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Short Communications

The Protective Effect of Oleate on Metabolic Changes Produced by Halothane in Rat Liver

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Halothane (2-bromo-2-chloro-1,1,1-trifluoroethane) has been shown to stimulate the rate of glycolysis and to cause a major inhibition of gluconeogenesis and urea synthesis in the isolated perfused rat liver (Biebuyck *et al.*, 1972). These effects of halothane are associated with, and probably caused by, an inhibition of respiration, which is more pronounced in livers from fed animals than in livers from starved animals. The inhibited biosynthetic processes are restored to normal rates within a short time of withdrawing the anaesthetic agent.

Experiments reported in the present paper show that the inhibitory effects of halothane on O_2 consumption and urea synthesis are largely abolished when a fatty acid (oleate) is added to the perfusion medium in concentrations that occur in the blood *in vivo* in starved rats. In addition, the presence of the fatty acid prevents the inhibition by halothane of urea synthesis, reverses the major changes in liver tissue metabolite concentrations brought about by halothane and decreases the degree of inhibition by halothane of gluconeogenesis from lactate.

Methods

Livers of fed or 48h-starved female rats were perfused, and exposed to halothane (2.5%, v/v), as described by Biebuyck *et al.* (1972). Solutions of oleate (0.1M) were prepared in albumin as described by Ross *et al.* (1967) [except that 10% (w/v) albumin was used] and added to the medium to a final concentration of 1 or 2mM. Oleate was determined as described by Itaya & Ui (1965). Other methods were as described by Biebucyk *et al.* (1972).

Results and discussion

Effect of halothane on ketone-body formation. Ketone-body synthesis by the perfused livers of 48h-starved rats was not inhibited by halothane, either from endogenous sources [19.3 ± 3.8 (3) μmol/h per g wet wt.] or from added oleate (2mM) [85.6 ± 6.6 (7) μmol/h per g wet wt.]. These rates are within the range of the control perfusions in the present work and of those reported by Krebs *et al.* (1969).

Qualitatively similar results were obtained in perfused livers of fed rats, in that exposure to halothane did not interfere with ketone-body production from added oleate (1mM).

When livers of either fed or starved animals were perfused with oleate (1mM, in order to determine more accurately fatty acid removal) the rate of fatty acid removal during exposure to halothane was within the same range as in the control experiments (10.7–19.0 μequiv./h per g wet wt.). Oleate did not prevent the stimulation of aerobic glycolysis by halothane observed during administration of halothane to perfused livers of fed rats (Biebuyck *et al.*, 1972). Thus, although oleate provides an oxidizable fuel under these conditions (see Table 1), it does not counteract the increased formation of lactate from glycogen in the presence of halothane.

Reversal by oleate of the inhibition by halothane of O_2 uptake. Biebuyck *et al.* (1972) showed that O_2 uptake by the perfused liver in the absence of added substrate is inhibited by 2.5% (v/v) halothane to a greater extent in the liver from fed animals (55%) than in that from starved animals (22%) (see Table 1). Halothane also prevented the increment of O_2 uptake of starved livers caused by the addition of lactate or NH_4Cl + ornithine + lactate to the medium, additions that promote gluconeogenesis and urea synthesis respectively.

When oleate (2mM) was included in the perfusion medium the increment in O_2 uptake brought about by the oleate was virtually the same in the control and halothane-treated livers (1.24 versus 1.02 μmol of O_2/min per g wet wt.; Table 1). Similarly when lactate (10mM) + oleate was present in the medium halothane had little effect on the increment of O_2 uptake due to the presence of the combined substrates (3.43 μmol/min per g wet wt. in the controls; 3.15 μmol/min per g wet wt. during halothane exposure). This is in marked contrast with the effect of halothane when lactate alone was present, when the increment in O_2 uptake caused by the presence of lactate was abolished.

In livers from fed rats the O_2 consumption rose from 2.09 to 2.33 μmol/min per g wet wt. on the

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Table 1. *Effect of oleate on the inhibition by halothane of O_2 uptake in the isolated perfused liver*

Livers from fed and 48h-starved rats were perfused as described in the text. Substrates were added to the medium at 30min. Halothane (2.5%, v/v) was administered during the 30–70min period. O_2 uptake was measured as described by Biebuyck *et al.* (1972). Results are expressed as μmol O_2/min per g wet wt., and are given as means ± S.E.M. with the numbers of observations in parentheses.

		O_2 uptake at 45min		O_2 uptake at 105min	
Source of liver	Substrate	Control	During halothane	Control	After halothane
Starved (48h) rats	None	1.83 ± 0.12 (7)	1.43 ± 0.33 (3)	1.86 ± 0.09 (7)	2.17 ± 0.18 (3)
	Lactate (10mM)	2.76 ± 0.13 (4)	1.63 ± 0.06 (3)	2.16 ± 0.22 (4)	2.53 ± 0.31 (3)
	Oleate (2mM)	3.07 ± 0.23 (5)	2.45 ± 0.08 (3)	2.30 ± 0.36 (5)	2.06 ± 0.21 (3)
	Lactate (10mM) + oleate (2mM)	5.26 ± 0.28 (3)	4.58 ± 0.51 (3)	4.67 ± 0.60 (3)	4.44 ± 0.59 (3)
Fed rats	None	2.09 ± 0.26 (3)	0.91 ± 0.06 (3)	2.16 ± 0.20 (3)	1.88 ± 0.29 (3)
	Oleate (2mM)	2.33 ± 0.43 (3)	1.94 ± 0.23 (5)	1.74 (2)	2.11 ± 0.20 (5)

Table 2. *Protective effect of oleate on liver metabolite changes produced by halothane during gluconeogenesis from lactate*

Data for the perfusions with lactate alone are from Biebuyck *et al.* (1972). In the experiments with oleate, livers from 48h-starved rats were perfused with lactate (10mM) + oleate (2mM). Substrates were added at 30min. Halothane (2.5%, v/v) was administered during the 30–60min period. Livers were freeze-clamped at 60min. Results are expressed as μmol/g wet wt., and are given as means ± S.E.M. of three experiments except where indicated otherwise. The results for the total number of metabolites determined are given in Table 4 of Biebuyck *et al.* (1972); only those significantly affected by halothane are included in the present table. The redox states of the mitochondria were calculated from the glutamate dehydrogenase system ([free NAD^+]$_m$/[free NADH]$_m$).

Substrate ...	Lactate (10mM)		Lactate (10mM) + oleate (2mM)	
	No halothane	Halothane	No halothane	Halothane
UDP-glucose	0.165 (2)	0.065 (2)	0.208 ± 0.003	0.177 ± 0.006
Glucose	1.57 (2)	0.57 (2)	2.10 ± 0.03	1.55 ± 0.09
Glucose 6-phosphate	0.064 ± 0.013	<0.01	0.079 ± 0.003	0.049 ± 0.010
Fructose 6-phosphate	0.015 (2)	<0.01	0.022 ± 0.002	0.015 ± 0.003
3-Phosphoglycerate	0.316 ± 0.012	0.771 ± 0.048	0.106 ± 0.009	0.151 ± 0.011
2-Phosphoglycerate	0.041 ± 0.002	0.086 ± 0.003	0.011 ± 0.005	0.018 ± 0.002
Phosphoenolpyruvate	0.194 ± 0.008	0.411 ± 0.025	0.088 ± 0.002	0.100 ± 0.010
Ammonia	0.610 ± 0.106	0.360 ± 0.044	0.45 (2)	0.700 ± 0.080
Glutamate	1.69 ± 0.09	0.930 ± 0.003	2.02 ± 0.11	2.03 ± 0.08
α-Oxoglutarate	0.341 ± 0.047	0.039 ± 0.011	0.333 ± 0.005	0.218 ± 0.018
[Free NAD^+]$_m$/[free NADH]$_m$	32 ± 8	4 ± 2	18 (2)	20 ± 4
ATP	1.70 ± 0.14	0.85 ± 0.02	1.97 ± 0.08	1.57 ± 0.08
ADP	0.84 ± 0.08	1.71 ± 0.11	0.83 ± 0.08	1.05 ± 0.07
AMP	0.17 ± 0.03	0.27 ± 0.02	0.19 ± 0.02	0.23 ± 0.01
Total adenine nucleotides	2.71 ± 0.24	2.30 ± 0.11	2.98 ± 0.16	2.85 ± 0.14
Citrate	0.524 (2)	0.081 (2)	2.05 ± 0.22	0.250 ± 0.050

addition of oleate, and the presence of oleate markedly decreased the inhibitory effect of halothane. In the absence of oleate the inhibition by halothane was 55%, whereas in its presence the inhibition was only 17%, i.e. of the same order of magnitude as in the livers from starved rats (see Table 1). Whenever there was a significant inhibition by halothane the rates of O_2 uptake had recovered to approximately the rate of the control at 105min (Table 1). These results support the conclusions of Biebuyck *et al.* (1972) that flavo-

protein-linked oxidation is relatively unaffected by halothane and that the site of inhibition by the drug is the reoxidation of NADH in the respiratory chain.

Effect of oleate on the inhibition by halothane of urea synthesis. The rate of urea synthesis by the isolated perfused rat liver has been shown (Biebuyck *et al.*, 1972) to be decreased during exposure to halothane to 0.28 ± 0.11 (5) from 1.91 ± 0.21 (4) μmol/min per g wet wt. in the presence of lactate (10mM) + NH_4Cl (10mM) + ornithine (2mM) in the perfusion medium. When oleate (2mM) was substituted for the lactate (10mM) in the present experiments the rate of urea synthesis [2.49 ± 0.11 (4) μmol/min per g wet wt.] was not significantly altered by exposure to halothane [2.20 ± 0.18 (3) μmol/min per g wet wt.].

Effect of oleate on the inhibition by halothane of gluconeogenesis from lactate. The fact that O_2 uptake with lactate + oleate as substrate was high even in the presence of halothane (see Table 1) suggested that oleate might prevent the inhibition by 2.5% (v/v) halothane of gluconeogenesis from lactate (10mM) (Biebuyck *et al.*, 1972).

Livers from 48h-starved rats were therefore perfused with lactate (10mM) + oleate (2mM). The presence of oleate increased the rate of glucose production from lactate from 0.95 ± 0.08 (12) to 1.44 ± 0.06 (3) μmol/min per g wet wt. Halothane administered during the 30–60min period caused a 50% inhibition of gluconeogenesis from lactate + oleate (0.72 μmol of glucose formed/min per g wet wt.) and only a 30% inhibition of lactate uptake. Both glucose synthesis and lactate removal had returned to the control rates during the 70–100min period (i.e. when the halothane was withdrawn). Thus, in spite of the fact that O_2 uptake with lactate + oleate was higher during exposure to halothane than in the control perfusions where lactate alone was the substrate, the rate of gluconeogenesis was lower than from lactate alone (0.72 versus 0.95 μmol/min per g wet wt.).

Effect of halothane on liver metabolites during gluconeogenesis from lactate + oleate. The inhibition of gluconeogenesis with lactate + oleate despite a high rate of O_2 uptake suggested some defect in the utilization, rather than in the supply, of ATP. Livers were freeze-clamped at 60min (i.e. after exposure to halothane for 30min) during perfusion with lactate (10mM) + oleate (2mM), and the tissue contents of intermediates related to gluconeogenesis were determined. Most of the changes in metabolite concentrations brought about by halothane on perfusion with lactate alone were abolished by the presence of oleate (Table 2). The presence of oleate prevented the decreases in UDP-glucose, glutamate and ATP that occurred when halothane was administered to perfusions with lactate alone, as well as preventing the eightfold decrease in α-oxoglutarate. The mitochondrial redox state as reflected by the calculated [free NAD^+]/[free NADH] ratio was maintained at the control value. The only major change produced by halothane when oleate was also present was an eightfold decrease in citrate content. The citrate concentration was very high (2.05 μmol/g wet wt.) in the presence of oleate, an observation that has been reported previously (Williamson *et al.*, 1969), and the effect of halothane was to decrease the citrate content to 50% of the control value of the experiments with lactate alone as a substrate. Thus there was little fall in the concentration of ATP during administration of halothane in the presence of oleate, and this indicates that halothane may have other effects besides inhibiting NADH dehydrogenase.

Discussion of this point and other new observations is premature. Questions that require further investigations are: Why does oleate fail to prevent the formation of lactate from glycogen in the presence of halothane? Is the increased lactate formation perhaps caused by an inhibition of fatty acid synthesis so that pyruvate, instead of forming acetyl-CoA, is reduced to lactate? Why is oleate more effective in abolishing the inhibition of urea synthesis than the inhibition of gluconeogenesis, although the ATP requirements for maintenance of normal rates of the two biosynthetic processes under the test conditions were almost the same?

The main outcome of the experiments is the demonstration that oleate can have a protective effect against metabolic changes produced by halothane, inasmuch as it maintains the ATP content of the liver and the normal rate of urea synthesis during halothane exposure (although it does not restore maximal rates of gluconeogenesis). The use of fatty acids as a protection against the adverse effects of halothane (particularly the interference with nitrogen metabolism) should be explored in patients undergoing prolonged or repeated clinical anaesthesia.

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