Inhibition of Lipogenesis by Halothane in Isolated Rat Liver Cells

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1. Halothane at clinically effective concentrations [2.5 and 4% (v/v) of the gas phase of the incubation flask] was found to inhibit significantly lipogenesis from endogenous substrates, e.g. glycogen, or from added lactate plus pyruvate. This was accompanied by a decrease in the ratio of the free $[NAD^+]/[NADH]$ of the mitochondrion and the cytoplasm, as shown by the $[3\text{-hydroxybutyrate}]/[\text{acetoacetate}]$ ratio and the $[\text{lactate}]/[\text{pyruvate}]$ ratio. 2. Acetoacetate or pyruvate decreased the inhibitory effect of halothane and restored lipogenesis to control rates. They were reduced rapidly by 3-hydroxybutyrate dehydrogenase or lactate dehydrogenase respectively, with the concomitant oxidation of NADH and the generation of NAD^+ . 3. These results suggest that the mechanism by which halothane inhibits lipogenesis from glycogen or lactate is by inhibition of the oxidation of NADH; this results in inhibition of flux of carbon through pyruvate dehydrogenase and a shortage of acetyl-CoA for fatty acid synthesis. Thus when NADH acceptors are added in the presence of halothane, the concentration of mitochondrial NAD^+ is raised so that the flux of carbon through pyruvate dehydrogenase increases and lipogenesis is restored,

Halothane (2-bromo-2-chloro-1,1,1-trifluoroethane), a widely used volatile anaesthetic, has been shown to inhibit the oxidation of NADH by decreasing the effectiveness of NADH reductase (EC 1.6.99.3) (Harris *et al.*, 1971; Cohen & Marshall, 1968; Miller & Hunter, 1970). This causes alterations in liver metabolism, which include inhibition of O_2 uptake (Hoëch *et al.*, 1966; Cohen & Marshall, 1968; Biebuyck *et al.*, 1972), inhibition of gluconeogenesis (Biebuyck *et al.*, 1972), and inhibition of urea synthesis (Biebuyck *et al.*, 1972). The inhibition of lipogenesis by halothane which is described in the present paper can also be attributed to the inhibition of NADH oxidation.

Materials and Methods

Rats

Female Wistar rats (180–220 g) were obtained from Charles River U.K. Ltd., Margate, Kent, U.K., and were 'meal-fed' from 10:00 h to 14:00 h each day on a high-sucrose–no-fat diet consisting (w/w) of 67% sucrose, 24% casein and 9% vitamin and mineral supplements.

Preparation of isolated liver cells and incubation conditions

Preparation of the cells was begun at 14:00 h and carried out by the method of Berry & Friend (1969) as modified by Cornell *et al.* (1973) and Krebs *et al.* (1974). Glucose (20 mM) was added to the perfusion

medium to minimize loss of glycogen. The glucose added to the perfusion medium was removed when the cells were washed to remove the cell debris and the digestive enzymes.

Incubations were carried out in 25 ml conical flasks with a gas phase of $O_2 + CO_2$ (95:5) with 4 ml of bicarbonate saline (Krebs & Henseleit, 1932), supplemented with 2.5% albumin (Pentex Biochemicals, Miles Laboratories Inc., Kankakee, IL, U.S.A.), which had been treated with charcoal to remove fatty acids (Chen, 1967). When halothane was tested, conical flasks with ground-glass joints and stoppers were used instead of a rubber bung, since halothane dissolves in the rubber. Halothane was introduced into the flask by a miniature vaporizer (Penlon Oxford Miniature Vaporizer, Radley Road, Abingdon, Oxfordshire, U.K.), which could be set to a precise concentration so that 0–5% (v/v) of the gas phase of the incubation flask could be made up of halothane. Incubations were maintained at 37.5°C in a shaking water bath (120 cycles/min) for 60 min unless otherwise indicated.

Analytical methods

Fatty acid synthesis was measured from 0.5 mCi of 3H_2O (The Radiochemical Centre, Amersham, Bucks., U.K.) with 90–110 mg wet wt. of cells per flask. The quantitative aspects of this method have been evaluated by Jungas (1968) and Windmueller & Spaeth (1966). Incubations were terminated with 0.4 ml of 60% (v/v) $HClO_4$, and fatty acids were ex-

tracted by the method of Kates (1972) as used by Harris (1975) for isolated liver cells.

The following metabolites were measured in the supernatant of KOH-neutralized HClO_4 extracts by the standard enzymic methods: lactate and pyruvate (Hohorst *et al.*, 1959), 3-hydroxybutyrate and aceto-

acetate (Williamson *et al.*, 1962), ATP (Lamprecht & Trautschold, 1965), and ADP (Adam, 1965).

Reagents

Enzymes and coenzymes were obtained from Boehringer Corp. (London) Ltd., London W.5, U.K. and halothane was from I.C.I. Ltd., Pharmaceuticals Division, Alderley Park, Macclesfield, Cheshire, U.K. Other chemicals were reagent grade.

Results

The rates of endogenous lipogenesis of the hepatocytes ($0.36 \mu\text{mol}$ of C_2 units/min per g) were comparable with values in the literature (Harris, 1975) and the rate was decreased significantly by halothane (Table 1). Addition of lactate plus pyruvate increased lipogenesis over control rates, and halothane decreased the rates of lipid synthesis from these substrates. Biebuyck *et al.* (1972) demonstrated in the perfused liver that the ratios $[\text{lactate}]/[\text{pyruvate}]$ and $[\text{3-hydroxybutyrate}]/[\text{acetoacetate}]$ were increased by halothane. This effect can also be demonstrated in the isolated hepatocytes (Table 2). A low mitochondrial $[\text{NAD}^+]/[\text{NADH}]$ ratio would be expected to inhibit the flux of carbon through pyruvate dehydrogenase to fatty acids. The addition of acetoacetate, which increases the $[\text{NAD}^+]/[\text{NADH}]$ ratio, would therefore be expected to return lipogenesis to normal rates. Table 2 shows that halothane indeed increased the production of 3-hydroxybutyrate from the added acetoacetate, implying a reduction of NADH to NAD^+ . In conjunction, Table 1 shows that acetoacetate reverses the inhibition of lipogenesis caused by 2.5% (v/v) halothane, but at 4% (v/v) halothane acetoacetate only partially

Table 1. *Effect of halothane on lipogenesis*

Isolated liver cells from 'meal-fed' rats were incubated as described in the Materials and Methods section. The initial concentration of lactate+pyruvate was $10\text{mM}+1\text{mM}$, of acetoacetate was 10mM and of pyruvate was 10mM . The initial concentration (v/v) of halothane is indicated. The values are means $\pm$ S.E.M. for four different cell preparations, and values that are significantly different by the paired Student's *t* test ($P < 0.05$) from their appropriate controls are indicated by * compared with the no-addition mean, by † compared with the lactate+pyruvate mean and by ‡ compared with the acetoacetate mean.

Additions	Fatty acid synthesis (μmol of C_2 units/min per g wet wt.)
None	0.36 ± 0.05
Halothane (2.5%)	$0.27 \pm 0.08^*$
Halothane (4.0%)	$0.19 \pm 0.03^*$
Lactate+pyruvate	$0.63 \pm 0.07^*$
Lactate+pyruvate+halothane (2.5%)	$0.51 \pm 0.06^{\dagger*}$
Lactate+pyruvate+halothane (4.0%)	$0.38 \pm 0.04^{\dagger}$
Acetoacetate	$0.43 \pm 0.04^*$
Acetoacetate+halothane (2.5%)	$0.47 \pm 0.10^*$
Acetoacetate+halothane (4.0%)	$0.29 \pm 0.05^{\ddagger}$
Pyruvate	$0.90 \pm 0.09^*$
Pyruvate+halothane (4.0%)	$0.85 \pm 0.07^*$

Table 2. *Effect of halothane on the redox state of the cell, the rate of production of 3-hydroxybutyrate from acetoacetate and the rate of production of lactate from pyruvate*

Isolated liver cells from 'meal-fed' rats were incubated as described in the Materials and Methods section. The initial concentration of lactate+pyruvate was $10\text{mM}+1\text{mM}$, of acetoacetate was 10mM , of pyruvate was 10mM , and of halothane as indicated (v/v). The intermediates were measured on the cells plus the medium. The values are means $\pm$ S.E.M. for three cell preparations, and values which are significantly different by the Student's paired *t* test ($P < 0.05$) from their appropriate control are indicated by * for no additions, by † for lactate+pyruvate, by ‡ for acetoacetate, and by § for pyruvate.

Additions	[Lactate] [pyruvate]	[3-Hydroxybutyrate] [acetoacetate]	3-Hydroxybutyrate production ($\mu\text{mol/h}$ per g wet wt.)	Lactate production ($\mu\text{mol/h}$ per g wet wt.)
None	4.0 ± 0.2	0.41 ± 0.08	—	—
Halothane (2.5%)	$14 \pm 2.4^*$	$0.87 \pm 0.15^*$	—	—
Halothane (4.0%)	$20 \pm 1.2^*$	$1.40 \pm 0.09^*$	—	—
Lactate+pyruvate	13 ± 1.6	0.31 ± 0.08	—	—
Lactate+pyruvate+halothane (2.5%)	$24 \pm 1.4^{\dagger}$	$0.56 \pm 0.09^{\dagger}$	—	—
Acetoacetate	3.4 ± 0.2	—	28 ± 2.4	—
Acetoacetate+halothane (2.5%)	$8.8 \pm 0.6^{\ddagger}$	—	$53 \pm 6.0^{\ddagger}$	—
Pyruvate	—	—	—	88 ± 20
Pyruvate+halothane (4.0%)	—	—	—	$268 \pm 42^{\S}$

restores lipogenesis. Thus the prediction appeared to be correct. However, more evidence on this matter was sought.

Addition of a high concentration of pyruvate is known to be followed by a rapid formation of lactate, involving the reduction of NADH to NAD⁺. As shown in Table 2, halothane more than doubles the availability of reducing equivalents for use in the reduction of pyruvate, and at the same time pyruvate completely reverses the inhibition of lipogenesis by halothane (4%, v/v) (Table 1).

Discussion

The mechanism by which halothane or other anaesthetic agents bring about their action is not known. Therefore studies on the response of metabolic pathways to these drugs are warranted. However, extrapolation to the situation *in vivo* cannot necessarily be made. Biebuyck & Lund (1974) showed that the metabolic intermediates of the freeze-clamped liver from halothane-anaesthetized rats did not give results comparable with those obtained with the halothane-perfused rat liver *in vitro*. This was attributed to the interaction of the other tissues of the rat. The experiments of Biebuyck & Lund (1974) show that in studies *in vivo*, the action of a compound on a tissue is complicated by the overall effect of the compound on the organism. In contrast, experiments on isolated organs or cells demonstrate the potential effect of an agent on a particular tissue.

The present study demonstrates that halothane inhibits lipogenesis from endogenous substrate (e.g. glycogen) or from added lactate plus pyruvate. The mechanism of the inhibition of lipogenesis by halothane may be explained by its inhibition of electron transfer from NADH to cytochrome *b*, as shown by Harris *et al.* (1971), Cohen & Marshall (1968) and Miller & Hunter (1970). The inhibition of NADH oxidation would lead to an inhibition of pyruvate dehydrogenase by limiting the availability of NAD⁺ and possibly by increasing the phosphorylation of pyruvate dehydrogenase (Kerby *et al.*, 1976). This concept is supported by the results that show that the NADH-accepting molecules, pyruvate and acetoacetate, reverse the inhibition of lipogenesis by halothane.

Pyruvate relieves the inhibition of lipogenesis by halothane more effectively than does acetoacetate. This is due to the much more rapid rate at which NADH is removed in the presence of pyruvate than of acetoacetate (see Table 2). Therefore an increased rate of removal of NADH would supply more NAD⁺ for the production of acetyl-CoA, the precursor of lipogenesis. The fact that the NAD⁺ is generated in the cytoplasm in the presence of pyruvate by lactate dehydrogenase and that pyruvate dehydrogenase uses mitochondrial NAD⁺ is of little consequence, since mitochondrial NADH can be oxidized very

rapidly, with the reducing equivalent being transferred indirectly into the cytoplasm for the reduction of pyruvate (Lardy *et al.*, 1965; Krebs *et al.*, 1967; Rognstad, 1975; Mapes & Harris, 1976). Therefore, my present experiments are in agreement with the proposed theory to explain the action of halothane on lipogenesis.

Halothane decreases the [ATP]/[ADP] ratio in the perfused liver (Biebuyck *et al.*, 1972), and it could be argued that the fall in [ATP]/[ADP] ratio could lead to an inhibition of lipogenesis. In the present experiments halothane (4%, v/v) decreased the [ATP]/[ADP] ratio from 2.7 ± 0.27 to 1.71 ± 0.08 , and in the presence of pyruvate from 2.34 ± 0.13 to 1.83 ± 0.07 (means $\pm$ S.E.M. of three experiments). Thus the small decrease in [ATP]/[ADP] ratio and the fact that pyruvate did not reverse the decrease in [ATP]/[ADP] ratio but did reverse the inhibition of lipogenesis show that the [ATP]/[ADP] ratio cannot explain the inhibition of lipogenesis by halothane.

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