Title : METABOLITE BINDING TO LIVER NUCLEI DURING LOW LEVEL EXPOSURE TO HALOTHANE

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Introduction. The possibility of halothane metabolite binding to nuclear components is of interest due to persistent reports which suggest genetic alterations following long term exposure to anesthetics. This interest has been rekindled by a recent report which shows that exposure of rats to low levels of nitrous oxide and halothane results in significant dose dependent increases of chromosomal abberations in proliferating spermatogonial or bone marrow cells (1). Furthermore, new studies of chromosomal proteases(2,3) suggest that other previous studies may not have detected metabolite binding to nuclear components due to proteolysis during isolation procedures. Because liver cell growth is very slow in adults, metabolite binding after a single dose of halothane is unlikely to simulate conditions of patient exposure or longer term exposure of operating room personnel to halothane. Therefore, we have stimulated liver cell growth in adult animals by various techniques during halothane exposure and we have devised a simply-constructed capsule for linear low level halothane release rates in experimental animals. After such exposure to halothane, metabolite binding to chromosomal histones, non-histone chromosomal proteins, DNA and the inner nuclear membrane has been determined.

Methods. A halothane delivery capsule suitable for delivery of 30 ul of halothane was constructed from 6452 teflon tubing and #280 polyethylene tubing. A 2 cm piece of teflon tubing was inserted into 3 cm of polyethylene tubing and the polyethylene tube heat sealed at one end. The tube was cooled in dry ice and halothane introduced with a microliter syringe and the top end heat sealed leaving a 3-5 mm air space between halothane and the completed seal.

Adult rats were either partially hepatectomized or treated with triiodothyronine to stimulate liver growth. Halothane, (120 uCi, 12 ul) contained in an abdominally implanted capsule, was released over a six hour period. Rats were kept in 8-10 percent oxygen during exposure to halothane. Liver chromatin was isolated from Triton X-100 purified nuclei in the presence of EDTA, PMSF and sodium butyrate to inhibit nuclear proteases. Acid extraction of chromatin yielded histones and non-histone chromosomal proteins which were isolated and counted after SDS-polyacrylamide gel electrophoresis.

Results. The release rate of halothane from the capsule was linear with time for a period of hours to several days. The release rate is proportional to temperature, capsule length, cross sectional area and tube wall thickness. Halothane diffusion may be slowed by adding propylene glycol to the halothane or increased by omitting the teflon sleeve before final sealing of the capsule. With the capsule constructed as described (32 ul, 60 mg) halothane with propylene glycol concentrations of 0, 10 or 30 percent, was released at 1.4, 0.8 or 0.4 mg/hr, respectively. When the teflon sleeve was omitted, the halothane release rates from capsules of #90, #190 or #240 polyethylene tubing were 9.8, 7.5 or 2.2 mg/hr, respectively.

Of the total covalently-bound halothane metabolites (2 x 10^6 dpm) found in livers of either untreated controls or growth-stimulated rats exposed to C-14 halothane, only approximately one percent (20,000 dpm) was associated with the purified nuclear fraction. Of this remaining radioactivity, 2000 dpm was found in nuclear membrane lipids, 8000 dpm was found in purified chromatin and of this, none were in DNA, 45 dpm were in histones and 2200 dpm were associated with non-histone chromosomal proteins.

Discussion. Histones were isolated free of contaminating cytoplasmic protein and lipid by SDS gel electrophoresis. The majority of halothane metabolites bound to chromatin is associated with non-histone chromosomal proteins, which, however, cannot be distinguished from similar molecular weight cytoplasmic protein contaminants.

The only nuclear components clearly labeled are lipids of the nuclear membrane, yet labeling of these membranes may also arise from cytoplasmic microsomal lipids during isolation of nuclei. These results indicate that minimal specific nuclear or chromosomal modifications result from halothane metabolism.

References.

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