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Title : HALOTHANE STABILIZES THE OUTER MITOCHONDRIAL MEMBRANE

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Introduction. Techniques for separating outer and inner membranes of liver mitochondria have contributed to the study of biochemical function and submitochondrial localization of enzymes. It is established that monoamine oxidase (MAO) activity is a marker enzyme for the outer mitochondrial membrane (OM). Treatment of liver mitochondria with a specific concentration of digitonin removes the outer membrane (OM) from the inner membrane + matrix (IMM) (1). This laboratory is investigating the effects of halothane on the redistribution of enzymes among compartments within mitochondria. The data reported in this abstract show that halothane stabilizes the outer mitochondrial membrane to the effects of digitonin.

Methods. Adult male rats were decapitated and liver mitochondria were prepared by conventional techniques in H-medium (sucrose 0.7M; mannitol 0.21M; HEPES 0.002M; BSA 0.05% (1). Respiratory control ratios (state 3:state 4 rates) with glutamate substrate were always greater than 5 before further treatment of mitochondria.

Suspensions of mitochondria in ice cold H-medium (50 mg protein/ml) were treated with digitonin (0.1 to 0.18 mg digitonin per mg protein) for 15 min and subjected to differential centrifugation to separate outer membranes (OM), inner membranes + matrix (IMM) and inter-membranal fluid compartments according to established techniques (1). MAO activity was assayed by the method of Tabor (2).

For controls, compressed air was perfused over the suspensions of mitochondria for 15 min before and during the 15 min after the addition of digitonin. Halothane 4% in compressed air was delivered from a calibrated vaporizer and perfused over mitochondrial suspensions for 15 min before and during the 15 min after the addition of digitonin. The concentration of halothane in the suspensions (measured by gas chromatography) reached equilibrium before the addition of digitonin and was constant throughout the incubation period. Centrifugations of halothane treated suspensions were performed in sealed tubes so that the anesthetic did not vaporize off until after separation of membrane fractions. MAO activity (total activity and specific activity) was assayed in each fraction after treatment with digitonin and centrifugation.

Results. Table 1 shows that at each concentration of digitonin, much more MAO activity remained with the IMM when mitochondria were equilibrated with 4% halothane vapor. The abrupt change in MAO distribution with a small increase of digitonin concentration is consistent with reported techniques (1). The total MAO activity (OM + IMM) did not change among preparations, so halothane did not affect intrinsic activity of the enzyme.

Table 1. MAO activity (% of total) remaining attached to inner membrane (IMM)

mg digitonin per mg protein :	0.14	0.15	0.18
mitochondria (control)	44.1 46.0 (45.0)	10.8 14.7 (12.8)	1.7 2.6 (2.2)
mitochondrial (4% halothane vapor)	84.6 71.6 (78.1)	49.9 64.5 (57.2)	8.6 13.3 (11.0)

Each experiment was done in duplicate with different mitochondrial preparations. Mean values are given in parentheses. Appropriate amounts of total MAO activity appeared in corresponding OM fractions so the total activity was unchanged regardless of digitonin or halothane concentration.

Discussion. Decreased MAO activity in the low speed pellet (IMM fraction) is an accepted index for the separation of outer and inner mitochondrial membranes (1). Halothane does not completely inhibit the effect of digitonin. However, when halothane is present, higher concentrations of digitonin are required to achieve separation of the inner and outer membranes. It is known that digitonin has a specific affinity for cholesterol. The data suggest that halothane interferes with the normal interaction of digitonin with membrane lipids (probably cholesterol).

The observations may be significant for several reasons. Molecular interactions of halothane with lipids may have a common basis in other membranes (e.g. nerve and cardiac cells). Digitonin is derived from foxglove and has a molecular structure analogous to the cardiac glycosides (i.e. aglycone ring + carbohydrate side chain). Therefore, molecular actions of cardiac glycosides with membranes of cardiac cells might be influenced by halothane in a related mechanism. Continuing experiments will investigate effects of halothane on redistribution of other mitochondrial enzymes and will employ a range of halothane concentrations.

References.

1. Schnaitman C, Greenawalt JW: Enzymatic properties of the inner and outer membranes of rat liver mitochondria. J Cell Biol 38:158-175, 1968.
2. Tabor CW, Tabor H, Rosenthal SM: Purification of amine oxidase from beef plasma. J Biol Chem 208:645-661, 1954.

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