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Alteration by Halothane of Glucose and Glycogen Metabolism in Rat Skeletal Muscle

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Exposure of resting rat diaphragm for one hour *in vitro* to halothane (1-1.5, 2-2.5 and 4-4.5 per cent in oxygen) produced significant alterations of intracellular glucose disposition. Glycolysis (as measured by lactate production) increased, while glycogen formation was inhibited in a dose-related fashion. Net glucose uptake was unaffected by the anesthetic except during exposure to 4-4.5 per cent halothane, when 14 per cent depression of uptake was found. Total glycogen content decreased, due mainly to the inhibition of glycogen synthesis and to some extent to a stimulation of glycogenolysis. The anesthetic did not interfere with the effect of insulin on glucose uptake or the intracellular disposition of glucose. Creatine phosphate concentrations decreased following exposure of diaphragm to 1-1.5, 2-2.5 and 4-4.5 per cent halothane, while the adenosine triphosphate concentration declined after exposure to 4-4.5 per cent only. Although the mechanism(s) whereby halothane alters glucose and glycogen metabolism are unknown, it is possible that the anesthetic acts primarily by affecting membranes containing enzymes involved in the metabolism of glycogen. (Key words: Anesthetics, volatile, halothane; Metabolism, glucose; Metabolism, glycogen; Hormones, insulin; Muscle, skeletal, metabolism.)

ALTHOUGH INHALATIONAL ANESTHETICS often produce hyperglycemia, the mechanism of this

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phenomenon is still controversial. Greene¹ recently summarized the often conflicting data concerning the effects of anesthetic agents on insulin release and action and glucose transport. It seems clear that agents such as diethyl ether, cyclopropane and halothane can produce "glucose intolerance" in man and animals, but the data obtained from glucose tolerance tests are difficult to interpret. During anesthesia, factors such as surgical stress, depth of anesthesia, and tone of the autonomic nervous system are hard to control. Alterations in any of these may affect carbohydrate metabolism and the release and/or action of insulin (and other hormones). Results from studies of model systems *in vitro* are frequently easier to evaluate and are helpful in the interpretation of data from clinical studies.

We have examined the effects of halothane on glucose disposition, insulin action, and high-energy phosphate levels in resting striated muscle *in vitro*. Skeletal muscle is an important site of insulin action, a large sink for glucose utilization, and a source of lactate. Because of its responsiveness to insulin and its large mass, skeletal muscle plays a dominant role in the regulation of blood glucose concentration.

In muscle the oxidation of carbohydrate is a major source of adenosine triphosphate (ATP) and creatine phosphate, high-energy compounds needed for muscle contraction. In rare cases, anesthetics cause muscle to behave aberrantly, as in the syndrome of malignant hyperthermia. A better understanding of the effects of anesthetic agents on metabolism in normal muscle is important for our eventual understanding of this unusual phenomenon.

The results presented indicate that net glucose uptake and the action of insulin in resting muscle

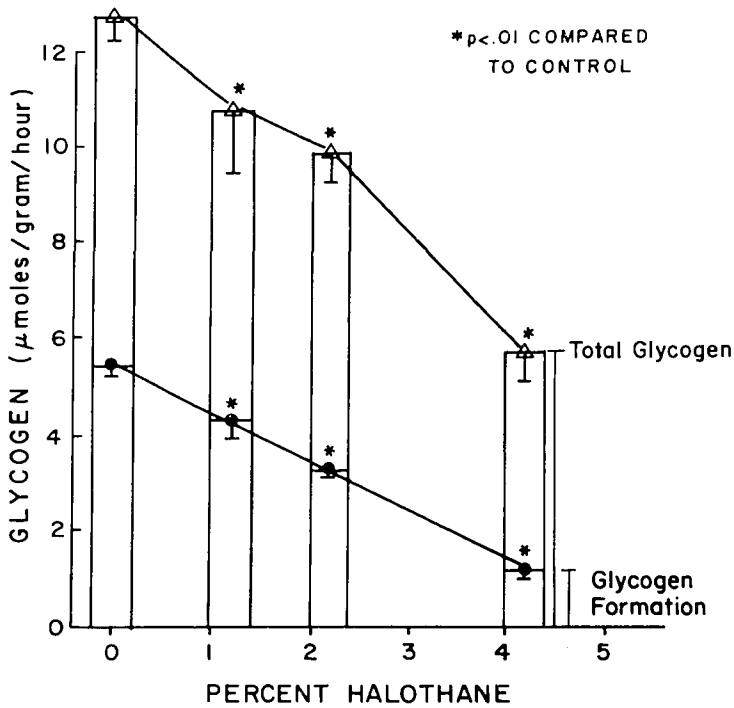


FIG. 1. Effects of a one-hour exposure to halothane on total glycogen and glycogen formation (in the absence of insulin).

are minimally affected by concentrations of halothane in oxygen as high as 4–4.5 per cent. However, during exposure to halothane there is a large decrease in glycogen synthesis with a concomitant increase in lactate formation from glucose. These metabolic changes are associated with a diminution of the tissue content of high-energy phosphate bonds.

Materials and Methods

Male Wistar rats weighing 150–200 g, fasted overnight, were decapitated and the diaphragms removed. After blotting and weighing, each hemidiaphragm was incubated for 15 minutes at 37 C in 2 ml of medium of the following composition: 0.04 M HEPES buffer, 5 mM MgCl₂, 5 mM KCl, 105 mM NaCl, 6 mM glucose (pH 7.4). This was followed by a 60-minute incubation at 37 C in a similar medium containing 6 mM U-¹⁴C-glucose. Insulin when added was 60 milliunits/ml.

Incubations were carried out in 25-ml Erlenmeyer flasks. Halothane, vaporized in oxygen, was blown into the flask for the first 2 minutes of the incubation and for 2 minutes after 30 minutes of incubation. At all other times, the flasks were tightly stoppered. In preliminary experiments this technique produced consistent gas-phase anesthetic concentrations. Samples of liquid or gas were taken periodically for the determination of halothane concentrations by gas chromatography.

Glucose uptake was determined by the difference between initial and final glucose concentrations

in the medium using the glucose oxidase method. Lactate, glucose-6-phosphate, ATP, and creatine phosphate were assayed by specific enzymatic methods.² Glycogen was determined as glucose after digestion of the tissue in hot KOH, precipitation with alcohol, and hydrolysis. Incorporation of ¹⁴C-glucose into glycogen was measured by the filter-paper technique of Thomas et al.,³ as described in an earlier article from this laboratory.⁴

In some experiments, lactate from the medium was separated by ion-exchange chromatography and the radioactivity of the isolated lactate determined.^{4,5} In this way, the amount of lactate derived from glucose in the medium was calculated.

Results

GLUCOSE UPTAKE

Halothane, except at the highest concentration used (4–4.5 per cent), had no significant effect on glucose uptake. At that concentration there was a small decrease (about 15 per cent) in uptake both in the absence and in the presence of insulin (table 1).

The stimulatory effect of insulin on glucose uptake was not affected by halothane.

GLYCOGEN FORMATION AND TOTAL GLYCOGEN

Net glycogen formation from glucose, as measured by incorporation of isotope into glycogen,

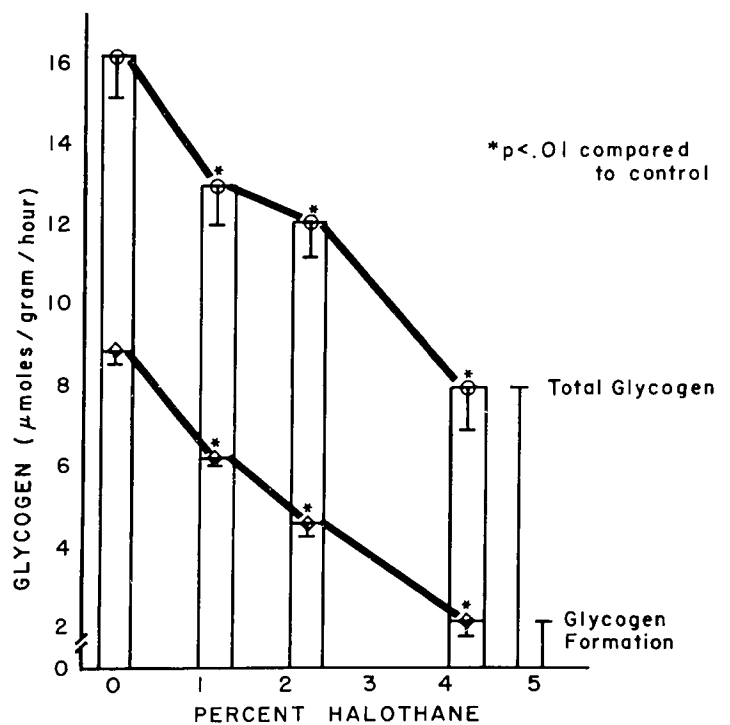


FIG. 2. Effects of a one-hour exposure to halothane on total glycogen and glycogen formation in the presence of 60 milliunits/ml insulin.

TABLE 1. Effect of Halothane on Glucose Uptake in the Presence and Absence of Insulin

	Glucose Uptake ($\mu\text{mol/g/hr} \pm \text{SEM}$) in the Presence of			
	No Halothane	Halothane 1-1.5 Per Cent (V/V)	Halothane 2-2.5 Per Cent (V/V)	Halothane 4-4.5 Per Cent (V/V)
No insulin	13.70 \pm 0.53 (n = 35)	14.60 \pm 1.39 (n = 9)	12.90 \pm 0.44 (n = 27)	11.81 \pm 0.41* (n = 16)
Insulin	21.92 \pm 0.74 (n = 11)	23.48 \pm 2.18 (n = 7)	21.64 \pm 1.01 (n = 7)	18.57 \pm 1.76* (n = 10)
Increase due to insulin	60 per cent	61 per cent	68 per cent	58 per cent

* $P < .01$ compared with control.

TABLE 2. Effect of Halothane on Lactate Formation

	Lactate Formation in the Presence of			
	No Halothane	Halothane 1-1.5 Per Cent (V/V)	Halothane 2-2.5 Per Cent (V/V)	Halothane 4-4.5 Per Cent (V/V)
Total lactate formation ($\mu\text{mol/g/hr} \pm \text{SEM}$) No insulin	14.86 \pm 0.32 (n = 35)	20.58 \pm 1.53* (n = 9)	21.00 \pm 0.66* (n = 27)	25.21 \pm 1.11* (n = 16)
Insulin	18.03 \pm 0.87 (n = 11)	26.27 \pm 2.73* (n = 7)	26.75 \pm 2.25* (n = 7)	33.39 \pm 1.95* (n = 10)
Increase due to insulin	21 per cent	28 per cent	27 per cent	32 per cent
Lactate from ^{14}C -glucose (as glucose equivalents†) No insulin	1.74 \pm 0.40 (n = 6)	2.77 \pm 0.38† (n = 10)	3.73 \pm 1.21† (n = 4)	5.68 \pm 0.71* (n = 7)
Insulin	3.02 \pm 0.51 (n = 7)	6.82 \pm 0.82* (n = 6)	5.55 \pm 1.32† (n = 5)	6.52 \pm 1.19* (n = 8)

* $P < .01$ compared with control.

† $P < .05$ compared with control.

‡ *i.e.*, the μmol of glucose converted to lactate. The actual lactate is twice this amount.

TABLE 3. Balance Sheet for the Effects of Halothane on Glucose Metabolism by Rat Diaphragm

	Insulin	Compared with No Halothane				
		A Change in Glucose Uptake ($\mu\text{mol/g/hr}$)	B Change in Glycogen Synthesis ($\mu\text{mol/g/hr}$)	C Change in Total Glycogen† ($\mu\text{mol/g/h}$)	D Change in Lactate Output†† ($\mu\text{mol/g/hr}$)	E Lactate ($\mu\text{mol/g/hr}$) Derived from Sources Other than Glucose Uptake*
Halothane						
1-1.5 per cent	0	+0.90	-1.17	-0.99	+2.86	0.79
2-2.5 per cent	0	-0.80	-2.15	-2.78	+3.07	1.72
4-4.5 per cent	0	-1.89	-4.28	-6.96	+5.18	2.79
1-1.5	+	+1.56	-2.87	-3.01	+4.12	0
2-2.5	+	-0.28	-4.67	-4.16	+4.36	0
4-4.5	+	-2.35	-7.31	-8.27	+7.68	2.72

* Calculated as follows: Change in lactate output-(change in glucose uptake-change in glycogen synthesis)

† Glycogen and lactate expressed as glucose equivalents.

TABLE 4. Effects of Halothane on Glucose-6-Phosphate, ATP, and Creatine Phosphate Levels

	Concentration ($\mu\text{mol/g hr} \pm \text{SEM}$ after 1 hour of incubation) in the Presence of			
	No Halothane	Halothane 1-1.5 Per Cent (V/V)	Halothane 2-2.5 Per Cent (V/V)	Halothane 4-4.5 Per Cent (V/V)
G-6-P	.245 \pm .011 (n = 8)	.226 \pm .007 (n = 5)	.227 \pm .01 (n = 6)	.206 \pm .008 (n = 10)
ATP	2.86 \pm 0.15 (n = 6)	2.21 \pm .018 (n = 5)	2.69 \pm 0.19 (n = 6)	2.01 \pm .15* (n = 11)
Creatine phosphate	9.63 \pm 0.38 (n = 6)	6.17 \pm .75* (n = 5)	6.32 \pm 0.60* (n = 6)	4.35 \pm 0.8* (n = 11)

* $P < .01$ compared with control.

was depressed in linear fashion by halothane beginning at the lowest concentration studied (fig. 1). In the presence of insulin a similar effect was observed (fig. 2).

Total tissue content of glycogen was also diminished during exposure to halothane.

LACTATE PRODUCTION

As indicated in table 2, total lactate output increased significantly at all halothane concentrations. Insulin itself produced a small increase in lactate formation, as has been observed previously.^{4,6}

Lactate derived from glucose, estimated from the amount of lactate containing ¹⁴C, increased significantly (table 2).

NET METABOLIC CHANGES PRODUCED BY HALOTHANE

The alterations in glucose disposition produced by halothane are summarized in the balance sheet shown in table 3.

By comparing columns B and C it can be seen that, at the lowest halothane concentration, the decrease in glycogen synthesis was essentially equal to the lowering of total glycogen. As the halothane concentration increased, there was a tendency for the breakdown of glycogen to become larger than the decrease in synthesis. Without insulin this was seen with 2-2.5 and 4-4.5 per cent halothane. With insulin it was seen with 4-4.5 per cent halothane only.

Halothane increased lactate production in a dose-related manner (column D). In column E we report the increase in lactate that cannot be accounted for by changes in glucose uptake (column A) and incorporation of glucose into glycogen (column B). This lactate fraction probably represents an action of halothane on glycogenolysis. In the absence of insulin there was a steady increase in this fraction with increasing halo-

thane concentrations. With insulin the increase was apparent only at 4-4.5 per cent halothane.

Overall, halothane inhibited glycogen synthesis and directed more glucose to lactate. As the concentration of halothane increased, changes in the rate of glycogenolysis became more prominent.

GLUCOSE-6-PHOSPHATE, ATP AND CREATINE PHOSPHATE

The tissue concentrations of glucose-6-phosphate were not significantly affected by halothane. ATP levels were also well maintained, and only in the present of 4-4.5 per cent halothane was there a statistically significant decrease. However, creatine phosphate concentration declined markedly upon exposure of the diaphragm to the anesthetic (table 4). The decrease in creatine phosphate was evident at the lowest concentration of halothane used.

Discussion

The experiments presented here show that in resting skeletal muscle incubated *in vitro*, halothane, at clinically useful concentrations, produces significant and specific effects on glucose metabolism. Although net glucose uptake is hardly affected by the anesthetic, the disposition of glucose after entering the cell is altered markedly. As the concentration of halothane is raised, there is a dose-dependent decrease in the synthesis of glycogen, concomitant with an increase in the proportion of glucose metabolized to lactate.

The observation that tissue uptake of glucose is unaffected by halothane is in agreement with results of investigations with intact animals and *in-vitro* preparations. In dogs anesthetized with 2 per cent halothane, Camu⁷ found that glucose uptake by hind limb musculature and adipose tissue was, if anything, slightly increased during anesthesia. Glucose flux was determined in these experiments according to the Fick principle, blood flow being measured with flowmeters. As in our experiments, it

was also found that halothane did not significantly influence the action of insulin in promoting tissue glucose uptake.

Greene showed that 2.3 per cent diethyl ether, 0.7 per cent halothane, and a number of other anesthetic agents did not affect glucose permeability of human erythrocytes.⁸ However, CO₂-stimulated glucose entry into erythrocytes was inhibited by these agents.⁹ Morrow and Paradise¹⁰ reported that halothane failed to influence net glucose uptake by non-working atrial tissue *in vitro*.

Biebuyck *et al.*¹¹ studied the action of halothane on metabolism of the perfused rat liver and observed that in this preparation the anesthetic markedly inhibited gluconeogenesis and stimulated glycolysis. In a second study by Biebuyck and Lund,¹² effects of halothane on rat liver metabolism were measured *in vivo*, and it was found that 1.5 per cent halothane promoted the formation of glucose from glycogen, an action that was reflected in an increase in blood glucose.

It is interesting to compare the action of halothane with those of other agents that influence glucose metabolism in muscle. Epinephrine and phosphodiesterase inhibitors, substances that increase the tissue content of cyclic AMP and elevate blood glucose, markedly decrease the formation of glycogen from glucose. However, in contrast to the depression produced by halothane, the depression of glycogen synthesis produced by these agents is associated with a decrease in glucose uptake by the tissue equal in magnitude to the effect on glycogen formation.⁴ It would be important to determine in future experiments whether the influx into the cell of a nonmetabolized sugar, such as 3-O-methylglucose, is altered by halothane. This process is unaffected by epinephrine.⁴

The acceleration of glycolysis produced by halothane may be related to the decrease in high-energy phosphate compounds, as shown by the lowering of creatine phosphate levels seen even at the lowest concentration of halothane studied. This, in turn, may reflect inhibition by halothane of mitochondrial energy production, as has been demonstrated with liver,¹³ brain,¹⁴ and muscle mitochondria¹⁵ incubated *in vitro*. It is suggested that the decrease in creatine phosphate and expected increase in inorganic phosphate would contribute to activation of the glycolytic control enzyme, phosphofructokinase, as has been shown in preparations from rabbit muscle.¹⁶ This would lead to increased lactate formation.

In some preparations, halothane has also been found to interfere with the ability of mitochondria to take up calcium.¹⁴ This might lead to an increase in the cytoplasmic concentration of free calcium, with subsequent effects on carbohydrate metabolism. For example, elevation of cellular calcium is known to accelerate phosphorylase

TABLE 5. Effects of 4–4.5 Per Cent Halothane and 15 Per Cent Ether* on Glycolysis in Rat Diaphragm

	No Insulin	Insulin
Glucose uptake (change from control)		
Ether†	-18 per cent	-9 per cent
Halothane‡	-14 per cent	-16 per cent
Total Lactate (change from control)		
Ether*	+45 per cent	+100 per cent
Halothane‡	+70 per cent	+85 per cent
Change in total glycogen (compared with incubation in absence of anesthetic)		
Ether*	-6.1 $\mu\text{mol/g}$	-8.8 $\mu\text{mol/g}$
Halothane‡	-7.0 $\mu\text{mol/g}$	-9.3 $\mu\text{mol/g}$

* 20 μl ether liquid vaporized in 25-ml Erlenmeyer flasks at 37 C.

† Data from Brunner's study.¹⁸

‡ Data from this study.

The values for glucose uptake and total lactate are expressed as percentage changes from control since the absolute values in Brunner's study differ from ours because he employed different incubation medium.

Although initial tissue glycogen content was *not* measured in this study as it was in Brunner's, the original glycogen content was calculated by subtracting the glycogen formed during the control hour of incubation from the total glycogen measured at the end of this hour.

activity. Although Reynolds¹⁷ failed to observe stimulation of phosphorylase activity by halothane in the rat diaphragm, the concentrations of halothane he employed were less than those at which we observed significant glycogenolysis.

The action of halothane on carbohydrate metabolism of muscle appears to be similar in many respects to that of diethyl ether. Brunner¹⁸ studied the effects of diethyl ether on glucose metabolism of rat diaphragm incubated *in vitro* and observed that this anesthetic promoted glycogen breakdown and increased tissue output of lactate. For comparison, we summarize in table 5 the main findings of Brunner, together with some of our own results. The experiments demonstrate that at relatively high concentrations of both halothane and ether there is a breakdown of glycogen in muscle with a marked increase in lactate formation. The action of insulin, however, is not interfered with.

Our data, with those obtained by Porsius and Van Zwieten¹⁹ using purified enzymes involved in glycolysis, and others²⁰ do not support the hypothesis of Paradise¹⁰ and co-workers that halothane effects a block of the Embden-Meyerhoff pathway at the phosphoglucose isomerase step. An inhibition by halothane of this enzyme could be expected to increase the glucose-6-phosphate content of the tissue. However, this did not occur.

Henneman and Vandam²¹ observed that the creatine phosphate content of human rectus muscle

decreased significantly during surgical anesthesia with ether. It would be important to know whether anesthetic agents cause more severe disturbances of glycolytic and mitochondrial reactions in patients susceptible to malignant hyperthermia than in normal individuals. For example, Harrison *et al.*²² and Nelson *et al.*²³ found that after 30 minutes of exposure to 4 per cent halothane *in vitro*, the ATP content of skeletal muscle from malignant hyperthermia-susceptible swine was diminished to a far greater extent than in muscle from normal pigs.

The studies of the action of halothane presented here were carried out with muscle preparations in a resting state. It is possible that in contracting muscle, anesthetic agents may have even more pronounced effects on metabolism. Studies on the effects of halothane on contractility and carbohydrate metabolism of the working nerve-diaphragm preparation are in progress.

In previous studies it has been well demonstrated that halothane and other anesthetic agents interfere with the action of enzymes situated on mitochondrial membranes. Since there is evidence that enzymes involved in the synthesis and breakdown of glycogen in muscle are situated on membranes^{6,24} also, *i.e.*, the sarcoplasmic reticulum, the action of halothane on muscle metabolism described here may also be related to an influence of the anesthetic on properties of membranes.

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