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Differences in Attachment Between Herpes Simplex Type 1 and Type 2 Viruses to Neurons and Glial Cells

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Fractions of nerve cell perikarya, synaptosomes, and astrocytic glia were prepared from human, monkey, rabbit, rat, and mouse brain tissue. The herpes simplex virus type 1 (HSV-1) and type 2 (HSV-2) binding capacity of these fractions was studied. Pretreatment of fractions with one type of HSV and the subsequent testing of adsorption of homotypic and heterotypic virus was employed to reveal type selectivity of virus binding receptors. A higher density of HSV-1 than of HSV-2 selective receptors was found on synaptosomes and glial cells, except with mouse-derived preparations. Synaptosomal and glial cell preparations of mouse brains adsorbed both types of HSV well. Little or no adsorption was observed with HSV-1 and HSV-2 to neuronal perikarya. The type selectivity of HSV binding receptors on brain cells was demonstrated on preparations of human synaptosomes and mouse glial cells. Some possible implications of the observations on the HSV infection of the nervous system are discussed.

Ganglionic infection by retrograde intra-axoplasmic transport of virus to sensory ganglia (4, 7, 14, 19) seems decisive for establishment of latent, reactivable herpes simplex virus (HSV) infection (2, 6, 20). Preceding the intra-axonal transport, HSV must adsorb to the surface of nerve terminals. Hence, the finding that preparations of enriched synaptosomes adsorbed HSV significantly more efficiently than corresponding preparations of neuronal perikarya became of particular interest (24). Cellular receptors for HSV are type-selective and capable of discriminating between HSV type 1 (HSV-1) and type 2 (HSV-2) (25). The present study reports the occurrence of HSV type-selective receptors on nerve cell perikarya, synaptosomes, and astrocytic glial cells. Human, monkey, rabbit, rat, and mouse brain tissues were used as sources for cell fractions obtained after dissociating and separating cells and synaptosomes by means of isopycnic centrifugation in Ficoll gradients (3, 5). The adsorption of HSV to these cellular preparations was then investigated.

MATERIALS AND METHODS

Viruses. Five strains of both HSV-1 and HSV-2 and one strain of each type resistant to trisodium phosphonoformate (21) were used. The strains were typed by using several different methods (23), including immunoelectroosmophoresis against type-specific antisera (12). In most experiments, strains F (HSV-1; B. Roizman) and B 4327 UR (HSV-2; S. Jeansson) were employed.

Plaquing of infective virus was performed on monolayer cultures of green monkey kidney (GMK) cells in 5-cm plastic dishes, using Eagle minimal essential medium supplemented with 2% calf serum and antibiotics.

Brain tissue. Brains of 22 albino rats, weighing 200 to 300 g, 8 albino rabbits (2 to 3 kg), or 100 5-week-old Swiss albino mice were used per experiment. In addition, four experiments were performed with monkey brains (two Cynomolgous and two African green monkeys), using one brain for each experiment. Animals were anesthetized with ether or barbiturates and perfused with Ringer solution through the left heart ventricle before the brains were removed. Three human brain materials were obtained from cases of traffic casualties within 48 to 72 h postmortem. Before exposure to the preparatory procedures, all tissues were kept at ice-bath temperature.

Preparation of cellular fractions. Neuronal perikarya and astrocytic glia enriched fractions were prepared as described previously (3). Briefly, the brains, excluding the cerebellum, were sliced and disrupted by passage through nylon mesh. After filtering the tissue fragments through nylon meshes down to 50 μm, the filtrate was centrifuged 5 min at 150 × *g*, and the pelleted cells were separated on a discontinuous Ficoll gradient with steps of 42, 35, 19.5, and 16% Ficoll. The cell suspension in 26% Ficoll was layered between 35 and 19.5% Ficoll, and the gradient was run at 83,000 × *g* for 110 min. The glial fraction was collected between 19.5 and 16% Ficoll and the perikaryal fractions between 42 and 35% Ficoll. The fractions were diluted with 0.32 M sucrose, pelleted at 2,000 × *g* for 20 min, and suspended in Eagle medium before exposure to HSV.

Synaptosomal fractions (5) were prepared with a glass-Teflon homogenizer, and nondisrupted brain cells were removed by centrifugation. The resulting supernatant was centrifuged at 10,000 × *g* for 20 min, and the washed crude mitochondrial pellet was sus-

pended in sucrose and layered on a gradient of 7.5 and 13% Ficoll in 0.32 M sucrose. The gradient was centrifuged at 83,500 × *g* for 45 min. The synaptosomal fraction, layering between 7.5 and 13% Ficoll, was pelleted in 0.32 M sucrose and suspended in Eagle medium before use in HSV adsorption studies.

Protein was determined by the procedure of Lowry et al. (16). One milligram of protein corresponds to approximately 20,000 glial cells or 50,000 neuronal perikarya. The appearance of fractionated cells from brain tissue in the electron microscope and the estimation of purity by means of morphological studies and assays of marker enzymes have been discussed in detail elsewhere (9, 24). In support of relatively intact plasma membrane functions of the fractionated cells are observations of their accumulation of amino acids and potassium against a concentration gradient in a ouabain sensitive fashion (8, 10).

Kidney and liver cell suspensions were prepared from organs of perfused mice and rats by squeezing the organs through stainless-steel grids. The suspended cells were washed three times in Hanks balanced salt solution suspended in Eagle medium to a final density of 10^8 cells per ml before use in adsorption experiments.

Adsorption of HSV. If not stated otherwise, the adsorption studies were performed as follows. Cell fractions suspended in 0.9 ml of Eagle minimal essential medium in plastic tubes were agitated in a water bath at 37°C. To the cell suspension was added 0.1 ml of 10^5 plaque-forming units of HSV. Samples (100 μl) were drawn immediately after addition of the virus and after 15, 30, and 60 min. Each sample was added to 9.9 ml of cold medium and centrifuged for 10 min at 1,000 × *g*, and the infective virus in the supernatants was titrated. Residual infectivities of virus added to tubes with medium alone were used as controls. Adsorption curves were constructed by plotting percentages of remaining infectivity against time of adsorption, after correction for the amount of infective virus disappearing in cell-free controls.

To exclude the possibility that proteolytic enzymes released from cell fractions studied caused degrading effects on HSV with loss of infectivity, homogenates of disrupted nerve and glial cells, from which the plasma membranes were removed by centrifugation, were incubated with HSV for 60 min at 37°C. No inactivation of virus infectivity beyond that of cell-free controls was observed. The HSV adsorption patterns have been confirmed by means of radioactively labeled and purified virus (24).

Blocking of cellular receptors with homotypic HSV. Pretreatment of mouse glial cells with HSV-1 was performed in the following way. The cellular fractions were incubated five times for 20 min each at 37°C with 10^8 plaque-forming units of HSV or with cell culture medium only. After each 20-min incubation period, cells were pelleted and the supernatant was replaced with fresh virus suspension. In one experiment, pretreated synaptosomes were used. Human synaptosomes were pretreated with a concentrated HSV-1 suspension (10^{10} plaque-forming units) for 1 h.

After pelleting the pretreated cells by centrifugation at 150 × *g* for 5 min and the synaptosomes by centrifuging at 10,000 × *g* for 20 min, the cellular fractions were resuspended in Eagle MEM containing 10^6 plaque-forming units of phosphonoformic acid-resistant mutants (PFAR) of HSV-1 or HSV-2, and the adsorption of infective virus was observed as outlined above.

Nonadsorbed amounts of the mutants were tested by plaquing virus in the presence of 0.50 mM phosphonoformic acid, a concentration known to reduce infectivity of phosphonoformic acid-sensitive HSV by 4 log units (21). More detailed information about procedures adopted is presented elsewhere (25).

RESULTS

Attachment of HSV-1 and HSV-2 to nerve cells and glia of rat, rabbit, and monkey brain tissue. The attachment of HSV-1 and HSV-2 to preparations of dissociated and enriched neuronal perikarya, fractions of synaptosomes, and astrocytic glial cells of rat, rabbit, and monkey brain tissues is illustrated in Fig. 1. Synaptosomes adsorbed HSV most efficiently, and type 1 adsorbed better than type 2 virus. The glial cells, although less effective than the

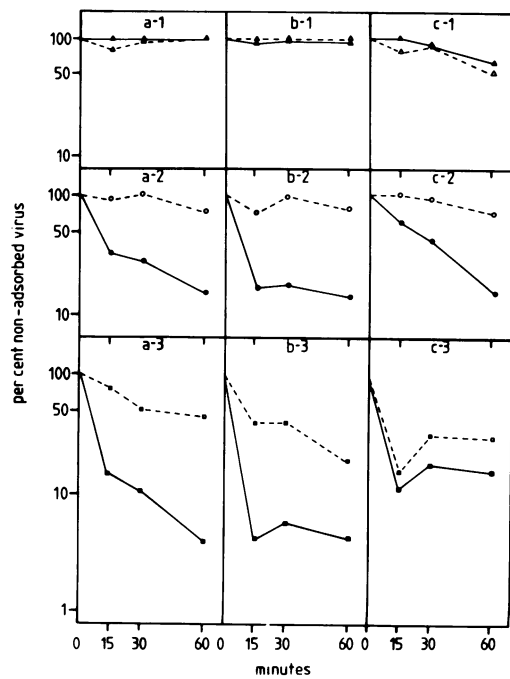

FIG. 1. *Adsorption of HSV-1 and HSV-2 to cell fractions of brain tissue from rat (a), rabbit (b), and monkey (c). Solid lines represent results with HSV-1 (strain F), and dashed lines represent results with HSV-2 (strain B4327UR).* ▲ *and* △, *Neuronal perikarya;* ○ *and* ●, *astrocytic glia;* ■ *and* □, *synaptosomes. Protein contents of suspensions of the neuronal perikarya: 4.8 mg/ml (rat), 2.9 mg/ml (rabbit), and 0.65 mg/ml (monkey); protein content of suspensions of astrocytic glia: 4.1 mg/ml (rat), 2.5 mg/ml (rabbit), and 0.6 mg/ml (monkey); and protein content of suspensions of synaptosomes: 2.8 mg/ml (rat), 4.4 mg/ml (rabbit), and 0.7 mg/ml (monkey).*

synaptosomal fractions, displayed a similar pattern; they adsorbed HSV-1 better than HSV-2. This difference in adsorption rates between HSV-1 and HSV-2 was found in all of six separate experiments performed with rat brain tissue and was statistically significant at the 1% level with the Willcoxon-two sample test. Neuronal perikarya, on the other hand, exhibited little or no HSV-adsorbing capacity for either of the two subtypes. Essentially the same results were obtained with cells from all three species examined.

The discrepancy in adsorbing capacity between the preparations of nerve and glial cells did not result from fewer cells in the fractions of neuronal perikarya, because the nerve cell fractions contained at least twice as many cells as the glial cell fractions. The activity per milligram of protein of Na^+ K^+ adenosine triphosphatase in astrocytic glial fractions has been reported to be two to three times higher than that of neuronal perikarya fractions (11, 17). The specific enzyme activity of a glial plasma membrane fraction is reported to be two to three times higher than that of a neuronal membrane fraction (11). Therefore, the observed differences in virus adsorption between neuronal perikarya and glial cells seemed not to be due to different amounts of plasma membranes of the preparations, but was considered to reflect differences in density, avidity, or both of HSV-binding receptors on the cellular membranes available.

Attachment of HSV to mouse nerve cells and glia. With preparations derived from mouse brain tissue, the adsorption of HSV followed a pattern which was similar to those for the cellular fractions of rat, rabbit, and monkey tissues. Synaptosomes adsorbed virus better than glial cells which, in turn, were superior to fractions of nerve cell perikarya (Fig. 2). However, in contrast to rat, rabbit, and monkey cells, preparations of mouse astrocytic glia adsorbed type 1 and type 2 virus at the same rate. This observation was studied further.

Table 1 demonstrates that the patterns of type 1 and type 2 virus attachment to glial cells were highly reproducible and that the rate of adsorption was essentially the same for all the four different virus strains studied of both types. Two of each type were strains newly isolated from patients with herpetic infections. In contrast to the rat cells, the mouse glial cell fractions adsorbed all the strains of both types of HSV equally well. Since liver and kidney cell fractions revealed no difference in capacity of HSV-1 and HSV-2 adsorption between rat and mouse, the discrepancy seen with glial cell fractions suggested that mouse astrocytic glial cells exhibited more of HSV-2-binding receptors than did glial cells of rat brain (Fig. 3).

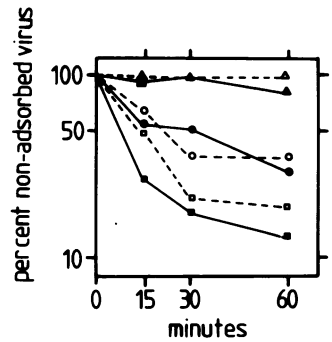

FIG. 2. *Adsorption of HSV-1 and HSV-2 to cell fractions of mouse brain tissue. Solid lines represent results with HSV-1 (strain F), and dashed lines represent results with HSV-2 (strain B4327UR).* ▲ *and* △, *Neuronal perikarya;* ○ *and* ●, *astrocytic glia; and* ■ *and* □, *synaptosomes. The protein content of neuronal perikarya suspension was 2.1 mg/ml, that of astrocytic glia suspension was 1.8 mg/ml, and that of synaptosomal suspension was 0.9 mg/ml.*

TABLE 1. *Adsorption (1 h) of HSV-1 and HSV-2 to mouse and rat glial cells*

Virus	Strain	Adsorption (%)[a]	
		Mouse[b]	Rat[c]
HSV-1	F	96	71
	KJ502	93	75
	90160	86	93
	90600	86	90
HSV-2	B4327UR	77	1
	B4181CX	77	0
	90128	75	0
	90114	80	1

[a] Corrected for infective virus lost in cell-free controls.

[b] Protein content, 2.2 mg/ml.

[c] Protein content, 3.1 mg/ml.

Attachment of HSV to cells of human brain tissue. Three human brain materials were available for investigation. The tissues were received at autopsy performed within 48 to 72 h postmortem. Although shortage of tissue limited the experimentation, the results of the HSV adsorption studies performed suggested that synaptosomal, perikaryal, and glial fractions were binding HSV-1 and HSV-2 according to a pattern closely similar to that described above for the rat-, rabbit-, and monkey-derived preparations (Fig. 4).

Demonstration of HSV type-selective receptors on plasma membranes of fractionated brain cells. By pretreating cellular fractions with HSV-1 and subsequently testing the adsorption of homotypic and heterotypic virus, the HSV type-selective receptors were exam-

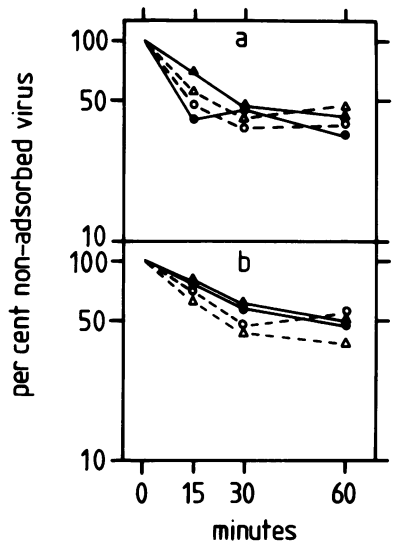

FIG. 3. *Adsorption of HSV-1 and HSV-2 to cell fractions of liver (a) and kidney (b) from mouse (dashed lines) and rat (solid lines). ▲ and △, HSV-1 (strainF); ○ and ●, HSV-2 (strain B4327UR).*

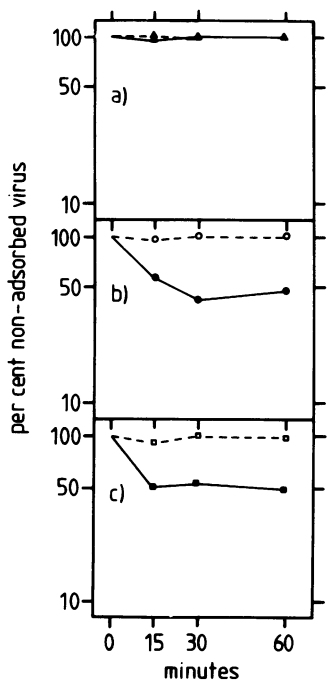

FIG. 4. *Adsorption of HSV-1 and HSV-2 to cell fractions of human brain tissue; suspended neuronal perikarya (a), astrocytic glia (b), and synaptosomes (c). Solid lines, HSV-1 (strain F); dashed lines, HSV-2 (strain B4327UR). The protein content of neuronal perikarya suspension was 1.1 mg/ml, that of astrocytic glia suspension was 0.7 mg/ml, and that of synaptosomal suspension was 0.4 mg/ml.*

ined. Mouse glial cells and synaptosomes prepared from human brain tissue were studied. The results are shown in Fig. 5. The HSV type-selective receptors were demonstrable with both kinds of preparations. HSV-1 but not HSV-2 receptors were blocked by exposure of cell fractions to HSV-1. Treatment of synaptosomal fractions with cytochalasin B or by lowering the temperature to 4°C during virus adsorption did not significantly affect the adsorption rate of HSV-1 (Fig. 6).

DISCUSSION

HSV locally produced in primary mucocutaneous infections may reach the nervous system through neural and hematogenous pathways. The retrograde intra-axonal transport of HSV (4, 14) has been demonstrated experimentally to be far more efficient than a hematogenous passage for establishment of latent, reactivable ganglionic HSV infection (19). As viral access to intra-axoplasmic flow must be preceded by adsorption of virus to structures of nerve terminals, it appears that HSV is either non-specifically accepted or more specifically attached to virus selective receptors on the nerve endings. Although proteins of the size of albumin, experimentally injected, are readily taken up by nerve terminals and transported axonally (13), entities of the size of HSV virions most probably require mediation of virus-specific receptors for attachment to peripheral nerve terminal structures and penetration into the axoplasmic flow. As a substitute for assays on HSV attachment to

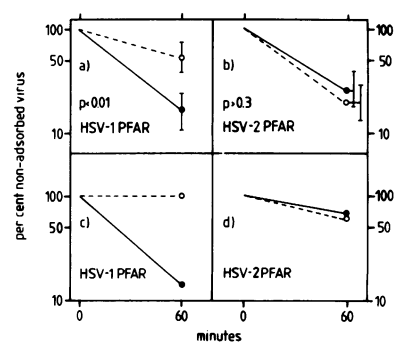

FIG. 5. *Adsorption of HSV-1 PFAR (a and c) and HSV-2 PFAR (b and d) to mouse glial cells (a and b) and human synaptosomes (c and d). Dashed lines represent HSV PFAR adsorption to cellular fractions pretreated with phosphonoformic acid-sensitive HSV-1; solid lines represents HSV PFAR adsorption to untreated cellular fractions. Bars represent standard deviation; n = 4. Protein contents of the mouse astrocytic glia suspensions were 2.8 mg/ml (a and b); and of the human synaptosomal suspensions 1.2 mg/ml (c and d). Plaque assays were performed in cultures with fluid medium containing 0.50 mM PFA.*

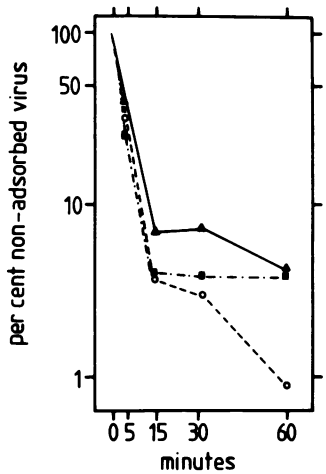

FIG. 6. *Adsorption of HSV-1 to rat synaptosomes. Effect of pinocytosis inhibition by cytochalasin B and decreased temperature.* ■, *Adsorption at 37°C;* ▲, *adsorption at 4°C;* ○, *adsorption in presence of 0.25 mM cytochalasin B.*

peripheral nerve terminals—no method for the isolation of peripheral nerve terminals is known to the authors—the methods for enrichment of cells by fractionation of dissociated cells of brain tissue in discontinuous Ficoll gradients (3, 5) were used.

The results obtained suggest that HSV-adsorbing receptors were present on the membranes of cellular fractions derived from brain tissues. These receptors were HSV type-selective; that is, they demonstrated a higher affinity for one type of HSV than for the other. Evidence for the occurrence of HSV type-selective receptors on both the plasma membrane of cells of primary cultures and established cell lines has been reported previously (25). Blocking of receptors was achieved with homotypic but not with heterotypic virus. Thus, the specificity of the adsorption reaction was indicated and the interference of cellular pinocytosis seemed unlikely. Moreover, treatments of cellular fractions with cytochalasin B or by lowering the temperature to 4°C during virus adsorption, both of which should impede the pinocytosis, did not significantly interfere with HSV adsorption.

There seemed to be relatively few HSV receptors on the nerve cell perikarya, whereas synaptosomal fractions were found to carry an abundance of HSV-adsorbing receptors. This finding is interesting because it suggests that virus-binding receptors are unevenly distributed along the plasma membrane of the neuron. Lack of receptors on the neuronal perikarya might reduce the spread of the HSV infection from cell to cell in peripheral ganglia and contribute to the development of latency by maintaining HSV infections without a rapid destructive progress. The presence of virus receptors in the synapses, on the other hand, would contribute to the spread of the infection following the neuronal pathways (1). Peripheral nerve endings might, as synaptosomes of the brain, be well-provided with virus binding receptors and facilitate the initiation of the HSV infection of the nervous tissue.

In adult humans HSV encephalitis is almost exclusively caused by type 1 infections (18). The reason for the dominance of the type 1 infection is unknown. Possibly the innervation of mucous membranes of the eyes and the naso-oral area by the cranial nerves simplifies spread of infection to the brain. All the species we examined (human, monkey, rabbit, rat, and mouse) revealed relatively more receptors for HSV-1 than for HSV-2 in the synaptosomal fractions of the brain tissues. The importance of this finding remains to be elucidated.

Infection of glial cells of the central nervous system may offer another possibility for the efficient replication and spread of virus within the central nervous system. The importance of infected glial cells for development of a necrotizing encephalitis has also been emphasized elsewhere (15, 22). Observations of the widespread tissue destruction in the central nervous system part of the transitional region of the trigeminal root (15, 22) may be explained, in part, by infection of astrocytes in the central nervous system portion. We found more receptors adsorbing type 1 than type 2 virus in fractions of astrocytic glia. This pattern of HSV adsorption was essentially the same for all species observed, with one exception. Mouse glial cell fractions behaved differently, displaying an affinity for HSV-2 equal to that for HSV-1. The mouse is more susceptible to HSV-2 central nervous system infection than most other mammals. In fact, it has been stated that type 2 strains should be more neurotropical than type 1 strains after inoculation of mice and that this observation could be regarded as an example of biological type differences (18).

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