ANESTHETIC ACTION

Title: HALOTHANE ALTERATIONS IN VIRAL RNA-PROTEIN SYNTHESIS

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Introduction. Previously we reported the inhibition of measles virus replication in halothane-treated cells. We elected to study the effects of this anesthetic on synthesis of the viral nucleocapsid, a ribonucleic acid-protein complex. Analysis of this viral component allowed us to look at the effects of anesthesia on measles virus protein and RNA synthesis and the interaction between them. In this way we could study the intracellular molecular perturbation produced by halothane.

Methods. Vero cells, a continuous primate cell line, were grown to confluency in sealed glass roller bottles. Cultures were exposed to 95% air-5% CO₂, with or without 1.8% halothane. Anesthetic vapor concentrations were assessed by gas chromatography; losses from the sealed containers were approximately 5% over a 24-hr period. Virus was inoculated (0.05 PFU/cell) in these cultures and allowed to incubate for one hour just prior to the exposure of the cells to the anesthetic. Cells were exposed to 20 μg of actinomycin D per ml 24 hours after infection and 2 hours later 20 μCi of [5-3H] uridine label per ml was added. At the end of the labeling period, 40 hrs following infection, cells were harvested. A cytoplasmic extract was prepared as follows: medium was removed and the cells were scraped into 50 ml of Tris-buffered saline. The cells were then pelleted at 1,500 x g for 10 min, and resuspended in 1 to 3 ml of a hypotonic buffer. Cells were disrupted by 15 strokes of a Dounce homogenizer. Nuclei were pelleted at 800 x g for 10 min. Labelled structures in the decanted cytoplasmic extract were separated by rate-zonal centrifugation in 15 to 40% (wt/vol) sucrose gradients. Gradients were fractionated and radioactivity incorporation into RNA determined by liquid scintillation counting.

Results. Incorporation of uridine label into measles virus was markedly enhanced by exposure of the cells to halothane (figure 1). This occurred in Vero cells using two different strains of measles viruses. Analysis of the nucleocapsid RNA was performed by disrupting the nucleocapsid protein and sizing of the RNA on sucrose gradients. These results indicated that most of the RNA produced during halothane treatment is substantially shorter than the typical 50S genomic RNA of wild type measles virus.

Discussion. Anesthetics are known to influence the intracellular biochemical milieu. We have provided evidence using measles virus nucleocapsid production, that RNA and protein synthesis undergo interesting changes in the presence of halothane. Despite inhibition of infectious virus progeny, synthesis of complete and defective viral products is enhanced. Since the viral nucleocapsid requires a membrane component to become infectious, one could postulate halothane disturbance of the nucleoprotein-RNA-membrane interaction. The increased defective nucleocapsids may have biological significance. These entities have been suggested as having role in the pathogenesis of slow viral diseases and viral carcinogenesis. Our data demonstrate that halothane anesthesia greatly affects the molecular events during measles virus replication. These studies suggest a molecular probe for the investigation of the intracellular perturbations produced by halothane.

Figure 1. Nucleocapsid profile of measles virus-infected Vero cells. The CC strains of measles virus was used to infect Vero cells either in the presence (●) or absence (○) of 1.8% halothane. Profiles were determined as described in Methods with the bottom (B) of the gradient to the left.

References.