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METABOLIC SITES OF ACTION OF HALOTHANE IN RAT ATRIA*

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Abstract Rat atrial metabolism was monitored by measuring the production of $^{14}CO_2$ from ^{14}C-labeled substrates. D-Glucose and D-mannose metabolism were depressed by low concentrations of halothane (1 mM) which did not significantly affect the metabolism of pyruvate, D-fructose, DL-beta-hydroxybutyrate or octanoate. Halothane (1 mM) did not alter the uptake of 3-*O*-methyl glucose by rat atria. It is concluded that halothane blocks an early step(s) in glycolysis. The most likely sites are the phosphoglucose isomerase (PGI) and phosphomannose isomerase (PMI) steps. The incorporation of D-glucose, D-mannose and D-fructose into glycogen were significantly inhibited by 1 mM halothane, although the total glycogen content was not affected. We conclude that halothane inhibits glycogen turnover. Higher concentrations of halothane (8 and 16 mM) were required to inhibit the metabolism of pyruvate and D-fructose. This action of halothane is attributed to the known inhibition by halothane of electron transport processes. Neither DL-beta-hydroxybutyrate nor octanoate metabolism to CO_2 was affected by 1 mM halothane, although higher concentrations of halothane produced an inhibition. It is concluded that some of the steps in fatty acid oxidation are unaffected by low concentrations of halothane.

HALOTHANE has been shown to inhibit an early step in glycolysis, probably the phosphoglucose isomerase step, if a single site is involved, based on the following observations. Halothane, as well as other inhalation anesthetics, depresses the force of contraction of the heart.[1] Depression of the contractile force of the isolated rat auricle to 50 per cent of the original force requires about 6 mg/100 ml (0·3 mM) halothane,[2] which is equivalent to a partial pressure of 5·7 mm Hg, just sufficient to prevent response to a skin incision in humans (MAC 1).[3] This depression can be partially overcome by the metabolizable substrates—pyruvate, lactate, acetate—but not additional D-glucose.[2] D-Fructose is metabolized via a nonspecific hexokinase and phosphofructokinase in yeast,[4] rapidly growing liver tumors,[5] skeletal muscle[6–8] and apparently also in rat atrial muscle,[9–12] and is also capable of partially restoring the reduced force of contraction of halothane-depressed atria.[13] In atria not exposed to halothane but containing D-glucose, neither D-fructose nor pyruvate has any significant positive inotropic effect, although D-glucose does. In substrate-free treated atria, all three substrates have a positive inotropic action.[10]

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The observations up to this point indicated that the negative inotropic action of halothane was due to an interference with utilization of D-glucose at an early step in glycolysis. The possible steps were: D-glucose uptake, phosphorylation of D-glucose to glucose 6-phosphate (G-6-P) or isomerization of G-6-P to fructose 6-phosphate (F-6-P). D-Glucose uptake or phosphorylation was ruled out because halothane produced its usual depressant effect in the absence of external D-glucose.[14] The atria apparently rely on endogenous glycogen as the major supplier of energy in the absence of externally supplied substrate.[11] Also, the fact that halothane produced a much faster decrease in force of contraction than merely omitting D-glucose tended to support the concept that D-glucose uptake or phosphorylation was not the site of halothane's depressant action.[12,14] These latter observations point to the phosphoglucose isomerase step as the step inhibited by halothane, if only a single site is affected.

An attempt was made to verify some of the observations in the isolated human atrial preparation. D-Glucose and pyruvate were found to be much better utilized for contractile force than D-fructose, lactate or acetate.[15] D-Glucose and pyruvate were thus tested in halothane-depressed preparations. D-Glucose had no effect, while pyruvate had a marked positive inotropic action.[16] This supported the concept that halothane blocked glycolysis in human as well as rat atria. The partial pressure of halothane required to depress the force of human atria by 50 per cent was, as in the rat, equivalent to MAC I. Since human cardiac function *in vivo* is apparently not as markedly affected by such low levels of halothane,[17,18] it is probable that hormonal and nervous adjustments are made to compensate partially for any changes in cardiac function. An additional possibility is that fatty acids supply the bulk of the energy for human cardiac function *in vivo* and that utilization of fatty acids is not interfered with by halothane. Atria from starved rats required almost twice the concentration of halothane to produce 50 per cent depression as atria from fed rats.[19] Since starvation is known to increase the endogenous lipid content of the heart[20] and apparently also its utilization,[21,22] it is probable that this lipid can be used to support the force in the presence of halothane.

The present investigation had three major goals: (1) to attempt to evaluate, using metabolic studies, the hypothesis that halothane blocks an early step in aerobic glycolysis in the mammalian heart; (2) to attempt to define further the exact site of this block in glycolysis; and (3) to attempt to determine whether halothane interferes with certain steps in lipid metabolism in the rat heart.

METHODS

Determination of halothane concentration. Extraction and determination of halothane concentrations in the incubation medium in contact with the tissues have been described previously.[23]

Metabolism of ^{14}C-labeled stubstrates to $^{14}CO_2$. Fed male Sprague–Dawley rats weighing between 225 and 325 g were killed by decapitation. The heart was quickly excised and was placed in chilled, constantly oxygenated medium. The atria (both the right and left atria with connecting tissue) were removed and trimmed free of excess venticular and vascular tissue.

The incubation medium had the following composition (mM): NaCl, 120; KCl, 6; $MgSO_4$–$7H_2O$, 1·34; NaH_2PO_4–H_2O, 1·21; $CaCl_2$–$2H_2O$, 1·22; and Tris [tris

(hydroxymethyl) aminomethane], 25·3, as the buffer with the pH adjusted to 7·4. One of the following compounds served as the substrate (mM): D-glucose, 5·55; D-mannose, 5·55; D-fructose, 30; DL-beta-hydroxybutyrate, 5; octanoate, 1·5; or pyruvate 2·5. Each of these substrates was tracer labeled as follows (μCi/flask): 6-^{14}C-glucose, 0·2; 6-^{14}C-mannose, 0·66; 6-^{14}C-fructose, 0·5; 2-^{14}C-beta-hydroxybutyrate, 0·5; 1-^{14}C-octanoate, 0·33; or 1-^{14}C-pyruvate, 0·5. The medium to which the tissue was exposed was bubbled continuously prior to the start of incubation with 100% O_2.

One atrial pair was placed in 2 ml of well oxygenated medium in a Warburg flask. The flasks were immediately sealed with glass stoppers, coated lightly with Cello-grease (Fisher), immersed in the water bath of a Gilson differential respirometer and incubated for 1 hr at 30°. When halothane was administered, it was injected into the medium just prior to sealing. The center well of these flasks contained 0·2 ml of 2 N KOH and the side arms contained 0·2 ml of 70% $HClO_4$. At 60 min, the $HClO_4$ was tipped into the medium to stop all enzymatic processes and to liberate CO_2. The CO_2 was trapped in the KOH in the center well of the Warburg flask by shaking for an additional hr. A 0·1-ml aliquot of this KOH was placed in a plastic scintillation vial containing 10 ml of Bray's solution and was counted in a Packard Tri-Carb liquid scintillation spectrometer.

A time study was carried out in which atria were incubated in the presence of 5·6 mM D-glucose for 30, 60 and 120 min. The production of $^{14}CO_2$ from the 6-^{14}C-glucose was 110, 305 and 815 cpm/g of tissue dry wt respectively. Since the CO_2 production continued to increase over this time span, it was decided that 60 min would be the incubation time.

All experiments with halothane contained a similar number of controls, run at the same time, to which the halothane experiments were statistically compared using Student's *t*-test.

Although the previous functional studies of Ko and Paradise[2,13,14,16,19] employed bicarbonate and 5% CO_2 as the buffer system, this could not be used in the present study. The CO_2 trapping agent could not be added by injection through a rubber stopper, since halothane has a great affinity for rubber. For this reason, an all-glass closed system containing KOH in the center well of the Warburg flask was used to trap CO_2 as it was formed. This necessitated a non-CO_2 buffer. Tris was used because the prior functional studies of Ko *et al.*[24] on rat atria have shown Tris to behave similarly to bicarbonate.

Incorporation of ^{14}C-labeled substrates into glycogen. The atria were incubated as previously described. At 60 min, they were removed, lightly blotted and immersed in tared centrifuge tubes containing 30% KOH. The glycogen was isolated via alcoholic KOH extraction,[25] hydrolyzed with sulfuric acid and neutralized with NaOH. An aliquot was taken for counting via liquid scintillation; a second aliquot was analyzed for μg of glycogen using the glucose oxidase colorimetric assay for glucose.[26]

Effect of halothane on phosphorylase a. After incubation for 1 hr, the atria were homogenized and the ratio of phosphorylase *a* to total phosphorylase was determined according to the method of Cahill *et al.*[27]

Effect of halothane on 3-O-*methyl glucose uptake.* Atria were isolated from 225 to 300 g male rats and placed in 2 ml Tris-buffered medium as previously described, except that it contained no substrate but did contain 1·0 mM mannitol and 5·0 mM 3-*O*-methyl glucose and either 1·0 μCi 1-^{14}C-mannitol or 1·0 μCi U-^{14}C-3-*O*-methyl

glucose. The tissues were incubated as previously described for 10, 20, 40, 60 or 80 min in the presence and absence of halothane. After the period of incubation, the atria were blotted either 2 or 20 times,[28] weighed, dried to constant weight and re-weighed. These atria were then pulverized and digested for 1 hr in 0·5 ml Soluene-100 (Packard Instrument Company), placed in 10 ml of Bray's scintillation solution and counted. An aliquot of medium was also taken for counting. From the amount of 1-^{14}C-mannitol in the tissue and its concentration in the medium, the volume of distribution of this extracellular marker in the total tissue water (extracellular space) was determined. The total water volume minus the extracellular water was considered to be the intracellular water space. In different atrial preparations, the volume of distibution of 3-*O*-methyl glucose was determined and the rate of 3-*O*-methyl glucose uptake in the presence and the absence of halothane was calculated.

Calculation of 3-O-*methyl glucose uptake.* One must assume that 1 mg medium = 1 μl medium.

(1) Determine total tissue water:

(total mg wet wt) − (total mg dry wt) = total μl water.

(2) Determine how much of total tissue water is equilibrated with the medium (mannitol or extracellular space):

$$\frac{\text{(dis./min/total mg dry wt)}}{\text{(dis./min/}\mu\text{l medium)}} = \frac{\mu\text{l medium}}{\text{total mg dry wt}}.$$

(3) Determine intracellular (non-mannitol) space:

(total μl water) − (water equilibrated with medium) = intracellular water.

The intracellular water to dry wt ratio was constant for the conditions of these experiments and unaffected by halothane. (For further information on the validity of using this ratio, see Paradise and Morrow.[29]) Since it is a constant, this intracellular water/dry wt was used in calculating 3-*O*-methyl glucose uptake in other atrial pairs.

(4) Determine intracellular water for tissue in which 3-*O*-methyl glucose uptake is to be measured:

$$\frac{\text{(intracellular water)}}{\text{(dry wt)}} \times \text{(dry wt)} = \text{intracellular water (constant for mannitol).}$$

(5) Determine non-3-*O*-methyl glucose space for this tissue: see steps 1, 2 and 3.

(6) Determine how much intracellular water is equilibrated with 3-*O*-methyl glucose:

(intracellular water) − (non-3-*O*-methyl glucose water) = ml of intracellular water equilibrated with 3-*O*-methyl glucose.

(7) Determine μmoles of 3-*O*-methyl glucose taken up by the tissue in 5 mM solution:

$$\frac{\text{ml intracellular water equilibrated with 3-}O\text{-methyl glucose}}{\text{g dry wt}} \times \frac{(5\ \mu\text{moles})}{\text{(ml)}} = \frac{\mu\text{moles}}{\text{g dry wt}}.$$

RESULTS

Production of CO_2 from glucose as a function of glucose concentration. The production of $^{14}CO_2$ from 6-^{14}C-glucose as a function of glucose concentration was measured in rat atria in the presence and absence of 1·0 mM halothane (Fig. 1). The range of glucose concentrations was from 12·5 to 400 mg/100 ml (0·7 to 22·2 mM). The specific activity of the glucose remained constant at 0·022 μCi/m-mole at all glucose concentrations. Halothane significantly inhibited CO_2 production at every glucose concentration studied, except at 50 and 400 mg/100 ml. The amount of $^{14}CO_2$ evolved/mg dry wt/hr reached a maximum and leveled off at 100 mg/100 ml (5·6 mM) glucose, regardless of whether halothane (1·0 mM) was present or absent.

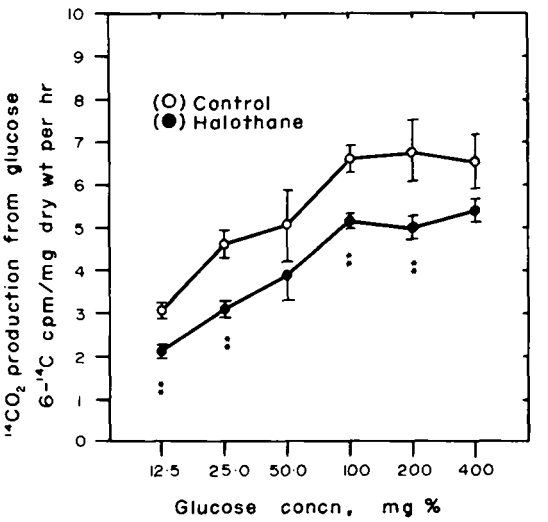

FIG. 1. Production of $^{14}CO_2$ from 6-^{14}C-glucose as a function of D-glucose concentration in rat atria in the presence and absence of halothane (1·0 mM). Values represent means ± S.E. for four atria. ** $P < 0·01$.

Effect of increasing concentrations of halothane on $^{14}CO_2$ production from various ^{14}C-labeled substrates. Ko and Paradise[2,13] presented evidence which suggested that the functional utilization of 5·6 mM glucose was blocked by low halothane concentrations, but that the functional utilization of 2·5 mM pyruvate or 30 mM fructose was only partially blocked. In order to substantiate these findings, rat atria were incubated in the presence of one of these substrates (mM): D-glucose, 5·6; pyruvate, 2·5; or D-fructose, 30 (Fig. 2). The data are expressed as the per cent inhibition of $^{14}CO_2$ production from each substrate, so that the effects of halothane on the different substrates can be compared. Carbon dioxide production from the atria at each halothane concentration was compared to control data of the same day. In the curve depicting $^{14}CO_2$ production from 6-^{14}C-glucose (Fig. 2, upper left), $^{14}CO_2$ production was depressed by all concentrations of halothane. Concentrations of halothane, 1 mM and higher (except for 4 mM), all significantly inhibited CO_2 production.

A different pattern occurred when pyruvate served as the substrate (Fig. 2, upper right). The production of $^{14}CO_2$ from 1-^{14}C-pyruvate was significantly inhibited only at 8 and 16 mM halothane.

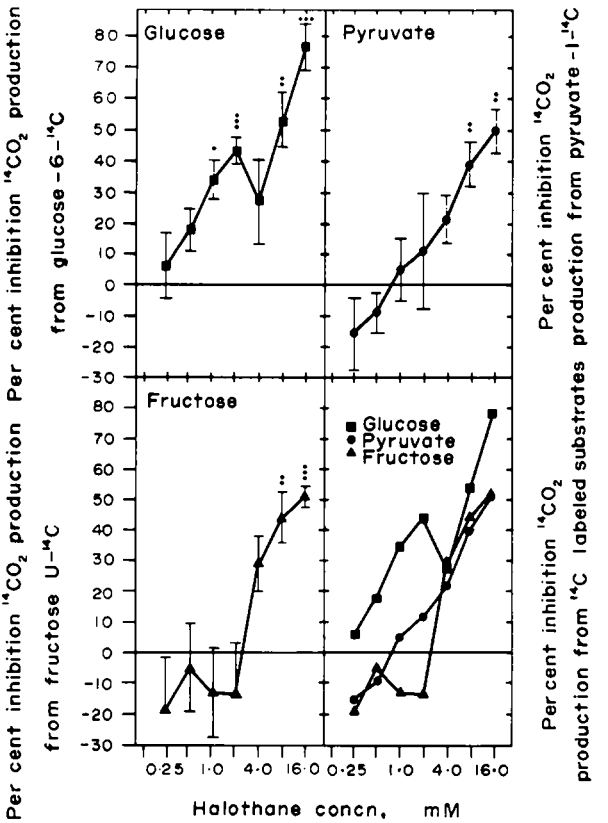

FIG. 2. Effect of increasing concentrations of halothane on $^{14}CO_2$ production from various ^{14}C-labeled substrates in rat atria. Values represent means ± S.E. for at least seven atria. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. By taking into account appropriate correction factors,[30] mean control values for CO_2 production from glucose, fructose and pyruvate were found to be 680 ± 28, 1020 ± 60 and 1190 ± 59 nmoles/g dry wt/hr respectively.

The graph of the effect of halothane on U-^{14}C-fructose conversion to $^{14}CO_2$, when expressed as per cent inhibition by halothane, very closely resembled the pyruvate data (Fig. 2, lower left). Only the 8 and 16 mM halothane concentrations significantly depressed $^{14}CO_2$ production.

When these three sets of data are plotted on the same graph (Fig. 2, lower right), the contrasts in the pattern of CO_2 production from the three substrates are more easily pointed out. Glucose metabolism to CO_2 was inhibited more than pyruvate metabolism to CO_2 or fructose metabolism to CO_2 at almost every halothane concentration. The most striking differences occurred at the halothane concentrations of 1 and 2 mM, where glucose metabolism was significantly inhibited but pyruvate and fructose metabolism were not significantly affected.

Effect of halothane on 3-O-*methyl glucose uptake in rat atria.* In order to determine whether the inhibitory action of low concentrations of halothane on glucose metabolism was due to an interference with glucose transport, we investigated the effect of halothane on a nonmetabolizable analogue of glucose which is also transported by the same mechanism as glucose, 3-*O*-methyl glucose. Mannitol was used as a marker to define the extracellular space.

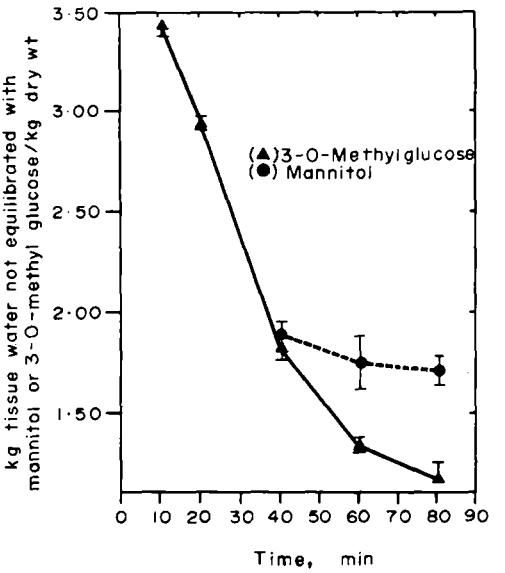

FIG. 3. Time course of tissue water not equilibrated with mannitol or 3-*O*-methyl glucose. Values represent means ± S.E. for at least four atria.

The amount (kg) of tissue water/kg of dry weight not equilibrated with mannitol or with 3-*O*-methyl glucose was determined and is graphed as a function of incubation time (Fig. 3). Mannitol essentially reached a state of equilibrium with the extracellular water by 40 min, since very little further equilibration occurred at 60 or 80 min. 3-*O*-methyl glucose became distributed in progressively more tissue water from 10 to 80 min. At 40, 60 and 80 min, there was less tissue water not equilibrated with 3-*O*-methyl glucose than with mannitol. Hence, if mannitol is a reliable indicator for extracellular water, 3-*O*-methyl glucose was taken up at these three time intervals.

The effects of halothane (1 mM) were studied on 3-*O*-methyl glucose uptake at 40, 60 and 80 min (Table 1). The effect of a known inhibitor of hexose transport, phlorizin (3 mM), was determined at 80 min.

Halothane had no effect on hexose uptake at the concentration utilized; however, the system was sensitive to 3 mM phlorizin, since a marked decrease in 3-*O*-methyl glucose uptake was observed in the presence of this inhibitor.

Effect of halothane on mannose metabolism. Another hexose which enters the glycolytic cycle above phosphofructokinase is D-mannose. The production of $^{14}CO_2$ from

TABLE 1. EFFECT OF HALOTHANE OR PHLORIZIN ON 3-*O*-METHYL GLUCOSE UPTAKE IN RAT ATRIA

Treatment	N	3-*O*-methyl glucose uptake (m-moles hexose/kg tissue dry wt/hr)		
		40 min	60 min	80 min
Control	4	0·53	2·00	2·06
Halothane (1 mM)	4	0·53	2·10	2·02
Phlorizin (3 mM)	4			0·41

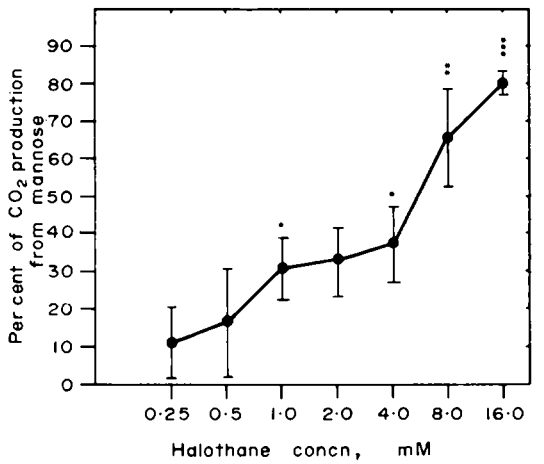

FIG. 4. Effect of increasing concentrations of halothane on $^{14}CO_2$ production from U-^{14}C-mannose in rat atria. Values represent means ± S.E. for at least seven atria. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. The mean control value of CO_2 production from mannose was 830 ± 60 nmoles/g dry wt/hr.

5·6 mM U-^{14}C-mannose was studied at varying halothane concentrations (Fig. 4). Halothane appears to inhibit D-mannose metabolism to CO_2 in a manner very similar to the way in which it inhibits D-glucose conversion to CO_2. Halothane concentrations of 1 mM or higher (except 2 mM) significantly depressed mannose metabolism.

Effect of halothane (1 mM) on the incorporation of ^{14}C-labeled hexoses into glycogen. Most of the previous studies in this investigation concerning the action of halothane on carbohydrate metabolism involved studying the oxidation of these substrates to CO_2. Many of the enzyme steps which are potential sites of action for the postulated halothane glycolytic block are also enzymes which are required for the synthesis of glycogen from different hexoses. Therefore, the effects of halothane (1 mM) on the

TABLE 2. EFFECT OF HALOTHANE ON HEXOSE INCORPORATION INTO GLYCOGEN AND ON GLYCOGEN CONTENT OF RAT ATRIA

Hexose	Treatment	N*	Hexose incorporated (m-moles/g tissue wet wt/hr)	Glycogen (μg/g wet wt)
Glucose	Control	20	641 ± 59	262 ± 19
	Halothane (1·0 mM)	12	321 ± 29†	229 ± 13
Mannose	Control	8	169 ± 11	429 ± 84
	Halothane (1·0 mM)	8	86 ± 9†	418 ± 49
Fructose	Control	8	93 ± 7	441 ± 53
	Halothane (1·0 mM)	8	63 ± 6‡	402 ± 29

* N = number of experiments.
† $P < 0.001$ compared to control.
‡ $P < 0.005$ compared to control.

incorporation of ^{14}C-labeled hexoses (5·6 mM) into glycogen and on glycogen content of rat atria were examined. Hexose incorporation into glycogen was expressed as nmoles of hexose incorporated per g tissue wet wt per hr. On an nM basis, considerably less fructose and mannose were incorporated than glucose (Table 2). Halothane significantly blocked incorporation of all three hexoses into rat atrial glycogen. Glycogen content (expressed as μg glucose/g of tissue wet wt) of atria was not significantly affected by halothane regardless of which hexose was present (Table 2).

Effect of halothane on phosphorylase activity. Phosphorylase activation and inactivation is one regulatory site for glycogen turnover. The per cent of phosphorylase found in the phosphorylase *a* or active form was determined in atria incubated in the presence and in the absence of 1 mM halothane (Table 3).

TABLE 3. EFFECT OF HALOTHANE ON THE PER CENT OF RAT ATRIAL PHOSPHORYLASE IN THE ACTIVE FORM

N	Treatment	Phosphorylase *a* (% ± S.E.)
4	Control	21·3 ± 1·4
4	Halothane (1 mM)	19·3 ± 2·4

Halothane had no significant effect on the per cent of phosphorylase which was in the active form, phosphorylase *a*.

Effect of pentobarbital on CO_2 production from glucose and pyruvate. Halothane has been demonstrated to block electron transport.[31-33] The effects of a known inhibitor of electron transport, pentobarbital,[34,35] were studied at increasing concentrations on D-glucose (5·6 mM) or pyruvate (2·5 mM) metabolism to CO_2 (Fig. 5). 6-^{14}C-glucose conversion to $^{14}CO_2$ was significantly stimulated at 30 mg/100 ml (1·2 mM) and inhibited at 60 mg/100 ml (2·4 mM) pentobarbital, while 1-^{14}C-pyruvate conversion to $^{14}CO_2$ showed only inhibition.

Effect of halothane on the oxidation of 1·5 mM octanoate or 5 mM DL-beta-hydroxybutyrate. Neither DL-beta-hydroxybutyrate, a ketone body, nor octanoate should have its metabolism blocked by low concentrations of halothane, which exerts its inhibitory action at some early step in glycolysis. In order to substantiate these contentions and to eliminate the possibility of still another site of halothane blockade in lipid metabolism, rat atria were incubated in the presence of either 1·5 mM octanoate or 5 mM DL-beta-hydroxybutyrate. The data are expressed as the per cent inhibition of $^{14}CO_2$ production from each substrate, so that the effects of halothane on the different substrates can be compared. Carbon dioxide production from the atria at each halothane concentration is compared to control data of the same day. In the curve depicting $^{14}CO_2$ production from 1-^{14}C-octanoate (Fig. 6, left). $^{14}CO_2$ production was first inhibited at 2 mM halothane and was significantly inhibited at this and all higher halothane concentrations. Significant depression in the metabolism of DL-beta-hydroxybutyrate was recorded at 4, 8 and 16 mM halothane (Fig. 6, right).

DISCUSSION

The first goal of this investigation was to evaluate the hypothesis that halothane blocks an early step in glycolysis in the mammalian heart.[13] This goal was pursued

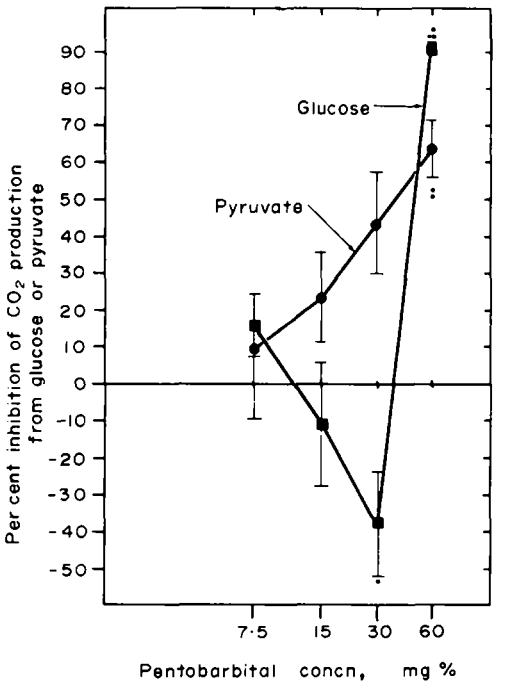

FIG. 5. Effect of increasing concentrations of pentobarbital on $^{14}CO_2$ production from 6-^{14}C-glucose and 1-^{14}C-pyruvate in rat atria. Values represent means ± S.E. for at least seven atria. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

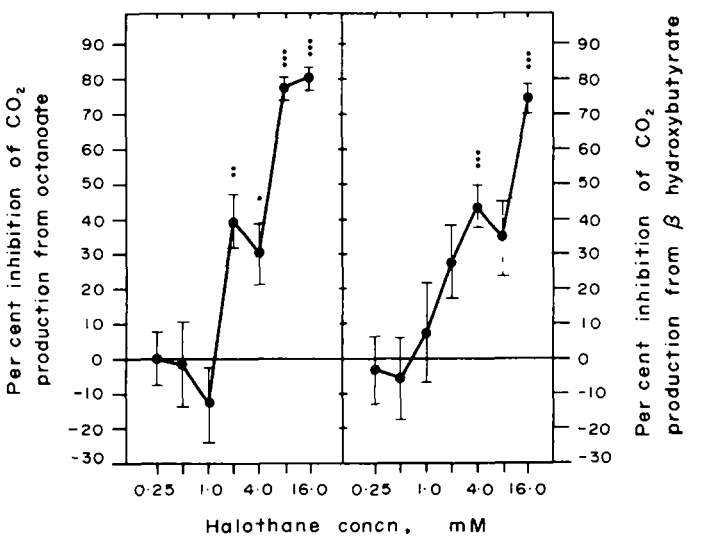

FIG. 6. Effect of increasing concentrations of halothane on $^{14}CO_2$ production from 1-^{14}C-octanoate and 3-^{14}C-beta-hydroxybutyrate in rat atria. Values represent means ± S.E. for eight atria. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. Mean control values for CO_2 production from octanoate and beta-hydroxybutyrate were 1700 and 2820 nmoles/g dry wt/hr respectively.

by studying the effects of increasing halothane concentrations on the production of $^{14}CO_2$ from ^{14}C-labeled substrates in rat atria. The oxidative metabolism of D-glucose was depressed by low concentrations of halothane, which did not significantly affect the metabolism of pyruvate or D-fructose. These data strongly suggest that low halothane concentrations inhibit some metabolic steps early in the metabolism of D-glucose.

The second goal of this investigation was to define further the precise site of action of halothane in glycolysis. Halothane had no effect on the uptake of 3-*O*-methyl glucose in atrial tissue. These data tend to rule out the possibility that halothane exerts its block in glucose oxidation to CO_2 at the transport step (site 1, Fig. 7).

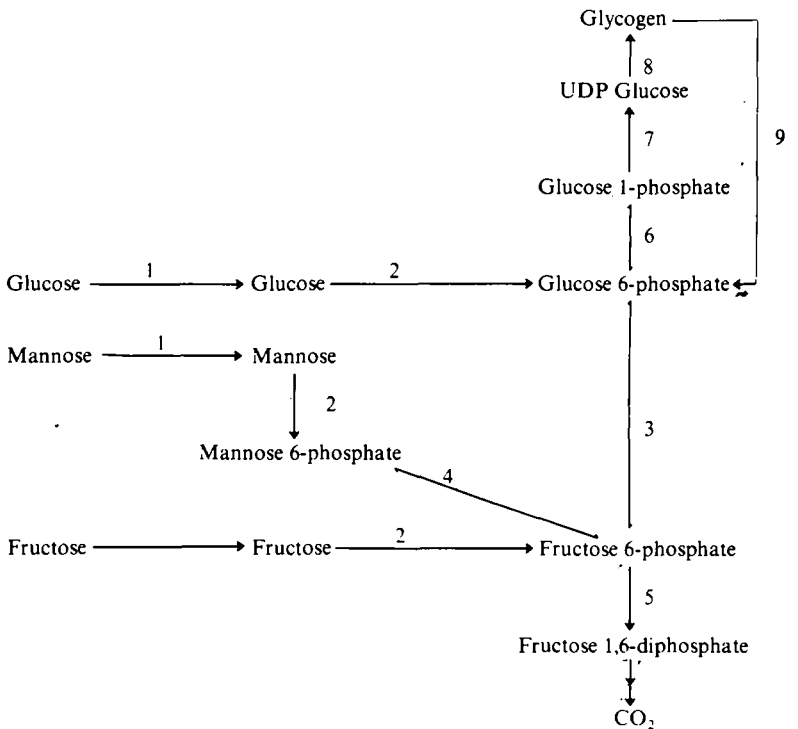

FIG. 7. Potential sites of action for halothane in myocardial metabolism: site 1, hexose transport; site 2, hexokinase; site 3, phosphoglucose isomerase; site 4, phosphomannose isomerase; site 5, phosphofructokinase; site 6, phosphoglucomutase; site 7, UDP-glucose pyrophosphorylase; site 8, glycogen synthetase; site 9, phosphorylase.

Knowledge of the pathway by which D-fructose is metabolized in rat atrial muscle is essential for elucidating further the site of halothane blockade. Liver contains a specific fructokinase which converts D-fructose plus ATP to D-fructose 1-phosphate. The latter is converted to D-glyceraldehyde and dihydroxyacetone phosphate by fructose 1-phosphate aldolase. D-Glyceraldehyde plus ATP yields D-glyceraldehyde phosphate in the presence of a triokinase.[6, 8,36,37] This is not a widely used pathway, however, as a specific fructokinase is absent in yeast and brain.[4] Rapidly growing liver tumors do not even use this pathway.[5] Evidence against the use of this pathway by D-fructose in muscle follows. Although a specific fructokinase has been described in muscle,[4,7,38] it has much less affinity for D-fructose than the corresponding

enzyme in liver. The k_m for the muscle enzyme is 190 mM;[7] that for liver is less than 0·5 mM.[36,39] Liver and muscle contain immunochemically distinct aldolases.[40] Extracts of liver cleave fructose diphosphate and fructose 1-phosphate at approximately equal rates. However, heart and muscle extracts from diffrent species, including the rat, catalyze the aldol cleavage of fructose disphosphate 30–50 times more rapidly than the cleavage of fructose 1-phosphate. Triokinase, needed for the conversion of D-glyceraldehyde to D-glyceraldehyde phosphate, is not present in muscle.[37] Furthermore, the distribution of ^{14}C in muscle glycogen formed from 1-^{14}C-fructose is not randomized between carbons 1 and 6 as it is in liver; more than 90 per cent of the radioactivity is found in the first carbon.[6] That a nonspecific hexokinase is involved in the first phosphorylation step for D-fructose in muscle is suggested by the experiments of Hers.[6] The metabolism of D-fructose to either glycogen or CO_2 was almost completely prevented by D-glucose. Since D-glucose inhibits the phosphorylation of D-fructose by nonspecific hexokinase, but not by specific muscle fructokinase,[4] the results of Hers[6] are probably due to an inhibition of D-fructose phosphorylation via a nonspecific hexokinase.

The arguments favoring the view that D-fructose is metabolized to D-fructose 6-phosphate via a nonspecific hexokinase and to fructose diphosphate via phosphofructokinase in atria (Fig. 7) are taken from functional studies. Known inhibitors of phosphofructokinase, citrate,[41–43] bicarbonate-free phosphate-buffered medium,[44,45] and low pH[46,47] decrease the force of contraction of isolated rat atria. Recovery of the force is achieved by addition of pyruvate but not glucose or fructose,[9,10] although all three substrates can be used for functional restoration of atria depressed by exposure to substrate-free medium.[10] Glucose uptake or phosphorylation was ruled out as a potential site of inhibition by citrate or bicarbonate-free phosphate-buffered medium.[11,12] The most likely explanation for the lack of effect of fructose in atria depressed by the above inhibitors is that fructose is metabolized via a pathway involving phosphofructokinase (Fig. 7) and this pathway is blocked by the above inhibitors of this enzyme.

Since fructose oxidation to CO_2 was not blocked at the low halothane concentrations which were required to block glucose oxidation to CO_2, hexokinase (site 2) has been eliminated as a site of action for halothane. Thus, phosphoglucose isomerase (site 3) is the most probable site of action for halothane in glycolysis.

D-Mannose oxidation to CO_2 was also inhibited by low concentrations of halothane. Hexose transport (site 1), apparently similar for D-glucose and D-mannose,[48,49] and hexokinase (site 2) have already been eliminated as potential sites of action for halothane. This necessitates the postulation that halothane must also exert a block at phosphomannose isomerase (site 4).

The incorporation of D-glucose, D-fructose or D-mannose into glycogen is significantly blocked by low concentrations of halothane. Hexose transport (site 1) and hexokinase (site 2) have previously been eliminated as sites of action. If halothane blocked only at phosphoglucose isomerase (site 3), D-fructose incorporation into glycogen and D-mannose incorporation into glycogen should be blocked, but D-glucose incorporation into glycogen should be unaffected. Since the incorporation of all three hexoses is blocked by halothane, an additional site of action for the anesthetic must be proposed at some point in glycogen turnover (sites 6–9). Halothane had no significant effect on the per cent of phosphorylase which was in the active form, phosphory-

lase *a*, the enzyme catalyzing site 9. However, the effect of halothane on the activity of that amount of enzyme which was in the active form was not tested.

Pentobarbital blocked the oxidation of D-glucose and pyruvate to CO_2 at high concentrations (60 mg/100 ml) in rat atria, which demonstrated that an agent which blocks electron transport[34,35] is capable of inhibiting the oxidation of these substrates to CO_2. At high halothane concentrations, the oxidation of D-glucose, pyruvate, D-fructose, D-mannose, octanoate and DL-beta-hydroxybutyrate to CO_2 was depressed in rat atria. This inhibition in the metabolism of all of these substrates by high halothane concentrations is believed to be the result of a block in electron transport. At a lower pentobarbital concentration (30 mg/100 ml), the production of $^{14}CO_2$ from 6-^{14}C-glucose was significantly stimulated. Webb and Elliott[50] reported that maximum stimulation of aerobic glycolysis in rat brain occurred at 45 mg/100 ml of pentobarbital with a concomitant inhibition of oxygen consumption. Apparently maximum glycolytic stimulation by pentobarbital occurs at roughly the same concentration in both rat brain and rat atria. If halothane, which also blocks electron transport,[33] would also stimulate glycolysis at higher concentrations, this might serve to explain the lessening of the halothane inhibition at 4 mM when D-glucose was the substrate (Fig. 2).

The third goal of this investigation was to study the effects of halothane on lipid metabolism in myocardial tissue. Low concentrations of halothane did not significantly inhibit the production of $^{14}CO_2$ from 1-^{14}C-octanoate or from 3-^{14}C-beta-hydroxybutyrate in rat atria. These data are consistent with the idea that halothane in low concentrations does not interfere with lipid metabolism in the rat heart.

Implications. Ko and Paradise[2] set out to determine the mechanism of the negative inotropic effect of halothane on the heart. One potential mechanism they pursued was the possibility that halothane interrupted the production of energy, thereby depressing contractility. Their functional studies[2,13,14,16,19] and these metabolic investigations just discussed support this hypothesis that halothane interferes with energy production in the heart. These metabolic studies indicate that halothane is capable of blocking glycolysis and electron transport. If a metabolic block is involved in the negative inotropic action of halothane, this block is surely at the glycolytic site, since this is the site of blockade most sensitive to halothane. The force of contraction of rat atria was depressed 50 per cent with 6 mg/100 ml of halothane.[2] However, 19 mg/100 ml (1 mM) halothane was required to depress the production of CO_2 from D-glucose by 34 per cent. Several possible explanations can be offered for the discrepancy between the concentration of halothane required to depress the force of contraction and the metabolism of rat atria: (1) The atria in which metabolism was measured were non-working and hence might be less sensitive to the actions of halothane than working atria. In working hearts, entry of glucose and CO_2 production are increased 3- to 4-fold over that of non-working hearts.[51] (2) Since there is no reason to suspect that x per cent inhibition of glycolysis will give rise to x per cent inhibition in force, possibly a small per cent inhibition of glycolysis would result in a large depression in force of contraction. (3) Halothane could act through more than one mechanism to exert its negative inotropic action on the heart. Recent functional studies by Ko and Paradise[52] suggest this latter possibility and further suggest that approximately half of the negative inotropic action of halothane can be accounted for by a block in glycolysis.

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