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The Effects of Halothane (2-Bromo-2-chloro-1,1,1-trifluoroethane) on Glycolysis and Biosynthetic Processes of the Isolated Perfused Rat Liver

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1. With reference to the post-operative dysfunction of the liver observed after halothane anaesthesia, the effects of the anaesthetic on some metabolic functions were studied in the isolated perfused rat liver. Oxygen uptake, glycolysis, gluconeogenesis and urea synthesis were affected by halothane at a concentration (2.5% of the gas phase) within the range used in clinical anaesthesia. 2. At this concentration of halothane uptake of oxygen was inhibited in livers from both fed and starved rats. 3. In livers from fed rats there was a 16-fold increase in lactate production. This was accompanied by a fivefold decrease in the tissue content of 2-oxoglutarate and a more than twofold decrease in citrate. The calculated [free NAD^+]/[free NADH] ratio in both cytoplasm and mitochondria was lower in the halothane-exposed livers than in controls. 4. In livers of starved rats the rate of gluconeogenesis from lactate was decreased by halothane to 30% of the control rate. 5. Halothane inhibited gluconeogenesis from alanine and propionate to the same extent as from lactate, whereas glucose formation from dihydroxyacetone, glycerol, fructose and sorbitol was relatively unaffected. 6. During gluconeogenesis from 10mM-lactate the tissue content of ATP was decreased by 50%, glutamate by 50% and 2-oxoglutarate was decreased eightfold in the halothane-exposed livers. 7. Halothane decreased urea synthesis in the presence of 10mM-NH_4Cl and 2mM-ornithine to 15% of the control rate. 8. The inhibitions of gluconeogenesis and urea synthesis were completely abolished within 15min of withdrawal of the anaesthetic. 9. The stimulation of uptake of oxygen brought about by the addition of lactate or precursors of urea was abolished by halothane. 10. Effects on gluconeogenesis similar to those of halothane occurred in livers exposed to the anaesthetic methoxyflurane, although normal rates were not restored on withdrawal of the drug. Other anaesthetic agents tested (ketamine–HCl and trichloroethylene) decreased gluconeogenesis to 66% of the control rate. 11. The inhibitory effects of halothane are consistent with an interference at the stage of the NADH dehydrogenase of the electron-transport chain.

Halothane (2-bromo-2-chloro-1,1,1-trifluoroethane), a widely used volatile anaesthetic agent, was introduced into clinical anaesthesia in 1956 (Bryce-Smith & O'Brien, 1956) after extensive investigation in animals (Raventos, 1956). It has been suggested that it may be responsible in some cases for post-operative dysfunction of the liver (for review see Little, 1968). This prompted us to investigate in the isolated perfused rat liver whether halothane has direct effects on the metabolism of the liver. Any harmful effects are most likely to be detected when the metabolic activity is maximal. The effects of halothane were therefore studied in the presence of excess of substrate when gluconeogenesis and urea synthesis are maximal. These biosynthetic processes require the intactness of the energy-transforming apparatus and were therefore used as criteria of the metabolic functions of the liver. The experiments reported in this paper indeed show that halothane at concentrations used in clinical anaesthesia can interfere with liver function. The effects observed are all reversible. Interference with metabolic functions presumably precedes changes seen on electron microscopy (Scholler, 1968) or cellular damage leading to leakage of enzymes (Brohult & Gillquist, 1969; Almersjö, 1971).

Materials and Methods

Tissue techniques

Rats. Female rats of the Wistar strain (180–220g) were obtained from Scientific Products Farm, Manston Research Centre, Margate, Kent, U.K., and were kept on Oxoid pasteurized breeding diet for rats and mice (Oxoid Ltd., London S.E.1, U.K.).

Liver perfusion. The basic perfusion method was as described by Hems *et al.* (1966). All animals were

anaesthetized with freshly prepared aqueous solutions of sodium pentobarbitone (60mg/kg) given intraperitoneally. The perfusion medium was also as described by Hems *et al.* (1966) except that albumin was a product of Pentex Biochemicals, Miles Laboratories Inc., Kankakee, Ill., U.S.A., and was dialysed before use. The albumin (10%, w/v) was dissolved in physiological medium (Krebs & Henseleit, 1932) and dialysed against three or four changes of the same medium during 48h in a cold-room. The dialysed albumin was stored frozen in plastic bottles, and used in the medium to a final concentration of 2.6g/100ml.

Substrates were added to the medium as 0.2M neutral solutions. Samples of medium were taken throughout the perfusion [0.5 or 2.0ml into 4.0ml of 2% (w/v) $HClO_4$ or 0.1ml of 60% $HClO_4$ respectively, the latter when urea and ammonia were to be determined in the neutralized (KOH) supernatant]. The volume changes of the medium caused by removal of samples were taken into account when calculating metabolic rates (μmol/min per g wet wt. of liver), which were obtained from the gradient of the plot of total metabolite in medium versus time. A cabinet was used that permitted the performance of two perfusions simultaneously, so that a control perfusion without anaesthetic could be carried out on each occasion. An exhaust tube attached to the oxygenator serving the halothane-treated liver removed the halothane-containing mixture from the cabinet.

In experiments in which tissue metabolites were measured, a piece of liver was quickly excised and pressed between aluminium clamps previously cooled in liquid N_2 (Wollenberger *et al.*, 1960). Neutralized extracts of the tissue were prepared as described by Williamson *et al.* (1967*a*).

Reagents

Urease and glutaminase IV were obtained from Sigma (London) Chemical Co. Ltd., London S.W.6, U.K., and L-alanine dehydrogenase was prepared as described by Yoshida & Freese (1964) as modified by Williamson *et al.* (1967*b*). All other purified enzymes were obtained from Boehringer Corp. (London) Ltd., London W.5, U.K. Coenzymes and other reagents used for the determination of metabolites were also obtained from Boehringer Corp. Florisil was obtained from Koch–Light Laboratories Ltd., Colnbrook, Bucks., U.K. Substrates used in the perfusion experiments were reagent grade. Halothane (Fluothane) and trichloroethylene (Trilene) were obtained from I.C.I. Ltd., Pharmaceuticals Division, Alderley Park, Macclesfield, Cheshire, U.K. Methoxyflurane (Penthrane; 2,2-dichloro-1,1-difluoroethyl methyl ether) and sodium pentobarbitone (Nembutal powder) were obtained from Abbott Laboratories Ltd., Queenborough, Kent, U.K. Ketamine hydrochloride [Ketalar; 2-(2-chlorophenyl)-2-methylaminocyclohexanone hydrochloride] was obtained in pure powder form from Parke, Davis and Co. Laboratories, Hounslow, Middx., U.K.

Anaesthetics

Anaesthetic vaporizers. The vaporizers used (Fluotec mk. III, Pentec and TriTec) were supplied by Cyprane Ltd., Keighley, Yorks., U.K.

Administration of anaesthetics. Halothane, methoxyflurane and trichloroethylene are volatile anaesthetics and were introduced into the perfusion medium by passing the flow of O_2+CO_2 (95:5, v/v) through the precision vaporizer before the gases entered the oxygenator. The concentrations of halothane in the final mixture are expressed as the number of volumes of halothane vapour in 100vol. of the mixture administered to the perfused liver (%, v/v).

Ketamine–HCl, dissolved in Krebs–Henseleit medium, was introduced directly into the perfusion medium.

Determination of metabolites

UDP-glucose was determined as described by Keppler & Decker (1970). Glucose was assayed by using glucose 6-phosphate dehydrogenase and hexokinase as described by Slein (1963). Urea was determined manometrically as CO_2 after treatment with urease at pH5 (Krebs & Henseleit, 1932). Other methods were as used by Hems & Brosnan (1970). A portion of neutralized extract was treated with Florisil as described by Williamson *et al.* (1967*a*) before assay of pyruvate, α-oxoglutarate and acetoacetate.

Oxygen consumption

The Van Slyke manometric technique (Van Slyke & Neill, 1924) for the measurement of oxygen content could not be used in these experiments because of the volatility of the halothane. Therefore a polarographic method (Solymar *et al.*, 1971), which is unaffected by volatile substances, was used. Samples (2ml) of perfusion medium were withdrawn from specific sampling sites on the 'arterial' and 'venous' sides of the perfused liver, after 20, 45 and 105min of perfusion.

The flow of medium through the liver was determined at the same time as sampling. The flow rate was measured as follows. The medium issuing from the liver was allowed to fall into a small container placed in the reservoir chamber of the perfusion circuit. A siphon tube was attached to this container in such a way that when 10ml of medium was collected the siphon began to flow and the container

emptied. The flow-rate was measured by timing the interval between the start of filling and the start of siphoning. Oxygen consumption of the liver was calculated by multiplying the arteriovenous difference in O_2 content (μmol/ml) by the flow rate of the medium (ml/min).

Results

Stimulation of glycolysis by halothane in perfused livers of fed rats

Livers of fed rats perfused in the absence of added substrate release glucose into the medium [0.65±0.05 (four observations) μmol/min per g wet wt. after the initial 40min perfusion period]. At the same time lactate production is low [0.12±0.05 (six observations) μmol/min per g wet wt. during the 40–70min period of perfusion]. Both the production of glucose and lactate removal are linear during this period. Halothane caused a major increase in lactate production to a rate of 2.05±0.39 (four observations) μmol/min per g wet wt. in the presence of 2.5% (v/v) halothane, a concentration within the range used in clinical anaesthesia. These rates of lactate production are comparable with rates obtained in anaerobic perfusions (Woods & Krebs, 1971). Glucose release was relatively unaffected during exposure to halothane. Lactate production virtually ceased within 15min of switching off the halothane; in some experiments there was an uptake of lactate that began during the recovery period.

Table 1. *Effect of halothane on metabolite content of perfused liver of fed rats*

Livers from fed rats were perfused in the absence of added substrate as described in the Materials and Methods section. Halothane (2.5%, v/v, in O_2+CO_2, 95:5) was administered from 15 to 45min. Livers were freeze-clamped at 45min. Values are expressed as μmol/g wet wt. of tissue (means±S.E.M.) and are the mean of four experiments except where stated. The redox state of the cytoplasm ([free $NAD^+]_c$/[free $NADH]_c$) was calculated from the lactate dehydrogenase system. The redox state of the mitochondria was calculated from the glutamate dehydrogenase ([free $NAD^+]_{m1}$/[free $NADH]_{m1}$) and 3-hydroxybutyrate dehydrogenase ([free $NAD^+]_{m2}$/[free $NADH]_{m2}$) systems.

	No halothane	Halothane-exposed
Uridine diphosphoglucose	0.20 (2)	0.17 (2)
Glucose	3.37±0.56	3.71±0.45
Glucose 6-phosphate	0.06±0.01	0.08±0.03
Fructose 6-phosphate	0.02 (2)	0.03 (2)
Dihydroxyacetone phosphate+glyceraldehyde 3-phosphate	0.04±0.002	0.04±0.005
α-Glycerophosphate	0.12±0.05	0.61±0.12
3-Phosphoglycerate	0.27±0.02	0.19±0.01
2-Phosphoglycerate	0.06±0.009	0.04±0.001
Phosphoenolpyruvate	0.12±0.01	0.07±0.005
Pyruvate	0.20±0.02	0.21±0.01
Lactate	1.29±0.17	5.82±1.01
[Lactate]/[pyruvate]	6.6±0.9	27.6±3.8
[Free $NAD^+]_c$/[free $NADH]_c$	1426±158	352±64
Ammonia	0.52±0.03	0.58±0.09
Glutamate	1.81±0.04	1.48±0.06
2-Oxoglutarate	0.32±0.03	0.07±0.003
[Free $NAD^+]_{m1}$/[free $NADH]_{m1}$	23.5±2.2	6.6±0.6
Acetoacetate	0.13±0.02	0.08±0.02
3-Hydroxybutyrate	0.06±0.01	0.20±0.07
[3-Hydroxybutate]/[acetoacetate]	0.55±0.18	2.87±1.04
[Free $NAD^+]_{m2}$/[free $NADH]_{m2}$	64.8±15.7	13.3±3.6
ATP	1.94±0.07	1.71±0.11
ADP	0.79±0.04	1.00±0.07
AMP	0.13±0.01	0.16±0.03
Total adenine nucleotides	2.86±0.09	2.87±0.20
Citrate	0.32±0.03	0.14±0.02
Malate	0.15±0.03	0.37±0.08
Alanine	0.90±0.14	2.61±0.26
[Alanine][2-oxoglutarate]/[glutamate][pyruvate]	0.80±0.12	0.57±0.04

Tissue content of metabolites during stimulation of glycolysis by halothane

The tissue contents of glycolytic intermediates and related metabolites, and of the reactants of 3-hydroxybutyrate dehydrogenase and glutamate dehydrogenase, were determined after exposure to 2.5% halothane for 30min, and in the controls after the same total perfusion time (Table 1). The results were used to calculate the redox state of the NAD couple because the two mitochondrial dehydrogenases and lactate dehydrogenase are known to be at near-equilibrium in the liver (Williamson *et al.*, 1967*a*).

The most significant change was a fivefold decrease in the tissue content of 2-oxoglutarate. This was accompanied by an approximately threefold increase in alanine, which is known to increase under anaerobic conditions (see Brosnan *et al.*, 1970). The concentration of ATP was relatively well maintained. The values obtained for the ratio [alanine][2-oxoglutarate]/[glutamate][pyruvate] were unaffected by halothane, which means that equilibrium in the glutamate–pyruvate aminotransferase system (Williamson *et al.*, 1967*b*) was maintained. The citrate content was decreased by halothane to 44% of the control, which may have contributed to the increased glycolysis because citrate is known to inhibit phosphofructokinase (Parmeggiani & Bowman, 1963; Passonneau & Lowry, 1963). In both cytoplasm and mitochondria, values of the ratio [free NAD^+]/[free NADH], as calculated from the tissue content of the reactants of lactate dehydrogenase, 3-hydroxybutyrate dehydrogenase and glutamate dehydrogenase, were lower in the halothane-exposed than in the control livers.

Inhibition by halothane of gluconeogenesis from lactate in the perfused liver

Gluconeogenesis from lactate in the perfused liver of rats starved for 48h was completely inhibited by 10% (v/v) halothane. [A concentration of 10% (v/v) was administered from a specially calibrated vaporizer; Longworth Scientific Instruments Ltd., Abingdon, Berks., U.K.] The dose–response curve (Fig. 1) shows that the inhibition decreased with decreasing halothane concentration: at 2.5% halothane the rate of gluconeogenesis was 30% of the control rate. At the same time the rate of lactate removal was 38% of the control rate. The inhibitory effect of halothane was exerted within 15min and was completely reversible; within 15min of cessation of halothane the rates of glucose synthesis and lactate uptake had returned to control values (Table 2). Although the inhibitory effects of halothane were lower at 2.5% than at 10%, the former concentration was used in all subsequent experiments because it is within the range used in clinical anaesthesia.

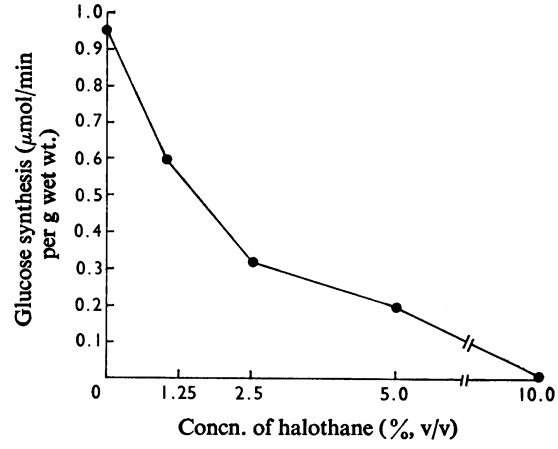

Fig. 1. *Effect of halothane concentration on rates of gluconeogenesis from lactate in the isolated perfused rat liver*

Halothane at the concentrations shown was administered during the 30–70min period of perfusion. Rates of gluconeogenesis were calculated during the 40–70min period. For other experimental details, see the Materials and Methods section.

Table 2. *Inhibition by halothane of gluconeogenesis from lactate in the perfused liver*

Liver from rats starved for 48h were perfused as described in the Materials and Methods section. Lactate (10mM) was added to the medium at 38min. Halothane was administered from 30 to 70min. Metabolite changes in the medium are expressed as μmol/min per g wet wt. of tissue. Values are means±S.E.M. with the number of observations in parentheses.

	Glucose production		Lactate removal	
Time (min) ...	40–70	85–130	40–70	85–130
Control (12)	0.95±0.08	0.88±0.05	2.11±0.15	1.91±0.13
Halothane (3)	0.31±0.05	0.80±0.25	0.78±0.26	1.89±0.30

Table 3. *Effect of halothane on gluconeogenesis from various precursors in the perfused liver*

The experimental conditions were as described for Table 2. Substrates were added to the medium to a concentration of 10mM. Rates of glucose synthesis are expressed as μmol/min per g wet wt. of tissue, with the number of observations in parentheses.

		Glucose formation		
Time (min) ...	(30–70)	40–70	85–130	Inhibition by
Substrate	Halothane (%)			halothane (%)
L-Lactate	0	0.95±0.08 (12)	0.88±0.05 (12)	
	2.5	0.31±0.05 (3)	0.80±0.25 (3)	67.4
L-Alanine	0	0.52±0.07 (4)	0.46±0.07 (4)	
	2.5	0.23 (2)	0.46 (2)	55.8
Propionate	0	0.56 (2)	0.56 (2)	
	2.5	0.21 (2)	0.63 (2)	62.5
Dihydroxyacetone	0	1.53±0.18 (3)	1.23±0.16 (3)	
	2.5	1.34±0.22 (3)	1.58±0.32 (3)	12.4
Glycerol	0	0.71±0.05 (5)	1.05±0.15 (5)	
	2.5	0.71±0.12 (3)	0.61±0.06 (3)	0
D-Fructose	0	2.04 (2)	1.87 (2)	
	2.5	2.13 (2)	2.46 (2)	0
Sorbitol	0	1.20 (2)	1.45 (2)	
	2.5	1.31 (2)	1.68 (2)	0

Inhibition by halothane of gluconeogenesis from other precursors

To locate the site of inhibition of gluconeogenesis by halothane, precursors that enter the gluconeogenic pathway at different stages were tested.

Gluconeogenesis from lactate, alanine and propionate was inhibited by halothane to approximately the same extent (Table 3). The fact that glucose formation from glycerol, dihydroxyacetone, sorbitol and fructose was relatively unaffected may be taken to suggest that there is some interference at a stage in the pathway between pyruvate and triose phosphate. Alternatively, inhibition by halothane could be due to an inhibition of energy supply, because each molecule of lactate, alanine or propionate requires the equivalent of three molecules of ATP to reach the stage of triose phosphate, whereas glycerol, dihydroxyacetone, sorbitol or fructose require only one molecule.

Effect of halothane on liver metabolites during gluconeogenesis from lactate

To gain further information on the inhibitory effects of halothane livers were freeze-clamped 30min after the addition of 10mM-lactate to the medium, i.e. during the period of maximal gluconeogenesis in the controls and during the period of inhibition in the livers exposed to halothane. The tissue content of phosphoenolpyruvate, 2-phosphoglycerate and 3-phosphoglycerate in the halothane-treated animals was at least twofold that in the controls (Table 4). Between the triose phosphate stage and glucose the concentrations of intermediates in the halothane-treated animals were the same, or lower, than those in the controls. The content of UDP-glucose was also lower in the halothane-treated livers (0.07μmol/g wet wt.; average of two experiments) than in controls (0.17μmol/g wet wt.; average of two experiments).

ATP was decreased by 50% during exposure to halothane [1.70μmol/g (three observations) in the controls; 0.85μmol/g (three observations) in the halothane-treated animals]. Halothane caused an eightfold decrease in the tissue content of 2-oxoglutarate, similar to the change seen in the perfused liver from fed rats (see Table 1). Both glutamate and ammonia decreased by almost 50%, with a 50% increase in alanine. The tissue content of citrate in the controls was high (0.52μmol/g; average of two experiments) and was decreased to approximately one-sixth by halothane.

Both the cytoplasmic and mitochondrial [free NAD^+]/[free NADH] ratios, as calculated from the tissue content of the reactants of lactate dehydrogenase and glutamate dehydrogenase respectively (see Williamson *et al.*, 1967*a*), were decreased in the presence of halothane.

Halothane also caused a large decrease in the 'phosphorylation state' of the liver (defined as the ratio [ATP]/[ADP][HPO_4^{2-}]; see Krebs & Veech,

Table 4. *Effect of halothane on metabolite content of perfused liver of rats starved for 48h*

Livers from rats starved for 48h were perfused as described in the Materials and Methods section. Lactate (10mM) was added to the medium at 38min. Halothane was administered from 38 to 68min. Livers were freeze-clamped at 68min. Values are expressed as μmol/g wet wt. of tissue (±S.E.M.) of three experiments: a single figure is the average of two experiments. The redox states of the cytoplasm ([free NAD^+]$_c$/[free NADH]$_c$) and mitochondria ([free NAD^+]$_m$/[free NADH]$_m$) were calculated from the lactate and glutamate dehydrogenase systems.

	No halothane	Halothane-exposed
UDP-glucose	0.17	0.07
Glucose 1-phosphate	<0.01	<0.01
Glucose	1.57	0.57
Glucose 6-phosphate	0.06±0.01	<0.01
Fructose 6-phosphate	0.015	<0.01
Fructose 1,6-diphosphate	0.04±0.004	0.04±0.007
Dihydroxyacetone phosphate+glyceraldehyde 3-phosphate	0.05±0.001	0.04±0.006
α-Glycerophosphate	0.22	0.17
3-Phosphoglycerate	0.32±0.01	0.77±0.05
2-Phosphoglycerate	0.04±0.002	0.09±0.003
Phosphoenolpyruvate	0.19±0.008	0.41±0.025
Pyruvate	0.41±0.05	0.28±0.01
Lactate	5.01±0.84	5.80±0.65
[Free NAD^+]$_c$/[free NADH]$_c$	765±139	443±68
Ammonia	0.61±0.11	0.36±0.04
Glutamate	1.69±0.09	0.93±0.003
2-Oxoglutarate	0.34±0.05	0.04±0.01
[Free NAD^+]$_m$/[free NADH]$_m$	32.0±7.8	4.1±1.6
ATP	1.70±0.14	0.85±0.02
ADP	0.84±0.08	1.71±0.11
AMP	0.17±0.03	0.27±0.02
Total adenine nucleotides	2.71±0.24	2.30±0.11
[ATP]/[ADP][P_i] measured	822	226
Total phosphate	3.87	5.42
Citrate	0.52	0.08
Malate	0.33±0.03	0.19±0.03
Alanine	0.99±0.07	1.50±0.27
[Alanine][2-oxoglutarate]/[glutamate][pyruvate]	0.49±0.02	0.22±0.02

1969) from 822 (mean of two experiments) in the controls to 226 (mean of two experiments).

The glutamate–pyruvate aminotransferase is at near-equilibrium in rat liver *in vivo*, where the average value found for the ratio [alanine][2-oxoglutarate]/[glutamate][pyruvate] is 0.53 (Williamson *et al.*, 1967*b*). The ratios in the perfused livers were 0.49 and 0.22 for the control and halothane-treated respectively, which shows that a near-equilibrium is maintained even in the face of major changes in the reactants.

Effect of halothane on urea synthesis

As gluconeogenesis depends on the supply of ATP, it was thought that the primary action of halothane might be an inhibition of ATP synthesis. An interference by halothane in ATP production would therefore be expected also to cause inhibition of urea synthesis.

To test this livers from rats starved for 48h were perfused with 10mM-NH_4Cl, 2mM-L-ornithine and 10mM-lactate added to the medium. Within 5min of administration of 2.5% halothane, urea synthesis decreased from 1.91±0.21 (four observations) to 0.28±0.11 (five observations) μmol/min per g wet wt., whereas ammonia uptake continued at one-third of the control rate. During this period of inhibition of urea synthesis the ammonia removed could not be fully accounted for as urea, and must have been temporarily stored, probably as amino acids such as glutamate, alanine and aspartate (Brosnan, 1968). This conclusion is supported by the results obtained during recovery from halothane, when the rate of ammonia removal [1.93±0.18 (three observations) μmol/min per g wet wt.] was too low to account for

the urea synthesized [1.23±0.14 (five observations) μmol/min per g wet wt.]. A typical experiment is shown in Fig. 2. After removal of halothane the rates of urea synthesis increased, but these experiments do not give a true picture of recovery from halothane exposure because a single addition to the medium of 10mM-NH_4Cl is removed too rapidly to maintain the control rate during the 70–90min period of recovery. However, it is clear that the effects of halothane on urea synthesis, as on gluconeogenesis, are reversible.

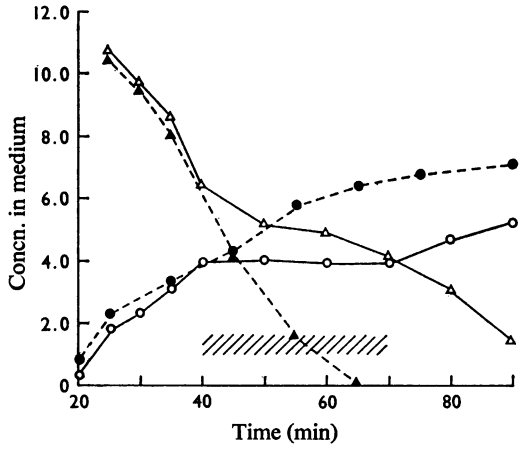

Fig. 2. *Time-course of urea synthesis from* 10*mM-*NH_4Cl *and* 2*mM-ornithine: effect of* 2.5% (*v/v*) *halothane*

Livers from rats starved for 48h were perfused as described in the Materials and Methods section, except that substrates were added to the medium at 20min and samples were taken at 5min intervals (not shown on the figure). The hatched area indicates the period of exposure to halothane. ---, Control; ——, halothane-treated. ▲, △, Ammonia removal; ●, ○, urea synthesis.

Effect of halothane on uptake of oxygen by the perfused liver

Because the effects of halothane suggested that it exerts an inhibitory effect on ATP supply, uptake of O_2 was measured at various stages during the perfusion. In the livers from starved rats uptake of O_2 was measured at 20min (i.e. before the addition of substrate), at 45min (i.e. 15min after the addition of substrate and 15min after the start of halothane) and at 105min (i.e. 75min after addition of substrate and 35min after the halothane was switched off). The same times of sampling were used for the livers from fed rats, which were perfused without any added substrate. A control perfusion was carried out in each case.

The basal uptake of O_2 by the livers from fed rats increased gradually during the perfusion (from 1.74 to 2.16μmol/min per g wet wt.). Halothane caused a 55% inhibition of respiration. At 105min the rate was not significantly lower than in the controls.

In the control perfusions of livers from starved rats, addition of 10mM-lactate increased the rate of consumption of O_2 from 2.27 to 2.76μmol/min per g wet wt. Under the same conditions halothane decreased

Table 5. *Inhibition by halothane of uptake of oxygen during gluconeogenesis and urea synthesis in the perfused liver*

The experimental conditions were as given in Table 2. The oxygen content of the medium entering and leaving the liver was as described in the Materials and Methods section. Rates of uptake of O_2 are expressed as μmol of O_2/min per g wet wt. (means±S.E.M.) with the number of perfusions in parentheses.

Nutritional state	Experimental procedure	Substrate added to medium (at 30min)	Uptake of O_2 during perfusion		
			20min	45min	105min
Fed	No halothane	None	1.74±0.06 (19)	2.09±0.26 (3)	2.16±0.20 (3)
	2.5% Halothane (30–70min)	None	1.74±0.06 (19)	0.91±0.06 (3)	1.88±0.29 (3)
Starved (48h)	No halothane	None	2.27±0.08 (37)	1.83±0.12 (7)	1.86±0.09 (7)
	2.5% Halothane (30–70min)	None	2.27±0.08 (37)	1.43±0.33 (3)	2.17±0.18 (3)
	No halothane	10mM-Lactate	2.27±0.08 (37)	2.76±0.13 (4)	2.16±0.22 (4)
	2.5% Halothane (30–70min)	10mM-Lactate	2.27±0.08 (37)	1.63±0.06 (3)	2.53±0.31 (3)
	No halothane	10mM-Lactate+ 10mM-NH_4Cl+ 2mM-ornithine	2.27±0.08 (37)	4.41±0.36 (3)	3.12 (2)
	2.5% Halothane (30–70min)	10mM-Lactate+ 10mM-NH_4Cl+ 2mM-ornithine	2.27±0.08 (37)	1.69±0.04 (3)	2.83±0.17 (3)

Table 6. *Effect of various anaesthetic agents on gluconeogenesis from lactate in the perfused liver*

The experimental conditions were as described in Table 2. The volatile anaesthetics were administered from 30 to 70min as described in the Materials and Methods section. Ketamine–HCl was added in solution at 30min. Rates of glucose production and lactate removal are expressed as μmol/min per g wet wt. of tissue.

	Glucose production		Lactate removal	
Time (min) ...	40–70	85–130	40–70	85–130
Control	0.95±0.90 (12)	0.88±0.06 (12)	2.11±0.17 (12)	1.91±0.14 (12)
Methoxyflurane (1.25%, v/v)	0.28, 0.31	0.47, 0.43	0.11, 0.20	1.24, 1.15
Trichloroethylene (1%, v/v)	0.61, 0.71	0.69, 0.71	1.15, 1.53	1.71, 1.53
Ketamine (10mg/100ml of medium)	0.67, 0.53	0.67, 0.53	1.36, 1.27	1.36, 1.27

the uptake of O_2 from 2.09 to 1.63μmol/min per g wet wt., i.e. to a rate in the presence of lactate that was lower than the endogenous rate, and only 59% of the control rate (Table 5). The inhibition by halothane was completely reversible; at 105min the uptake of O_2 was the same as in the controls.

In other control perfusions, in which gluconeogenesis and urea synthesis were occurring simultaneously (i.e. 10mM-lactate, 10mM-NH_4Cl and 2mM-ornithine added to the medium), the rate of consumption of O_2 increased from the endogenous rate of 2.27 to 4.41μmol/min per g wet wt. Under these conditions halothane decreased the uptake of O_2 to 1.69μmol/min per g wet wt., i.e. to a rate that was lower than the endogenous rate, and to the same basal rate as was found during halothane treatment when lactate alone was present in the medium (Table 5). At 105min the rate of uptake of O_2 in the control perfusions had decreased to 3.12μmol/min per g wet wt., probably because of limitation of substrate. At the same time the uptake of O_2 by the livers exposed to halothane had increased somewhat (to 2.83μmol/min per g wet wt.), but the recovery from the effect of halothane was not as complete as in the experiments where lactate alone was added.

Effect of other anaesthetic agents on gluconeogenesis from lactate

The rates of gluconeogenesis from lactate in perfused livers of rats starved for 48h were less affected by the presence of trichloroethylene (1%, v/v) or ketamine (10mg/100ml of perfusion medium) than by halothane. Methoxyflurane (1.25%, v/v) caused a 65% inhibition over the control rate of gluconeogenesis, with only a partial recovery when the methoxyflurane was switched off (Table 6).

Discussion

Primary effect of halothane

The effects of halothane in the isolated perfused liver can be explained on the assumption that inhibition of electron transport between NADH and O_2 is the primary event. As a consequence ATP production is decreased. This causes an inhibition of ATP-dependent biosyntheses such as gluconeogenesis and urea synthesis.

There are some differences in the responses to halothane of livers from fed and starved animals. In the livers of fed rats, which contain much glycogen, the decreased rate of ATP supply causes a production of lactate. This does not occur in the livers of starved rats, where glycogen is virtually absent. The inhibition of uptake of O_2 in the absence of added substrate was rather greater in the fed state (55%) than in the starved (22%), although consumption of O_2 in both was similar in the absence of halothane during the 30–70min period of perfusion under discussion.

The effect of halothane becomes much greater when precursors of glucose (lactate) or of both urea and glucose (lactate, NH_4Cl, ornithine) are added. The large increases in the rate of uptake of O_2 on addition of these substrates is virtually abolished by halothane. Thus the basal consumption of O_2, especially in the starved liver, is much less affected by halothane than is the oxidation of added substrates. This may be due to differences in the nature of the fuels of respiration. It is generally accepted that fatty acids provide the main oxidative fuel of the liver in starvation, and that oxidation of pyruvate (and hence carbohydrate), even in the fed state, represents a very small proportion of the oxidative metabolism (Glogner & Schurek, 1969). The implication is that fatty acid oxidation is relatively unaffected by halothane. The greater inhibition in the fed state suggests that some fuel other than fatty acids provides the main energy supply.

It should be emphasized that the inhibitory effects of halothane in the perfused liver are not due to a lack of oxygen. Although halothane causes a decreased hepatic blood flow *in vivo*, the decrease is secondary to a depressed cardiac output (Galindo *et al.*, 1966). In the isolated perfused liver halothane markedly increased blood flow, and hence oxygen supply, to the tissue.

Table 7. *Calculated requirements for NADH dehydrogenase activity in different oxidative reactions*

Oxidative reaction	O_2 required (mol)	NADH formed (mol)	Reduced flavoproteins formed (mol)
$\frac{1}{2}$ Glucose → 3 CO_2+3 H_2O	3	5	1
1 C_2-unit of fatty acids → 2 CO_2+2 H_2O	3	4	2
4 C_2-units of fatty acids → 2 3-hydroxybutyrate	3	2	4
3 C_2-units of fatty acids → 1$\frac{1}{2}$ acetoacetate	3	3	3

Site of inhibition by halothane

Inhibition of consumption of O_2 by halothane has been reported in isolated rat liver mitochondria (Cohen & Marshall, 1968), in mammalian cells in culture (Fink & Kenny, 1968), and in rat liver, heart and brain slices (Hoech *et al.*, 1966). Although re-oxidation of NADH through the electron-transport chain is inhibited by halothane, in isolated mitochondria succinate oxidation is not affected (Cohen & Marshall, 1968; Miller & Hunter, 1970; Harris *et al.*, 1971). The results of the present experiments and of those in isolated mitochondria suggest the NADH dehydrogenase stage as the site of inhibition by halothane, since both succinate oxidation and the β-oxidation of fatty acids are largely dependent on flavoprotein-linked enzymes. Thus when fatty acids are the fuel of respiration, especially when ketone bodies are major end-products, the NADH dehydrogenase activity required per mol of O_2 consumed is less than when carbohydrate is oxidized, as the calculations in Table 7 show. This suggests that a partial inhibition of NADH dehydrogenase can still be associated with an uptake of O_2 of a normal order of magnitude when β-oxidation of fatty acids circumvents to some extent the inhibited step.

The fact that gluconeogenesis from dihydroxyacetone, sorbitol, glycerol or fructose is much less inhibited by halothane than is glucose formation from lactate, alanine and propionate is not incompatible with the above conclusions, because the former substrates require two molecules of ATP and the latter six molecules for the formation of one molecule of glucose.

Barbiturates, substances of an entirely different chemical structure but with similar pharmacological effect, also block electron transfer between NADH and flavoproteins (Jowett & Quastel, 1937; for further references see Krebs *et al.*, 1961).

A toxic breakdown product of halothane has been suggested as the possible cause of liver damage after halothane anaesthesia (for review, see Cohen, 1971). The experiments in isolated mitochondria rule out a breakdown product as the inhibitory agent, because the drug is metabolized via the cytochrome *P*-450 system (Van Dyke, 1966). The similarity of the conclusions on the site of inhibition from experiments in the intact liver and in isolated mitochondria suggest that halothane itself is the inhibitor. It may be that the long-term effects of halothane are due to a breakdown product.

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