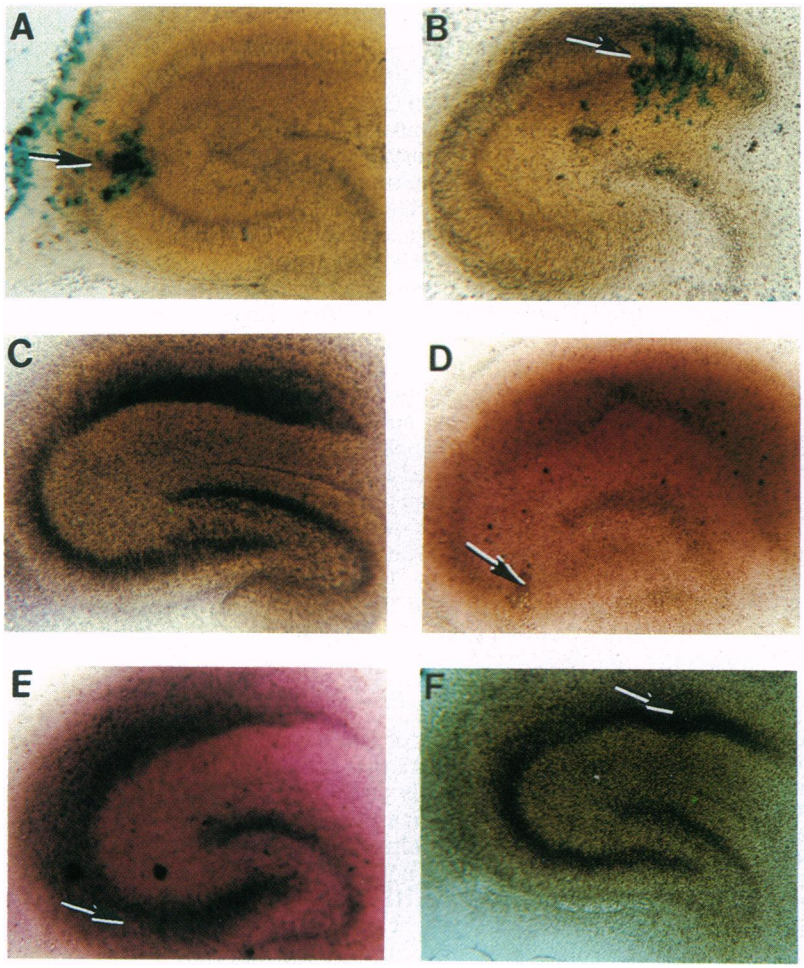

FIG. 3. Microapplication of HSVlac or HSVGluR6 to organotypic slice cultures. Slice cultures were transduced with 125 HSVlac virions, incubated for 24 hr, and stained with X-Gal (*A* and *B*); or nontransduced (*C*); or transduced with 100 HSVGluR6 virions (*D–G*), incubated for 24 hr, and stained with cresyl violet. A complete loss of pyramidal cells between the microapplication site and the hilus was scored (*D*). The large neuronal loss was not seen in cultures treated prior to infection with the NMDA antagonist CPP (50 μM) (*E*). Arrow indicates site of microapplication. (×4.5.)

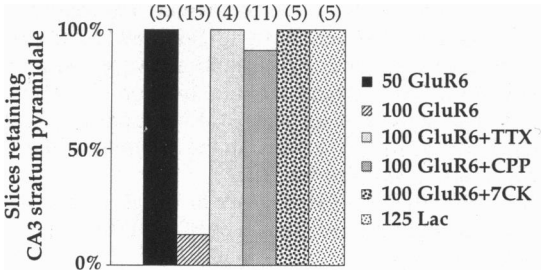

FIG. 4. Microapplication of HSVGluR6 induces a large neuronal loss in hippocampal slice cultures. The indicated number of HSV-GluR6 (GluR6) or HSVlac (Lac) virions was microapplied to CA3 stratum pyramidale, and the slice cultures were stained and scored for loss of CA3 pyramidal cells located between the microapplication site and the hilus. Some slice cultures were treated prior to transduction with tetrodotoxin (TTX, 0.2 mg/ml) or an NMDA antagonist, CPP (50 μM) or 7-CK (200 μM). Number of times each microapplication was performed is indicated in parentheses.

observed within 16–20 hr (data not shown). Six hours later, a complete loss of pyramidal cells between the microapplication site in CA3 and the hilus was observed in 12 out of 15 experiments (Figs. 3 *D* and 4). In the remaining 3 experiments, the neuronal loss was larger and included the remainder of CA3, the hilus, and the dentate gyrus (data not shown). The large loss of neurons induced by application of only 100 HSVGluR6 virions suggested that both HSVGluR6-transduced neurons and nontransduced neurons had been killed. A similar loss of CA3 and hilar neurons was obtained regardless of the location of the site of microapplication within CA3 stratum pyramidale. Loss of CA3 and hilar neurons required HSVGluR6 microapplication directly into CA3 stratum pyramidale; in a few cases where 100 virions of HSVGluR6 were inadvertently microapplied to CA3 stratum radiatum, a loss of cells was observed only at the microapplication site (data not shown).

The CA3 region of the hippocampus contains recurrent excitatory synapses that may contribute to the large loss of cells seen in HSVGluR6-transduced slice cultures. Tetrodotoxin (0.2 μg/ml), which blocks action potentials and consequently most neurotransmitter release, was used to test the role of synaptic activation in producing the large neuronal loss in HSVGluR6-transduced cultures. Tetrodotoxin blocked the loss of cells distant to the microapplication site, but not at the microapplication site, suggesting that it protected the non-

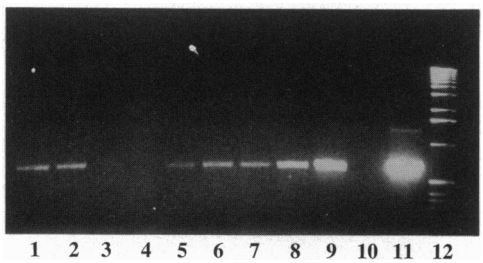

FIG. 5. PCR analysis of HSVGluR6-infected slice cultures. After microapplication of HSVGluR6 virus stock directly into test tubes or to slice cultures, DNA was extracted, amplified by PCR, and electrophoresed in 1% agarose gel with ethidium bromide-staining. A 653-bp amplified product indicates the presence of the HSVGluR6 genome. Amplified DNA was analyzed from tissue punches containing the microapplication site 30 min (lanes 1 and 2) or 16 hr (lanes 3 and 4) after microapplication or from the DNA extracted from 12 virions (lane 5), 25 virions (lane 6), 50 virions (lane 7), 100 virions (lane 8), or 200 virions (lane 9). A control amplification was performed with no added DNA (lane 10). Amplification of 10 ng of HSVGluR6 vector DNA was performed as a positive control (lane 11). Size markers (1-kb ladder, BRL) are shown (lane 12).

transduced cells but not the HSVGluR6-transduced cells (Fig. 4).

The activity of the HSVGluR6-transduced neurons may result in excessive release of glutamate and NMDA receptor activation. NMDA receptor activation has been implicated in spread of epileptic discharge and excitotoxicity (28–30). Two NMDA antagonists, neither of which was likely to block the GluR6 ion channel, were used to test the role of NMDA receptors in the cell loss induced by HSVGluR6. HSVGluR6-transduced slice cultures were treated with CPP (50 μM), a competitive antagonist of the NMDA receptor at the glutamate site, or 7-CK (200 μM), a noncompetitive antagonist that binds at the glycine site. Both antagonists blocked cell loss distant from the microapplication site, but not at the microapplication site (Figs. 3*E* and 4). These data suggests that loss of nontransduced cells, but not HSVGluR6-transduced cells, requires activation of NMDA receptors.

Microapplication of HSVGluR6 to CA1 Stratum Pyramidale Induces Cell Loss Only at the Microapplication Site. CA3 pyramidal cells, in contrast to CA1 pyramidal cells and the granule cells of the dentate gyrus, have recurrent excitatory synapses and intrinsic burst activity that initiates and synchronizes epileptiform discharge (31–33). It is possible that the transsynaptic neuronal loss induced by HSVGluR6 requires these characteristics of CA3 pyramidal cells. This was tested by microapplication of 100 HSVGluR6 virions to CA1 stratum pyramidale. In 8 out of 10 slices, a small loss of CA1 pyramidal cells at the microapplication site was observed (Fig. 3*F*). CA3 or hilar neuronal loss was not observed when HSVGluR6 was microapplied to CA1, suggesting that the large neuronal loss of CA3 and hilar neurons requires HSV-GluR6 infection of CA3 stratum pyramidale.

Loss of the HSVGluR6 Genome After Microapplication into Slice Cultures. HSVGluR6 uses the strong HSV IE 4/5 promoter to direct expression of the GluR6-encoded kainate receptor. When wild-type HSV-1 infects cells, the products of genes using the IE4/5 promoter can be detected as early as 2 hr after virus infection (34). This suggests that GluR6 protein may be able to accumulate and rapidly assemble into kainate receptors. It was important to determine how long transduced neurons persisted after the increase of GluR6 expression from the IE 4/5 promoter. Since HSVGluR6 persists as a multicopy episome in transduced cells, the presence of transduced cells could be assayed by PCR amplification (19). Tissue punches of slice cultures containing the microapplication site were isolated 30 min, 6 hr, or 16 hr after delivery of 100 HSVGluR6 virions. Total DNA was extracted and amplified by using primers specific for the GluR6 cDNA. HSVGluR6 viral genome was detected at the site of microapplication 30 min (Fig. 5, lanes 1 and 2) but not 6 or 16 hr (lanes 3 and 4; data not shown) after microapplication. These results suggest that HSVGluR6-transduced cells were rapidly lost between 30 min and 6 hr after microapplication. Six and 16 hr after microapplication, HSVGluR6 was not found in tissue punches of stratum pyramidale located 0.4 mm from the microapplication site (data not shown). The cellular gene for GluR6 was not amplified by the PCR in these experiments due to the presence of intronic sequences (16).

DISCUSSION

Increased expression of GluR6 in a small number of HSV-GluR6-transduced CA3 pyramidal cells induced a large loss of CA3 and hilar cells due to an NMDA-dependent transsynaptic process. Since no cell loss was induced in either fibroblasts or slice cultures with HSVlac, the HSVGluR6-induced cell loss was not due to a nonspecific toxic effect of the HSV vector, the HSV packaging cell line, or the replication-defective HSV helper virus which was used to prepare the virus stocks (18, 35). The large loss of cells also did not

result from HSVGluR6 replication in slice cultures. HSV-1 requires a minimum of 15 hr to replicate (34). HSVGluR6 DNA was not detected at the site of microapplication or at distant sites 6 or 16 hr after exposure to the viral vector. Further, the large loss of neurons was seen only after application of 100 virions of HSVGluR6 to CA3 stratum pyramidale. In contrast, microapplication of 50 HSVGluR6 virions to CA3 stratum pyramidale, or 100 HSVGluR6 virions to CA3 stratum radiatum or CA1 stratum pyramidale, resulted in cell loss only at the microapplication site. Tetrodotoxin and NMDA antagonists blocked the large neuronal loss, further suggesting that HSVGluR6 transduction was restricted to the microapplication site.

It is likely that the loss of HSVGluR6-transduced and nontransduced neurons occurred by separate mechanisms. The protective effect of tetrodotoxin or NMDA antagonists was limited to nontransduced cells. The transsynaptic loss of nontransduced neurons was relatively slow; signs of neurodegeneration were seen only 16 hr after microapplication, and an additional 6 hr was required for complete loss of nontransduced neurons. In contrast, 6 hr after microapplication no HSVGluR6 genomes remained, suggesting loss of HSVGluR6-transduced cells. Unlike the transsynaptic loss of nontransduced cells, loss of HSVGluR6-transduced cells required neither propagation of neuronal activity by action potentials nor NMDA receptor activation. Furthermore, GluR6 expression in fibroblasts resulted in kainate plus Con A-dependent cell loss. The likely cause of the loss of transduced neurons in slice cultures, therefore, was a direct effect of enhanced GluR6 expression. In transduced cells, GluR6 mRNA may accumulate as early as 2 hr after transduction, due to the rapid activation of the HSV IE 4/5 promoter (34). Therefore, the consequence of HSVGluR6 transduction may be a rapid and lethal increase in excitability.

The loss of nontransduced cells appeared to require a threshold of HSVGluR6-transduced CA3 pyramidal cells. This may reflect the minimum number of CA3 pyramidal cells required to initiate epileptiform activity due to recurrent excitation and burst firing (36). In the acute hippocampal slice, Miles and Wong (37) have reported that there is a 0.5% probability that two CA3 pyramidal cells have a monosynaptic excitatory connection. The number of recurrent excitatory connections is higher in neonates (38). Slice cultures are likely to have increased recurrent connections in CA3 because they are established from neonates and additional synaptogenesis has been reported to occur *in vitro* (20, 21). Despite the larger number of recurrent CA3 synapses, our slice cultures have rarely shown spontaneous epileptic activity (P.J.B., P.C.-B., H.J.F., and Armin Stelzer, unpublished results). The HSVGluR6-transduced neurons may be the source of a toxic epileptic discharge spreading throughout the CA3, hilar, and dentate gyrus regions. In preliminary experiments, epileptiform activity has been observed in HSVGluR6-transduced slice cultures which terminate with loss of all spontaneous and evoked activity in CA3, suggesting that the epileptiform discharge was the cause of the nontransduced cell loss (P.J.B., P.C.-B., H.J.F., and Armin Stelzer, unpublished results). In summary, enhanced expression of kainate receptors in a small number of CA3 pyramidal cells could induce a transsynaptic excitotoxicity in slice cultures that is similar to the pathological lesions in the hyperexcitable hippocampus.

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