Date :

Title : HALOTHANE & HYPOXIA: QUANTITATIVE E.M. ANALYSIS

Authors : W. T. Ross, Jr., M.D.

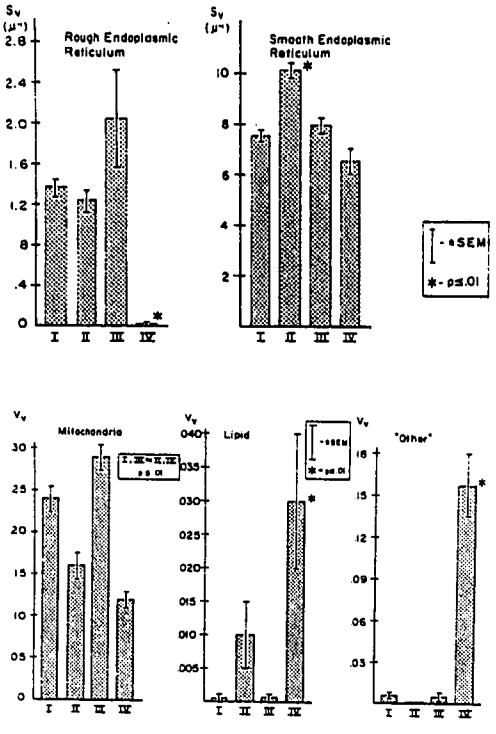
Affiliation: Department of Anesthesiology, University of Virginia Medical Center, Charlottesville, Virginia 22908

Introduction. Centrilobular liver necrosis occurs when phenobarbital treated rats receive halothane while hypoxic. Previous reports have described biochemical changes and light microscopic findings after such treatment. Quantitative electron microscopic studies have not been previously reported. We report here the effects of halothane and hypoxia on hepatic ultrastructure in phenobarbital treated rats and demonstrate quantitative assessment of electron microscopic images using the morphometric techniques of Weibel.[1]

Methods. 20 male Wistar rats weighing 250 to 300g were maintained in our vivarium and exposure to xenobiotics was avoided. Gas mixtures were administered in a 24 liter glass enclosure at flows of 10 L/m to ensure rapid washout and to prevent CO_2 accumulation. During halothane exposure the enclosure was warmed with radiant heating to maintain a neutral thermal environment. Four groups were established at random: Group I - Normal animals breathed air for one hour. Group II - Pretreated with phenobarbital (75 mg/kg i.p. q.d. x 4 days) and exposed to 0.6% halothane in 50% oxygen for one hour. Group III - Normal animals breathed 0.6% halothane in 8% oxygen for one hour. Group IV - Pretreated with phenobarbital (as above) and breathed 0.6% halothane in 8% oxygen for one hour. All were fasted for 24 hours following exposure and sacrificed at that time. The livers were prepared for electron microscopy using standard techniques. Tissue blocks were oriented and sections obtained so that the location of cells within the lobule was established. Electron micrographs were prepared of centrilobular cells with a final magnification of approxiamtely 16,000. Intracellular structures which occur as surfaces and appear in section as single lines (such as endoplasmic reticulum) were counted when they intersected reference lines on a test grid randomly placed over the micrograph. Intracellular structures occupying a volume within the cell (such as mitochondria) were counted when points on a test grid fell within the area of the structure as it appeared in the electron micrograph. Estimates of surface area per volume of cytoplasm for the two dimensional structures (surface density, Sv) were calculated and a volume estimate per volume of cytoplasm (volume density, Vv) was calculated for the three dimensional structures using standard formulae.[1] These data were compared for each of the four groups using the analysis of variance. When the F statistic was significant, significant differences between means were identified by calculating the least significant difference for $P \leq .01$.

Results. Organelles that were examined but were not different in the four groups were Golgi membranes, lysosomes, microbodies, and glycogen. Figure 1 displays the measurements for those organelles showing significant differences between groups. After halothane and phenobarbital, Group II, smooth endoplasmic reticulum was increased and mitochondria were decreased. Phenobarbital pretreatment followed by halothane and hypoxia, Group IV, resulted in almost complete absence of rough endoplasmic reticulum while lipids and "other" volume occupying structures were increased. These "other" structures were largely electron dense areas occurring in association with the endoplasmic reticulum in the severely injured cells. The mitochondria, while identifiable in Group IV, were abnormal and many contained electron dense areas consistent with severe damage.

Discussion. The increase in smooth endoplasmic reticulum and decrease in mitochondrial volume in the Group II animals is a response to the phenobarbital pretreatment and has been reported by others. In this model of halothane precipitated liver damage, the almost complete absence of identifiable rough endoplasmic reticulum in the Group IV animals and the occurrence of electron dense regions ("other") within membranes of the endoplasmic reticulum suggest that halothane and hypoxia affect ribosome function. This would be expected to interfere with protein synthesis and maintenance of proteins within cells and could account for the observation that the most severe damage appears to be to membranes of the endoplasmic reticulum.



References. 1. Weibel ER: Stereological principles for morphometry in electron microscopic cytology. Int Rev Cytol 26:235-302, 1969

Supported, in part, by NIH Grant R23 GM25117