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The Metabolic Effects of Halothane on Mammalian Hepatoma Cells in Vitro:

I. Inhibition of Cell Replication

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Suspension cultures of rat hepatoma cells growing logarithmically were exposed to 0.1–5.0 per cent halothane for 24 hours. Halothane inhibited cell multiplication in a dose-related manner. This effect was completely reversible for all doses tested upon removal of halothane from the cultures. Highly significant inhibition of growth was detected within six hours. Cell viability was unaffected. High, but nontoxic, concentrations of acetate, pyruvate, and lactate did not prevent halothane-induced inhibition of cell multiplication. (Key words: Cell multiplication; Halothane; Cell viability; Acetate; Pyruvate; Lactate; Growth kinetics.)

SEVERAL INVESTIGATORS have studied the effects of halothane on cells in tissue culture. Low concentrations of halothane administered for as long as three days were reported to have no effect on the morphology of human liver cells in culture.[1-3] However, it is recognized that in sufficient concentrations, most anesthetics, as well as some narcotics and tranquilizers, interfere with cell multiplication.[4] Indeed, Fink has demonstrated that four days of halothane treatment produced significant dose-related depression of the growth of mouse heteroploid and sarcoma Ia cultures.[5,6] In addition, very high concentrations of halothane have been shown to cause cytolysis of rat hepatoma cells in tissue cultures.[7]

The present paper describes the effects of halothane on the replication and viability of rat hepatoma cells exposed to 0.001–0.05 atmosphere halothane for periods as long as 24 hours, and demonstrates that intermediary carbohydrate substrates do not affect the observed reversible inhibition of growth.

Methods

Rat hepatoma cells (HTC) were maintained in suspension culture as described previously.[8,9] A closed system was employed to deliver voltatile anesthetics to the HTC cells. Within a 37-C warm room, premixed 98 per cent air/2 per cent carbon dioxide flowed through a temperature-compensated halothane vaporizer † at a rate of 550 ml/min. The anesthetic atmosphere was sterilized by passage through a 0.22-μm filter and partially humidified by passage over distilled water.‡ The resulting gas mixture was conducted through a short length of rubber tubing (in parallel) over 100 ml of stirred culture medium contained within a sealed 500-ml glass bottle. The gaseous exhaust was removed from the bottles via suction apparatus to eliminate laboratory contamination. An 18-gauge, 8-inch stainless steel needle inserted through a small rubber stopper was positioned 1) in the culture medium for injection and/or removal of non-gaseous material, or 2) in the atmosphere overlying the medium for sampling gases. The carbon dioxide and oxygen tensions of the culture medium and overlying gas were determined with appropriate electrodes, as was the *p*H of the culture. Concentrations of halothane in both the medium and the gas were measured by gas chromatography.

In a typical experiment, at zero time 10 ml of logarithmically-growing HTC cells (750,000 cells/ml) were added to 90 ml of medium pre-

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† Tropical model Vapor halothane vaporizer, North American Dräger, Telford, Pennsylvania.

‡ The volume of water lost from culture bottles during a 72-hour experiment was negligible.

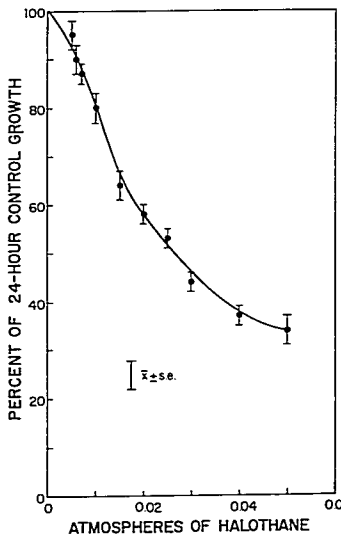

FIG. 1. Inhibition of cell culture growth by exposure to halothane for 24 hours. Growth inhibition is determined by expressing the growth rate of a drug-treated culture as a percentage of the growth rate of its control culture. Each point represents the mean, and the accompanying bars represent the standard error.

equilibrated § with carrier gas or halothane in carrier gas (four bottles in each control or treated group). Halothane was studied in the concentration range of 0.001–0.05 atmosphere (0.1–5.0 per cent). Cell count and viability (measured by erythrocin B exclusion technique [10]) were determined at zero time and after 24 hours of exposure to gas, at which time halothane was discontinued. Six hours were necessary for complete elimination of halothane from the bottles, and counts and viabilities were determined at 54 and 78 hours to test for reversibility. Each halothane concentration was tested on at least four separate occasions.

§ Pre-equilibration was achieved overnight, although 90 per cent equilibration was achieved within 180 minutes.

The growth rate of cell cultures is expressed as the inverse of the doubling time in generations per hours, and growth inhibition is expressed as the ratio of growth rates of the treated to control cultures.[8] Reversibility of growth inhibition was defined as a re-establishment of the control growth rate.

In order to outline precisely the time course of halothane's inhibition of HTC culture growth, cells were exposed to 0.025 or 0.04 atmosphere halothane (two experiments for each concentration), and cell counts and viabilities were determined every two hours.

In additional experiments, prevention of growth inhibition with acetate, pyruvate, and lactate was attempted. The experimental design was identical to that of the 24-hour growth study except that sodium acetate, sodium pyruvate, or sodium lactate was added to the pre-equilibrated medium to final concentrations of 10^{-4}–10^{-2} M prior to the addition of the cells.

Results

Twenty-four hours of treatment with 0.001–0.05 atmosphere halothane inhibited HTC cell replication in a dose-dependent manner (fig. 1). The maximum dose, 0.05 atmosphere, inhibited replication by 65 per cent. Throughout the range of halothane doses examined, this inhibition was completely reversible upon withdrawal of the anesthetic. In this concentration range of halothane, HTC cell viability was not affected. Examination of the initial growth kinetics indicate that significant ($P < 0.01$) inhibition of cell replication occurs within six hours at 0.025 atmosphere halothane (table 1).

Neither sodium acetate nor sodium pyruvate nor sodium lactate (10^{-4}–10^{-2} M) prevented the inhibition of HTC cell replication induced by 0.01 and 0.03 atmosphere halothane (table 2). Concentrations of these substances higher than 10^{-2} M markedly inhibited the growth of control cells.

Discussion

Previous investigations have demonstrated that treatment of human liver-cell cultures with lower concentrations of halothane (26 mg/100 ml culture medium) for three days

caused no morphologic changes, whereas 52 mg/100 ml for two to three days reversibly effected a moderate amount of cytoplasmic vacuolization, and 104 mg/100 ml caused a greater amount of only partially reversible cytoplasmic changes.[1, 2] The partial pressures of halothane corresponding to the last two concentrations mentioned were necessarily very high because of the low partition coefficient of halothane in culture medium. Similar effects with low concentrations of halothane were reported by Rees, who found that the morphology of differentiated human embryo liver cells in culture was unaffected by a single exposure to 1 per cent halothane for two to 24 hours.[3]

Fink described the effects of halothane on the multiplication of cells in culture. Administration of halothane for four days to mouse heteroploid and sarcoma Ia cultures caused dose-related inhibition of growth.[5, 6] Using another cell line (HTC) we have demonstrated a dose-dependent inhibition of multiplication of cells treated with halothane for one day, and this result correlates well with that of the four-day experiments. In addition, we have examined both the temporal course and the reversibility of the growth inhibition. The growth-kinetics data indicated that the inhibition was significant ($P < 0.01$) within six hours for 0.025 atmosphere halothane. For all doses examined, removal of the halothane resulted in reversal of the observed growth inhibition and re-establishment of the control growth rate. This is in agreement with our finding that HTC cell viability is unaffected by exposure to halothane concentrations as high as 0.05 atmosphere for 24 hours. The inconsistency with experiments demonstrating cytolytic effects of halothane on HTC cells [7] may be explained by the fact that in the experiments demonstrating cytolysis, liquid halothane was added to cultures to achieve initial concentrations exceeding 100 mg/ml, a technique which results in very high (> 0.1 atmosphere, unpublished data) partial pressures of halothane.

As our experiments were conducted with initially asynchronous cell cultures, we were unable to determine whether the inhibition of cell division was related to an effect on a specific stage of the cell cycle.

In his studies to elucidate the mechanism(s) underlying halothane's inhibition of cell culture growth, Fink showed that growth-inhibitory concentrations of halothane decreased aerobic metabolism while concomitantly in-

TABLE 1. Initial Growth Kinetics

Time (Hours)	0.025 Atmosphere Halothane		0.040 Atmosphere Halothane	
	Cell Count* ($\times 10^3$/ml)		Cell Count* ($\times 10^3$/ml)	
	Control	Treated	Control	Treated
0	101.1 ± 3.7	101.1 ± 2.5	79.9 ± 2.2	80.0 ± 3.4
2	104.1 ± 2.9	104.3 ± 2.9	84.5 ± 2.7	82.8 ± 2.6
4	111.6 ± 2.3	108.9 ± 3.0	90.1 ± 3.0	85.5 ± 4.7
6	119.0 ± 1.2	111.5 ± 2.7†	103.7 ± 3.1	87.4 ± 3.5†
8	134.3 ± 1.8	118.4 ± 4.2†	120.8 ± 5.5	92.1 ± 5.6†

* Mean ± SE.
† Difference between cell counts significant ($P < 0.01$): two-sided t test—figures obtained from Student's t table with 10 degrees of freedom.

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TABLE 2. Effects of Acetate, Pyruvate and Lactate on Growth

Supplemental Substrate	Control		0.01 Atmosphere Halothane		0.03 Atmosphere Halothane	
	Cell Count ($\times 10^3$/ml)*		Cell Count ($\times 10^3$/ml)*		Cell Count ($\times 10^3$/ml)*	
	Zero Time	24 Hours	Zero Time	24 Hours	Zero Time	24 Hours
None	78 ± 4	160 ± 7	72 ± 5	127 ± 9	76 ± 5	105 ± 8
Sodium acetate, 10^{-2} M	79 ± 6	166 ± 10	78 ± 5	135 ± 10	74 ± 6	100 ± 8
Sodium pyruvate, 10^{-2} M	76 ± 5	152 ± 10	76 ± 5	131 ± 9	76 ± 5	103 ± 8
Sodium lactate, 10^{-2} M	81 ± 5	162 ± 10	78 ± 4	135 ± 7	75 ± 5	103 ± 7

* Mean ± SE.

creasing glucose uptake and metabolism by anaerobic glycolysis.[5,6] Supplementation of the culture medium with additional glucose antagonized halothane's growth-inhibitory effects. In rat hepatoma cultures growth-inhibitory concentrations of halothane have been shown also to increase the cellular uptake of acetate.[7] An unspecified portion of the acetate is found in the cellular lipid fractions, but information concerning other metabolic fate(s) of the acetate is unavailable. Acetate, pyruvate, and lactate (and fructose) have been demonstrated to reverse the myocardial (atrial strips) contractility-depressant effects of halothane, an effect apparently secondary to bypassing the blocked glucose phosphate isomerase-mediated step of the glycolytic pathway.[11-12] Whether these intermediary carbohydrate substrates reverse other depressant effects of halothane has not been examined. Preliminary work in our laboratory showed that both treated and untreated HTC cells also take up exogenously administered pyruvate and lactate. However, high (10^{-2}–10^{-4} M), but nontoxic, concentrations of these three intermediary compounds failed to prevent the inhibition of HTC cell replication by halothane. This indicates that 1) pyruvate, lactate, and acetate reversal of inhibitory effects produced by halothane is not a generalized phenomenon, and 2) there is probably not a common mechanism underlying halothane's inhibitory effects on myocardial contractility and cell replication.

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