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Concentration-Dependence of Halothane Metabolism in Rabbits

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ABSTRACT

Effect of end-expiratory halothane concentration on its aerobic and anaerobic metabolism was studied in 20 male rabbits.

Biliary excretion of trifluoroacetic acid (TFAA), an aerobic metabolite of halothane, and the pulmonary excretion of CF_2CHCl (CDFF) and CF_3CH_2Cl (CTFE), anaerobic metabolites of halothane, were measured simultaneously during 5 hr of inhalation at various concentrations of halothane (0.02—1.5%).

The total amount of biliary TFAA excreted for 5 hr remained unchanged (38.4μmol~44.1μmol) at each concentration except 0.02%. However, the total amount of CDFE and CTFE excreted for 5 hr increased in a concentration-dependent manner up to 0.5% end-expiratory halothane, and remained unchanged (47.2μmol~64.7μmol) at more than 0.5%. Total biliary excretion of TFAA reached its maximum at a 0.05%.

These results suggest that the primary route of halothane metabolism at a subanesthetic concentration is the aerobic pathway which produces TFAA. However, at anesthetic concentration exceeding 0.5%, the anaerobic route which produces volatile CDFE and CTFE plays a role also as an aerobic route.

Halothane (2-bromo-2-chloro-1,1,1 trifluoroethane) metabolites can be detected *in vivo* as CF_2CHCl and CF_3CH_2Cl[15] in the expired gas and as trifluoroacetic acid (TFAA)[14] in the bile[10] and urine. *In vitro* studies have shown that these three metabolites are produced by NADPH-dependent liver microsomal enzyme system. The former two metabolites are produced under anaerobic condition[6,7], while the latter one under aerobic condition[9]. The concentration of the former two metabolites in the expired gas increases with decrease of inspired oxygen concentration and increases when animals are pretreated with phenobarbital but not with 3-methylcholanthrene[12,19].

Sawyer et al[18] reported that the ratio of the amount of halothane metabolized in the liver to that which entered it (fraction removed) increased as the alveolar halothane concentration decreased. Sada et al[17], measuring plasma bromine concentration, suggested that suppression of halothane metabolism occurred following inhalation of high concentrations of halothane. Sakai[16] and Mazze[13] reported that the serum inorganic fluoride level and urinary inorganic fluoride excretion increased during and after halothane anesthesia. Widger et al[21] demonstrated that halothane anesthesia under hypoxic conditions resulted in a significant elevation in plasma fluoride concentrations. Despite these many reports, there has been no study on the relationship of serum inorganic fluoride level, urinary inorganic fluoride excretion and inhalational halothane concentration. Although bromide and fluoride ion concentrations may serve as indices of halothane metabolism, measurement of these levels alone provides only limited grounds for a more detailed discussion of metabolism due to the involvement of both oxidation and reduction. Furthermore, endogeneous bromide and fluoride prevent an accurate determination of the amount of halothane derived

from them. Therefore, simultaneous measurement of TFAA produced by oxidation as well as CF_2CHCl and CF_3CH_2Cl generated by reduction is necessary for the study of halothane metabolism.

By capillary tube isotachophoretic analysis, a kind of electrophoresis originated by Kohlraush[11], Morio et al[8,14] quantitatively analyzed urinary and serum TFAA during and after halothane administration. Urinary TFAA was negligible during anesthesia, but after cessation it increased, reaching peak values in both groups on the following day. Kikuchi et al[10] attempted to clarify the cause of the delayed occurrence of peak urinary TFAA excretion by directly measuring the biliary TFAA level in an experimental model. TFAA was detected in the bile shortly after initiation of halothane inhalation and earlier than it appeared in urine, suggesting that bile is the primary route of TFAA excretion.

In the present study, the dependency of halothane metabolism on its concentration was examined by simultaneously measuring biliary TFAA, an aerobic metabolite, as well as expiratory CF_2CHCl and CF_3CH_2Cl, anaerobic metabolites.

METHODS

The femoral artery and vein of 20 rabbits weighing 2~2.5 kg were cannulated under regional anesthesia, and the arterial pressure was monitored throughout this study. Tracheostomy was performed under regional anesthesia and the animals were ventilated using a Harvard respiration pump (Model 607)(Fig. 1) with an oxygen supply of 5 liters/min, tidal volume of 5 ml/kg, and ventilation rate of 50 times/min using a nonrebreathing system.

Pentobarbital (20 mg/kg) and Gallamine (1.0 ml) were intramuscularly injected and a quarter dosage of these agents was administered every hour, followed by abdominal incision. After ligating the cystic duct, an external biliary drain was constructed by cannulation of the common bile duct. The bile was collected every 30 min. Halothane anesthesia was maintained with a Dräger Werk Lubeck vaporizer for 5 hr at 6 different end-expiratory halothane concentrations ranging from 0.02 to 1.5%. Bile, urine, expiratory gas, and arterial and venous blood samples were collected 30 min prior to the initiation of halothane inhalation (control) and at 30 min intervals after the initiation. Lactated Ringer's solution was infused during anesthesia at a rate of 20~30 ml/kg/hr to maintain constant arterial pressure. Expiratory gas samples were collected with a 20 ml syringe from a reservoir bag (100 ml) attached to the expiratory outlet of the system and were analyzed with a gas chromatograph (Shimadzu, GC-4A) to determine the concentration of halothane metabolites.

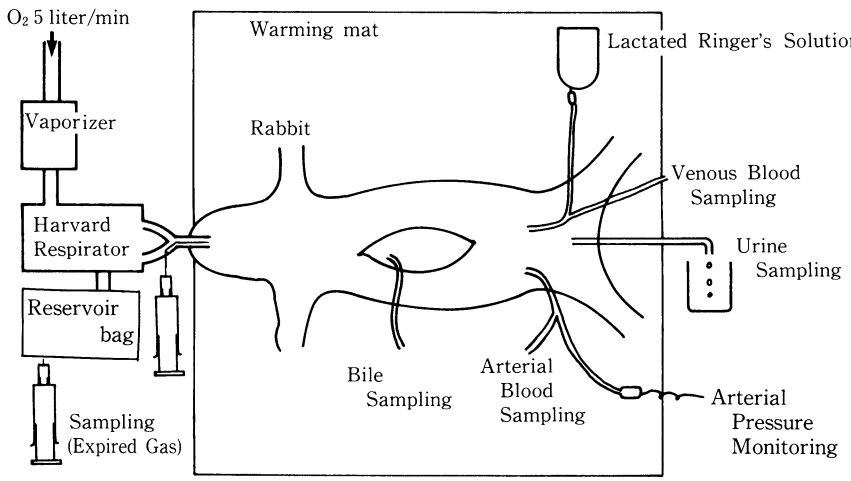

Fig. 1. Diagram of the nonrebreathing anesthetic circuit. Twenty rabbits were given halothane ranging from 0.02% to 1.5% end expiratory concentration with oxygen (5 liters/min), at a tidal volume of 5 ml/kg and a ventilation rate of 50 times/min for five hr.

Biliary, urinary, and blood TFAA contents following confirmation by mass spectrum analysis were quantitated with a capillary-type isotachophoretic analyzer (Shimadzu, Model IP-2A)[14]. CF_2CHCl and CF_3CH_2Cl in the expiratory gas, which were also confirmed by mass spectrum analysis, were quantitatively analyzed with a gas chromatograph (Shimadzu, GC-4A). Arterial halothane and end-expiratory halothane concentrations were determined with gas chromatograph, and arterial blood oxygen partial pressure with a Po_2 electrode (Radiometer BUS2 Uk2) was monitored every 30 min. The body temperature of the animal was maintained at 37°C with a heating pad (Gorman-Rupp Model K-KC-3). The blood flow rate and O_2 tension in the liver tissue were also measured by the hydrogen clearance method (MT-Giken, Model PHG-300) that is theoretically based on polarography[1]. The tissue flow rate was determined every hour after initiation of halothane inhalation, and the mean value at each halothane concentration was expressed as the percentage to the pre-inhalation values.

The data were statistically analyzed by using Student's t test. Data are expressed as mean values ± 1 standard deviation (SD). A probability of chance occurrence less than 5% was considered significant.

RESULTS

During the 5~6 hr duration of the experiment, bile flow did not significantly differ from the control values (6.2 ± 1.7 ml/30 min), though it tended to decrease in general, nor was it significantly affected by various end-expiratory concentrations of halothane. No decrease in flow was noted even at 1.0 and 1.5% end-expiratory concentrations of halothane. Hepatic blood flow measured by the hydrogen clearance method did not significantly decrease in any animal which in various end-expiratory concentrations of halothane, although the values decreased to 84.2 ± 18.5% (n=3) of the pre-inhalation value at 1.5% end-expiratory halothane.

The relationship between arterial and end-expiratory halothane concentration after one hr of halothane anesthesia was found to be directly proportional, showing a linear regression (r=0.984). Therefore, halothane concentration is expressed hereafter as end-expiratory concentration.

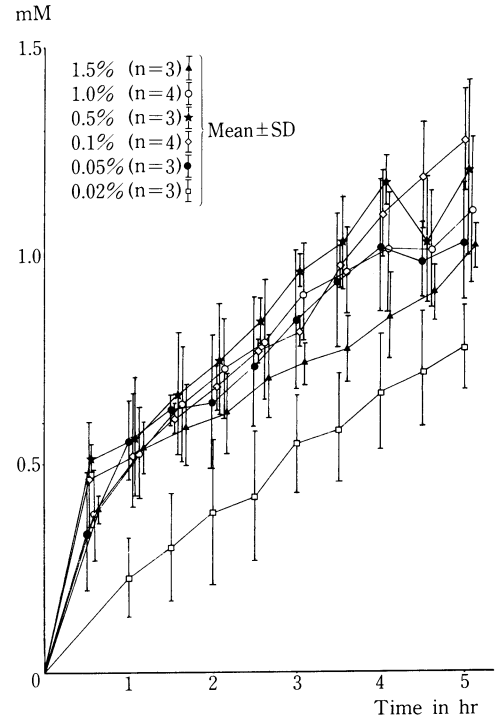

Fig. 2. Effect of end-expiratory halothane concentrations of 0.02%~1.5% on TFAA concentration in the bile (mM, mean ± SD) excreted via external biliary drain from twenty rabbits anesthetized with halothane for five hours.

1. Effect of end-expiratory halothane concentration on TFAA concentration in the bile.

In general, biliary TFAA concentration increased rapidly during the first hour of inhalation, but at slower rates thereafter, throughout the observation period (Fig. 2).

At 0.02%~0.5% end-expiratory halothane concentration, TFAA concentration in the bile tended to increase in an end-expiratory concentration-dependent manner, but statistically significant differences between 0.02% and 0.5% is partially demonstrated. On the contrary, the increments of end-expiratory halothane concentration from 0.5 to 1.5% reduced biliary TFAA concentration, but the difference was not significant (Fig. 2).

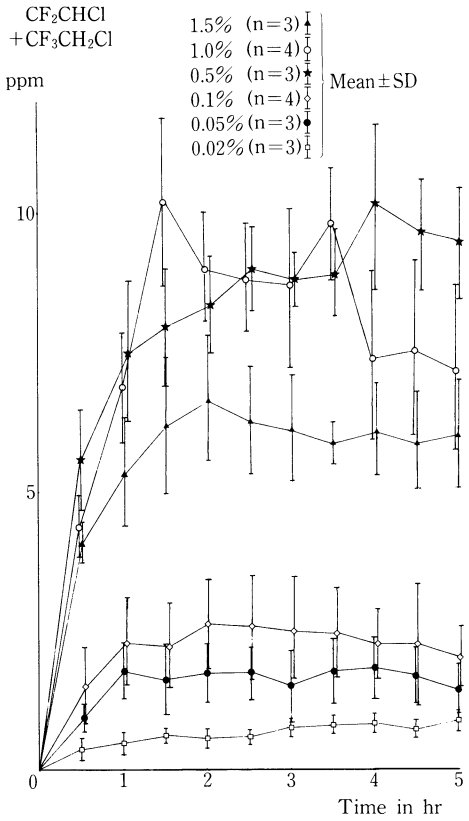

Fig. 3. Effect of end-expiratory halothane concentration on the anaerobic metabolites of CF_3CH_2Cl and CF_2CHCl concentrations in the expired gas from rabbits anesthetized with halothane for five hr.

The metabolite concentration reached a plateau within 1 or 2 hr of halothane anesthesia at each halothane concentration. They also increased with end-expiratory halothane concentration between 0.02% and 0.5%, but decreased at 1.0% and 1.5%.

2. Effect of end-expiratory halothane concentration on anaerobic metabolites (CF_3CH_2Cl and CF_2CHCl) of halothane.

The metabolite concentrations reached a plateau within 1 or 2 hr after halothane anesthesia at each halothane concentration (Fig. 3). The concentrations increased in concentration-dependent manner from 0.02 to 0.5% end-expiratory halothane, but decreased at 1.5%.

3. Effect of end-expiratory halothane concentration on the total amount of three excreted metabolites.

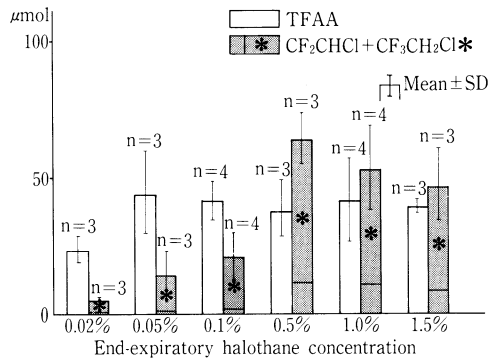

Fig. 4. Effects of end-expiratory halothane concentration on the total amount of TFAA in the bile and the total amount of volatile metabolites (μmol) in the expired gas for five anesthetic hours at various end-expiratory halothane concentrations (0.02%—1.5%).

This figure also demonstrates the relationship between the total amount of an aerobic metabolite (TFAA) and anaerobic metabolites (CF_2CHCl and CF_3CH_2Cl) at each end-expiratory halothane concentration.

The biliary TFAA level was the highest at an end-expiratory halothane concentration of 0.05% (44.1 ± 16.7 umol), but showed no notable changes at higher halothane concentration. The expiratory anaerobic metabolite level was lower at the lower concentration (0.02%～0.1%); however, it was the highest at 0.5% (64.7 ± 9.3 umol) and tended to decrease with an increase in halothane concentration.

Biliary aerobic metabolite (TFAA) and expiratory anaerobic metabolites (CF_3CH_2Cl and CF_2CHCl) excreted during the 5 hr halothane anesthesia were calculated at each halothane concentration (Fig. 4).

The excreted anaerobic metabolites of halothane can be expressed as the sum of these two metabolites, CF_3CH_2Cl and CF_2CHCl. The total amount of excreted expiratory anaerobic metabolites increased in concentration-dependent manner at subanesthetic concentration. The excretion of CF_2CHCl was nominal at halothane concentrations of 0.1% or less. The CF_2CHCl/CF_3CH_2Cl ratio was nearly constant at concentrations higher than 0.5%.

The total amount of excreted biliary TFAA re-

mained unchanged in each concentration except 0.02%.

DISCUSSION

The bile flow measured at 30-min intervals showed no significant change despite the differences in the end-expiratory halothane concentration. The literature has suggested that the bile flow reflects hepatic blood flow[2]. Thus, changes in hapatic blood flow are considered to have been minor in this study. Also the hepatic tissue flow, as determined by the hydrogen clearance method, showed no remarkable change. This relative constancy of hepatic blood flow even at a high end-expiratory halothane concentration may be due partly to sufficient fluid infusion during anesthesia to maintain a uniform circulatory condition. Therefore, transport of halothane and halothane uptake by the liver, which vary by circulatory changes caused by inhalation of anesthetics, might not have been affected in these experimental conditions, even at 1.5% halothane concentration.

Unlike the anaerobic metabolites, the increase in biliary TFAA concentration continued throughout the observation period, which indicated increased TFAA formation in liver cells. The mean biliary TFAA concentration was the highest at an end-expiratory halothane concentration of 0.5%. Above that concentrations, the biliary TFAA level tended to decrease (Fig 2). However, the bile flow in 1.5% of halothane showed not significantly but slightly higher than those in 0.5% of halothane. Therefore, the total excreted amount of TFAA during 5 hr remained unchanged (Fig 4).

Sawyer et al[18] have reported that halothane is more readily metabolized in the liver at lower concentrations. Halothane concentrations lower than 0.02 % was not obtained technically feasible in the study. However, our findings suggest that TFAA production may increase in proportion to the end-expiratory halothane concentration below 0.02% and may also demonstrate that the aerobic halothane metabolism is most prominent in the subanesthetic halothane concentrations.

Our results indicate that the total amount of halothane metabolism differs depending on the end-expiratory concentration (Fig. 4). At subanesthetic concentrations, the primary route of halothane metabolism is an aerobic reaction that produces TFAA, while at anesthetic concentrations, the anaerobic route yielding CF_3CH_2Cl and CF_2CHCl plays an unnegligible role. It is very interesting that a similar experiment done by Fiserova-Bergerova, V.[5] using rats which were exposed to subanesthetic concentration of halothane *in vivo* showed similar results at subanesthetic concentration, though the data did not show at anesthetic concentration.

No report has been made on changes in expiratory and blood halothane concentrations after termination of halothane anesthesia. However, Corbett[4] have detected the agent in the expired gas from 7 to 64 hr following termination of exposure in anesthesiologists. The result suggest that the agent remains in the body, and therefore, the production of TFAA continues for a considerable length of time following its administration. TFAA production is considered to be greater after than during halothane anesthesia, which would explain the delayed occurrence of the peak urinary TFAA excretion on the second postoperative day reported by Morio[11,14] and the presence of urinary TFAA for two postoperative weeks.

In our study, the metabolism of halothane was suppressed when halothane was inhaled at anesthetic concentrations. Sada et al[17] also noted a similar decrease in the metabolism of halothane measuring excreted bromide. Two possible mechanisms for this have been postulated[17]: 1) the amount of halothane reaching the liver, when it is metabolized, may decrease with a reduction in hepatic blood flow due to the anesthetic action of the agent, and aerobic metabolism may be inhibited by the hypoxic condition of the liver resulting from this reduced blood flow, and 2) hepatic metabolism of halothane may be inhibited by the agent itself.

The possibility of the first mechanism may be excluded in our study as mentioned earlier. As for the second possibility that the hepatic metabolism of halothane may be inhibited by the agent itself. Brown[3] has reported that halothane depresses the metabolism of Type I substrates by rat hepatic microsomal enzymes. This inhibition is dose-dependent, reversible, noncompetitive and independent of the liquid solubility of the substrates. In contrast, Type II substrate is enhanced by halothane. These ac-

tions may represent an effect of halothane on the terminal oxidase of the system, cytochrome P 450. Although the mechanism of inhibition of aerobic halothane metabolism could not be clarified by this study, the literature[20] suggests that halothane, a Type I agent, exhibits the absorption spectrum characteristic of Type II agents in an anaerobic condition and binds to the ligand of hemoferrum. It may, therefore, compete with oxygen for binding with hemoferrum, resulting in suppression of its oxidation.

Our results elucidated that the suppression of anaerobic halothane metabolism was shown in anesthetic concentration, and the aerobic metabolism was suppressed in subanesthetic concentration.

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