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Inhibition of Herpes Simplex Virus Type 1 Replication by Halothane

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Halothane (2-bromo-2-chloro-1,1,1-trifluoroethane) inhibited herpes simplex virus type 1 (HSV-1) replication, although HSV-1 DNA was synthesized at normal levels in Vero cells. Viral capsids and extracellular virions were inhibited, and HSV-1 protein synthesis decreased by 50%, although no specific HSV-1 protein failed to be synthesized. Hyperbaric pressure failed to reverse the halothane-induced inhibition of HSV-1 replication.

Halothane (2-bromo-2-chloro-1,1,1-trifluoroethane), a commonly used inhalational anesthetic, inhibits the replication of a number of animal viruses in cultured Vero cells (1a). Of the viruses studied, measles virus is most sensitive to halothane exposure. When Vero cells infected with measles virus at a multiplicity of infection of 0.1 are exposed to 1.8% halothane, the virus H, F, and P proteins are not detected (1). Hyperbaric pressure, which reverses clinical anesthesia (9, 10, 15), also reverses the inhibition of the F and H (but not the P) proteins (1). This probably accounts for the fact that although the antiviral effects of halothane are not completely reversed, more than 100 times as much measles virus is synthesized in the presence of 2.0% halothane at 100 atm absolute (atmA) (10,110 kPa) than at 1 atmA (101.1 kPa) (2).

Herpes simplex virus type 1 (HSV-1) replication in Vero cells is also inhibited in a concentration-dependent manner by halothane (1a). Exposure to 2.4% halothane decreases infectious HSV-1 titers by 3 orders of magnitude, although HSV-1 replication is never completely inhibited at clinical concentrations (less than 3.0%) of the anesthetic. After the removal of halothane, the inhibition of HSV-1 replication is reversed, and control levels of the infectious virus are recovered (1a). In this report we characterize some of the molecular events which occur when HSV-1-infected Vero cells are exposed to halothane, and we report that although both HSV-1 and measles viruses are inhibited in a concentration-dependent and reversible manner by halothane, the mechanism of this inhibition appears to be different for the two viruses.

Effect of halothane on HSV-1 DNA and protein synthesis. HSV-1 DNA replication was unchanged during halothane treatment (Table 1). When the amount of intracellular HSV-1 DNA synthesized in halothane-exposed cells (determined either as counts of [^{3}H]thymidine incorporated per milligram of cellular protein [14] or as the amount of HSV-1 DNA isolated from infected cells producing banding in CsCl [16]) was compared with that in unexposed cultures, there was no difference in the amount of HSV-1 DNA synthesized, regardless of the halothane concentration used.

We did, however, observe that only half as much [^{35}S]methionine or [^{3}H]leucine was incorporated into acid-precipitable material in infected cultures treated with 2.4% halothane as was incorporated in nonexposed controls (Table 1). When HSV-1 proteins were analyzed by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate (7), the banding patterns were unchanged if equal counts of total infected cell proteins were applied to each gel lane (data not shown). No change in the glycosylation pattern of HSV-1 proteins was observed, except that less of the precursor to glycoprotein C was detected in halothane-exposed cells. Since glycoprotein C is not essential for HSV-1 replication in cell cultures (17), this cannot account for the inhibitory effect of halothane.

Effect of halothane on capsid assembly. When Vero cell monolayers became confluent, they were infected with HSV-1 at a multiplicity of infection of 5 to 10 PFU per cell. The virus was adsorbed for 1 h; the cells were then washed once with TBS (50 mM Tris [pH 7.8] containing 150 mM NaCl). At 5 h postinfection, 10 μCi of [^{3}H]thymidine label (New England Nuclear Corp.) or 5 μCi of [^{35}S]methionine label (Amersham Corp.) per ml or both were added. Cells were harvested when cultures not exposed to the anesthetic revealed cytopathology in 100% of the cells. The cells were then pelleted at 1,500 × *g* for 10 min, washed once in TBS, and suspended in 1 ml of 10 mM Tris (pH 7.8) containing 25 mM NaCl. Infected cells were disrupted with 15 strokes of a loose-fitting Dounce homogenizer, and the nuclei were pelleted at 800 × *g* for 10 min. The nuclei were suspended in 1 ml of 50 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES) buffer (pH 7.5) containing 100 mM NaCl, 0.5% Brij (Atlas Chemical Industries, Inc.), 0.5% deoxycholate (Sigma Chemical Co.), 10 mM $MgCl_2$, and 100

TABLE 1. Effect of halothane on HSV-1 DNA and protein synthesis[a]

Halothane concn (%)	DNA synthesis[b] (%) ± SD	HSV-1 protein[b] (%) ± SD
0	100	100
2.4[c]	99.6 ± 8.4	48.9 ± 20.3
0[d]	95.2 ± 8.8	121.7 ± 26.7

[a] Average of three experiments, each done in triplicate.
[b] Counts per minute incorporated per milligram of cellular protein; expressed as percentage of nonexposed controls.
[c] Halothane present until cells unexposed to the anesthetic demonstrated cytopathology in 100% of cells (ca. 30 h).
[d] Twelve hours after removal of 2.4% halothane.

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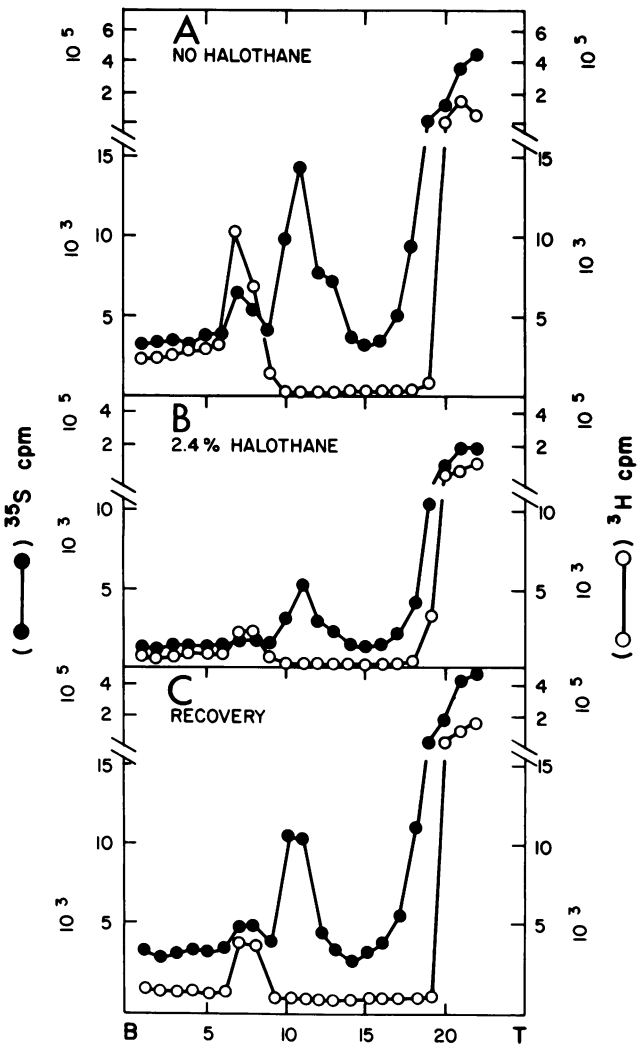

FIG. 1. Effect of halothane on nucleocapsid synthesis in Vero cells. Nucleocapsid proteins were labeled with [^{35}S]methionine, and the DNAs were labeled with [^{3}H]thymidine. Recovery time was 12 h after the removal of the 2.4% halothane. B, Bottom of gradient; T, top of gradient.

μg of DNase 1 (Sigma) per ml. Samples were incubated for 20 min at 37°C; reactions were then terminated by the addition of 15 mM EDTA. Nucleocapsids were isolated by rate zonal centrifugation (80 min at 18,000 × *g*) in 10 to 40% (wt/vol) sucrose gradients containing 100 mM NaCl and 50 mM HEPES buffer (pH 7.5). The assembly of HSV-1 capsids was inhibited during halothane exposure. Two distinct populations of nucleocapsids, which could be separated by rate zonal centrifugation (6), were synthesized in HSV-1-infected Vero cells (Fig. 1A). Comparison of the amounts of [^{35}S]methionine incorporated into the nucleocapsid material shows that during halothane treatment, the slowly sedimenting peak was approximately one-third the size of its control counterpart, whereas the rapidly sedimenting DNA-containing material was virtually absent (Fig. 1B). The DNA-containing nucleocapsid peak reappeared after removal of the halothane (Fig. 1C).

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis analysis (7) was performed on the material isolated from each of the nucleocapsid peaks shown in Fig. 1. There was no difference observed either in the number of protein species detected or in the amounts of each protein present when identical numbers of counts were compared (data not shown). This was true for peaks of both heavy and light capsids, whether infected cultures were or were not exposed to halothane. Therefore, although less nucleocapsid material was synthesized in the presence of halothane, there was no detectable difference in the protein composition of those capsids which were assembled.

Effect of halothane on Vero cell protein synthesis. When Vero cells were exposed to halothane, the amount of radiolabel incorporated into acid-precipitable peptides decreased as a function of both the concentration and duration of anesthetic exposure (Fig. 2). At a concentration of 1.0%, no significant halothane-induced depression of protein synthesis occurred during the first 24 h of exposure. However, by 48 h, the rate of protein synthesis was less than half that seen in the control cultures. A 2.0% concentration of halothane inhibited protein synthesis by 50% within 24 h, and 3.0% halothane depressed protein synthesis by 40% within 6 h and almost entirely after 48 h.

When the Vero cell proteins that were synthesized during halothane exposure were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (13), we observed that a generalized depression in protein synthesis occurred. When equal counts were run in each gel lane, protein banding patterns were identical, and very few protein bands decreased in intensity as a result of halothane exposure, regardless of the halothane concentration or exposure time (data not shown).

Effect of pressure on halothane-induced HSV-1 inhibition. Increases in ambient pressure in the range of 100 to 300 atmA (10,110 to 30,330 kPa) antagonize anesthetic-induced narcosis (8, 9, 10, 15) as well as several anesthetic-induced alterations in cell structure and function (2, 4, 5, 11). A set of experiments was performed to examine the effects of pressure on HSV-1 inhibition. For each experiment, six 48-mm glass petri dishes and two 8-oz (ca. 237 ml each) glass bottles

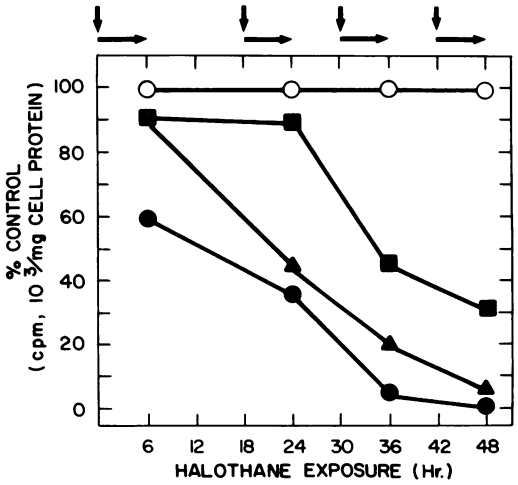

FIG. 2. Rate of Vero cell protein synthesis for cultures exposed to different concentrations of halothane. At the times indicated by the vertical arrows, 100 μCi of [^{3}H]leucine (10 μCi/ml) was added to the Vero cultures, which were incubated for 6 h (shown by horizontal arrows) and then harvested. Shown are the rates of Vero cell protein synthesis for cultures exposed to 0% (○), 1.0% (■), 2.0% (▲), and 3.0% (●) halothane. Datum points shown represent the means of three experiments.

TABLE 2. Effect of halothane and pressure on HSV-1 cultures grown in vero cells

Treatment	Pressure level (atmA)	No. of expts	Mean virus titers (PFU/ml) ± SD	% Control
Air (control)	1	4	$5.5 \times 10^8 \pm 0.8 \times 10^8$	100
Halothane	1	4	$1.2 \times 10^6 \pm 0.1 \times 10^6$	0.2
Pressure	100	4	$5.4 \times 10^7 \pm 0.5 \times 10^7$	9.7
Halothane and pressure	100	3	$2.5 \times 10^5 \pm 0.2 \times 10^5$	0.04

of Vero cells were inoculated with HSV-1 at a multiplicity of infection of 3 to 5. Two of the petri dishes were equilibrated with 95% air–5% CO_2 (control), and the two prescription bottles were equilibrated with 2.4% halothane in 95% air–5% CO_2 for 20 min. The other petri dishes were placed in a Parr cell disruption bomb which had the siphon tube removed. Halothane (2.4%) in 95% air–5% CO_2 was allowed to flow through one of the bombs for 20 min. The second bomb was prepared identically, except that halothane was omitted. Both bombs then were pressurized with helium (used because it is chemically inert, has low lipid solubility, and allowed us to keep the partial pressures of all other gases identical to nonpressurized controls) to 100 atmA for 10 min. The cultures remained under 100 atmA until the nonpressurized infected control (without halothane) demonstrated virus-specific cytopathology in 100% of the cells (approximately 30 h). Next, the pressurized cultures were allowed to decompress for 10 min. Before the harvest, cells were examined by phase-contrast microscopy to establish gross morphologic changes. All cultures then were harvested for the infectious virus, and the titers were determined by the plaque assay (12).

Hyperbaric pressure did not reverse the halothane-induced inhibition of HSV-1 replication (Table 2). HSV-1 replication was inhibited by the hyperbaric conditions (100 atmA) imposed in these studies, a finding consistent with observations from another laboratory (3), and the inhibitory effects of halothane and pressure were additive (Table 2).

The results suggest that the mechanism of the antiherpesvirus activity of halothane is different from the mechanism by which halothane inhibits measles virus replication. Unlike the selective inhibition observed in measles virus protein synthesis (1), halothane-induced inhibition of HSV-1 protein synthesis and of unanesthetized Vero cells reflected a generalized depression of protein synthesis. Another major difference between these two viruses is that the inhibition of measles virus replication by halothane is antagonized at 100 atmA with helium (2), whereas the anti-HSV-1 effect was not. In fact, the inhibition of HSV-1 replication induced by exposure at hyperbaric conditions was additive to the inhibitory effect of halothane. These differences may occur because the cell fusion (syncytia formation) step of measles virus replication in Vero cells is very sensitive to membrane changes induced by halothane (2), whereas HSV-1 maturation does not normally involve a syncytia-forming step (18).

The halothane-induced inhibition of HSV-1 replication appears to be due to a nonspecific block in virus maturation. The number of DNA-containing capsids assembled in the presence of halothane was decreased by nearly 90%, and infectious HSV-1 titers dropped by 3 orders of magnitude, more than would be expected to result from a 50% reduction in protein synthesis. A 50% reduction in protein synthesis might account for a much greater decrease in the amount of the infectious virus produced if the synthesis of (for example) a rate-limiting assembly protein was involved. Thus, although the failure of HSV-1 capsid and infectious virus particles to assemble may reflect an inhibition in protein synthesis, it is possible that other steps in the HSV-1 maturation process are affected during exposure to halothane as well.

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