Title: BINDING OF HALOTHANE METABOLITES TO RAT LIVER MICROSOMAL LIPID

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Introduction--Covalent binding of halothane metabolites to protein and lipid occurs in liver microsomes as a consequence of the biotransformation of the anesthetic.[1] The binding to lipid is evident when biotransformation takes place at low oxygen partial pressure[2] which is one of the conditions found to be necessary to produce centrilobular necrosis with halothane in vivo.[3] Thus, metabolite binding might play a role in this toxicity. Previous studies suggest that the lipid involved in binding is principally phospholipid[2] and Trudell et al[4] have reported the structure of a halothane metabolite bound to a fatty acid of a phospholipid in a reconstituted system containing human P-450. This investigation examines the nature and diversity of the lipid binding site of halothane metabolites in rat liver microsomes.

Methods--Liver microsomes were prepared from phenobarbital-treated male Sprague-Dawley rats, and in vitro incubations with 1-^{14}C halothane were carried out as previously described.[2] Microsomal lipids were extracted by the method of Bligh and Dyer.[5] Phospholipid classes were separated by thin layer chromatography (TLC) as described by Skipski.[6] Neutral lipids were separated by TLC in hexane, diethyl ether and acetic acid (70:30:1). Lipid bands were scraped from the TLC plates and ^{14}C content was determined by scintillation spectrophotometry. Phospholipid fatty acids were extracted into petroleum ether after alkaline hydrolysis in 90% methanol-NaOH. Lipid phosphate was determined by the method of Bartlett.[7] Results are expressed as mean ± standard error.

Results--Lipid extracted from microsomes after incubation with halothane bound 198.78 ± 1.34 nmole ^{14}C/gm of lipid (n=5). Table 1 lists the label content of the major phospholipid classes resolved by TLC. Table 2 lists the label content of the neutral lipid portions. Phosphatidyl choline was found to bind 358.88 ± 7.91 nmole ^{14}C/mmole lipid phosphate; phosphatidyl ethanolamine bound 517.13 ± 10.06 nmole/mmole lipid phosphate (n=10). The difference in binding is statistically significant, $p < 0.05$. Alkaline hydrolysis and extraction of phospholipid fatty acid (purified, labeled phosphatidyl choline) yielded 91.8 ± 0.5% of the radioactivity with the fatty acid. Autoradiography of TLC plates demonstrated multiple binding sites.

Discussion--Phospholipids are the major binding site for the metabolites of halothane in hypoxic liver microsomes. In general, the degree of binding to a phospholipid class appears proportional to the amount of that lipid in the membrane. Phosphatidyl choline is the most abundant phospholipid in rat liver microsomes and is found to contain the greatest amount of label. Phosphatidyl ethanolamine is bound with a higher specific activity than phosphatidyl choline and this may be due, in part, to a larger portion of unsaturated fatty acids present in rat liver phosphatidyl ethanolamine. Trudell et al[4] have indicated the importance of oleic acid in dioleoyl-phosphatidyl choline in reconstituted systems as the major site for binding of the halothane metabolites. However, the present study suggests that in addition to the phospholipids, neutral lipids may represent an important binding site. Also, of interest in this study is the discovery that a significant percentage of the binding (12.43%) occurs to an unidentified lipid (Table 1) which remains at or near the origin in TLC in a system designed to move the less changed molecules and not those with greater change. The nature of this lipid is not known. Obviously, one important question needs to be resolved; is the relatively broad spectrum of lipid binding important or is there a specific binding site which may play a role in the development of toxicity?

Table 1--Phospholipid Separation

Neutral lipid (solvent front)	11.49 ± 0.2
Phosphatidyl ethanolamine	16.40 ± 0.3
Phosphatidyl serine plus phosphatidyl inositol	11.08 ± 0.2
Phosphatidyl choline	38.82 ± 0.4
Sphingomyelin	3.99 ± 0.0
Lysophosphatidyl choline	3.66 ± 0.0
Origin (unidentified)	12.43 ± 0.9

Table 2--Neutral Lipid Separation

Band 1 (cholesterol ester)	0.26 ± 0.0
Band 2	2.71 ± 0.0
Band 3	1.45 ± 0.0
Band 4 (cholesterol)	0.86 ± 0.0
Band 5 (origin; phospholipid)	93.66 ± 0.1

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