The Effects of Substrates on Contractility of Rat Atria Depressed with Halothane

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A concentration of approximately 6 mg/100 ml halothane was necessary to maintain 50 per cent depression of contractility of rat atria suspended in a modified Krebs–Ringer bicarbonate glucose medium, pH 7.4, kept at 30 C for two hours. Sodium pyruvate, sodium acetate and lactic acid partially restored the contractility of the depressed atria. Maximally effective concentrations were 2.5 mM for pyruvate and acetate and 5 mM for lactic acid. Neither 5 nor 20 mM of additional glucose restored the force of contraction of halothane-depressed atria, although these concentrations markedly increased the contractility of normal atria not exposed to halothane. Contractility of normal atria was not increased by acetate or lactic acid and was increased only slightly by pyruvate. The results are consistent with the hypothesis that at least part of the negative inotropic action of halothane is the result of inhibition of glucose uptake or utilization in the glycolytic pathway. Blockade must occur prior to the conversion of pyruvate to acetyl CoA. (Key words: Halothane; Heart; Substrates; Contractility; Pyruvate; Lactate; Acetate; Glucose.)

ALTHOUGH numerous reports concerning the direct depression of myocardium by inhalation anesthetics have appeared, the mechanism of this depression has not been elucidated. A few investigations of functional properties of halo-

thane have been made in the intact heart,[1, 2] isolated perfused heart[3, 4] and isolated heart muscle.[5, 6]

Paradise and Griffith[7] reported that concentrations of halothane sufficient to depress the contractility of isolated rat atria at 29 C by 50 per cent for two hours had no significant effect on potassium and water content. A concentration of approximately 6 mg/100 ml halothane in the perfusate was necessary to maintain 50 per cent depression of the contractility of perfused rat ventricles at 29 C for two hours; 17 mg/100 ml to achieve 98 per cent depression of contractility and a significant decrease in tissue potassium content.[4] The authors concluded, however, that despite the similarity of alterations in force of contraction and potassium content occurring with halothane and with anoxia, the differences in perfusion rate and recovery of contractile activity indicate that different biochemical processes occur in the cells. We considered the possibility that the myocardial depression resulting from halothane might be related to metabolic behavior as well as to substrate utilization by the myocardium.

The present investigation was undertaken to elucidate the sequence of events that leads to the hypodynamic state induced by halothane in the rat heart. This paper advances the hypothesis that the cardiac depressant effect of halothane on rat atria is a manifestation of inhibition of glucose uptake or glucose utilization.

Methods

Male Sprague–Dawley rats, weighing 180 to 200 g, which had had *ad lib.* access to food and water, were employed. Atria were removed from decapitated rats and suspended in a modified Krebs–Ringer bicarbonate glu-
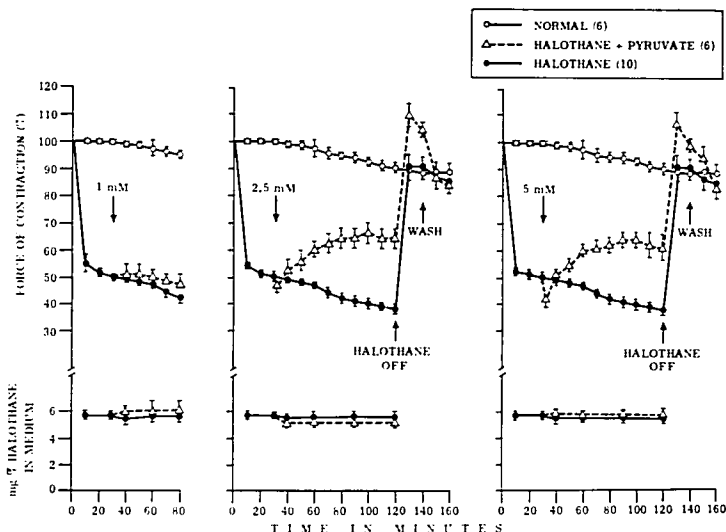
FIG. 1.   Effects of 1, 2.5 and 5 mM sodium pyruvate on halothane-depressed atria.   Pyruvate was added 30 min after the start of halothane administration.   In this and subsequent figures halothane was added at zero time (*i.e.*, following a 60-min equilibration period in modified Krebs–Ringer bicarbonate glucose medium).   Vertical bars represent ± one standard error of the mean.   Figures in parentheses represent number of experiments.

cose medium of the following composition (mM)[5]: NaCl 120; KCl 4.8; CaCl$_2$ 1.22; MgSO$_4$·7 H$_2$O 1.33; KH$_2$PO$_4$ 1.2; NaHCO$_3$ 25.3; glucose 5.55. The medium was aerated with 95 per cent O$_2$ and 5 per cent CO$_2$ at *p*H 7.4 and 30 C. A constant resting tension of 750 mg was maintained throughout the experiment. The developed tension was recorded with a Statham strain gauge, and the atria were stimulated electrically at a rate of 200 pulses/min. An equilibration period of 60 min was allowed before readings were taken. The experimental values of contractility (peak tension) were compared with control values obtained at zero time (following equilibration) and expressed as per cent change in developed tension. Halothane was administered to the medium by means of the anesthetistat previously described by Paradise and Griffith.[4, 9]   Halothane concentrations in

the medium were determined at 10- to 30-min intervals with a gas chromatograph throughout the experimental period.[4]   The sodium pyruvate, sodium acetate and L(+) lactic acid (grade L-I) employed in this study were obtained from Sigma Chemical Co.

Results

EFFECT OF HALOTHANE ON ATRIAL CONTRACTILITY

The behavior of atria in the presence of halothane was determined to provide data with which the responses to substrates of the depressed atria could be compared.   Halothane administration was begun at zero time (following a one-hour equilibration period).   During the first 30 min the anesthetist was adjusted to deliver enough halothane to achieve 50 per cent depression of contractility.
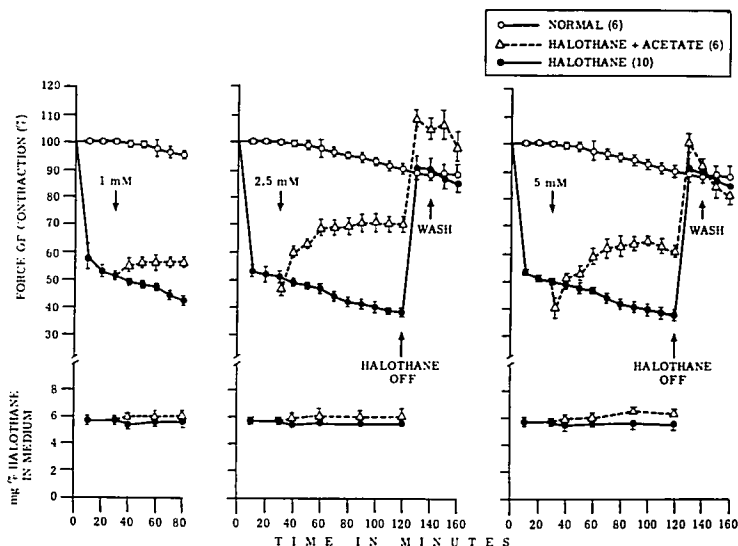
Fig. 2. Effects of 1, 2.5 and 5 mM sodium acetate on halothane-depressed atria.

Following this period no further adjustments were made. Relatively stable concentrations of halothane were present in the bathing medium during the following 90 min (5.6 to 5.8 mg/100 ml mean values). The force of contraction declined slightly at approximately the same rate as that in normal atria not exposed to halothane (fig. 1). After administration of halothane was stopped recovery was complete.

EFFECTS OF SUBSTRATES ON ATRIA DEPRESSED WITH HALOTHANE

Sodium pyruvate was added to the bathing medium 30 min after the start of halothane administration.   Despite the continued administration of halothane to maintain levels in the medium similar to those in the ten control experiments, the addition of pyruvate resulted in a slight decrease, followed by a gradual marked increase, in the force of contraction (fig. 1). The maximally effective concentration of pyruvate was 2.5 mM. Stopping

the administration of halothane resulted in recovery of the force of contraction to above the expected values.

The effects of sodium acetate on the halothane-depressed atria were essentially the same as those of sodium pyruvate (fig. 2). The maximally effective concentration was again 2.5 mM. No significant change in pH followed the addition of pyruvate or acetate.

The addition of 5 mM sodium chloride to six atrial preparations depressed 50 per cent with halothane did not change contractility. This indicates that the effects of sodium pyruvate and sodium acetate are due to the anions, not to the presence of the sodium ion.

The effects of lactic acid on the halothane-depressed atria are shown in figure 3. The maximally effective concentration was 5 mM. The initial decrease, rate of rise and extent of increase in force of contraction were more marked than with sodium pyruvate or sodium acetate. These effects may be related to the
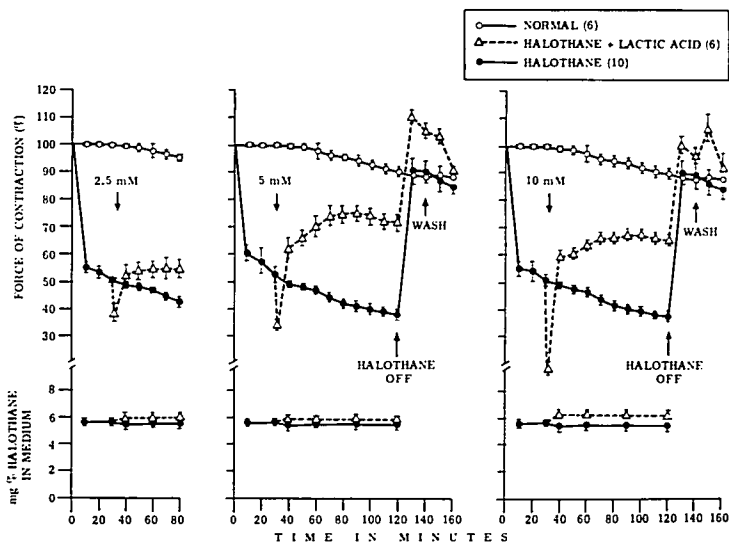
Fig. 3. Effects of 2.5, 5 and 10 mM lactic acid on halothane-depressed atria.

dose-dependent decrease in *p*H seen immediately after adding the lactic acid (table 1). An initial rapid drop in *p*H was followed by a gradual return to a stable value in about 5 min. This drop in *p*H was responsible for the decrease in contractility, since similar initial changes in both *p*H and contractility occurred after addition of hydrochloric acid to normal

TABLE 1. Effect of Lactic Acid on *p*H of Normal Medium

	Time After Addition of Lactic Acid										
	Seconds			Minutes							
	0	10	30	1	2	3	5	10	20	30	60
5 mM lactic acid											
Experiment 1	7.4	6.5	6.6	6.8	7.0	7.1	7.3	7.3	7.3	7.3	7.3
Experiment 2	7.4	6.4	6.6	6.8	6.9	7.1	7.3	7.4	7.4	7.4	7.4
Experiment 3	7.4	6.5	6.6	6.8	7.0	7.1	7.3	7.3	7.3	7.3	7.3
MEAN	7.4	6.5	6.6	6.8	7.0	7.1	7.3	7.3	7.3	7.3	7.3
10 mM lactic acid											
Experiment 1	7.4	5.5	5.8	6.0	6.4	6.5	6.7	6.8	6.8	6.8	6.8
Experiment 2	7.4	5.8	6.1	6.3	6.5	6.7	6.9	7.0	7.0	7.0	7.0
Experiment 3	7.4	5.6	5.9	6.1	6.3	6.6	6.9	7.0	7.0	7.0	7.0
MEAN	7.4	5.6	5.9	6.1	6.4	6.6	6.8	6.9	6.9	6.9	6.9

atria. Also, this change in pH would be expected to have an effect on the ionization of lactic acid. At low pH more lactic acid is in the unionized form and, presumably, better able to penetrate cells. This may account for the more rapid and greater increase in contractility. Suppression of ionization by lowering the pH from 7.6 to 6.2 permits the utilization of the dicarboxylic acids, succinic, malic and oxalacetic acids by the isolated rat ventricle strip.[10] The possibility that release of catecholamines is caused by the low pH, however, cannot be ruled out as an explanation for the increased rate of rise and maximum force development following the initial drop in contractility due to the lactic acid. (The pH effects are now being studied in another investigation.)

Figure 4 shows the effects of additional glucose on the halothane-depressed atria. Neither 5 nor 20 mM produced any appreciable effect, although termination of halothane administration was accompanied by marked dose-dependent increases in the force of contraction.

EFFECTS OF SUBSTRATES ON NORMAL ATRIA

Sodium acetate and lactic acid, in concentrations effective in the halothane-depressed atria, had no positive inotropic effect on normal atria (fig. 5). Sodium pyruvate, 2.5 mM, had a slight positive inotropic effect. Glucose produced marked dose-dependent increases in contractility not seen in the halothane-depressed atria. These findings confirm similar results seen with glucose, acetate and pyruvate in normal atria by Gimeno et al.[11]

Discussion

Although the effects of halothane on functional properties of the heart have been studied in isolated heart preparations,[3, 5, 6, 12] few investigations of the effects of halothane on metabolism have been made. Halothane has been shown, in hepatic studies in man, to diminish perfusion pressure, hepatic blood flow and venous oxygen tension without effect on the overall oxygen consumption and with no excess lactate liberation.[13] Investigations of the effects of halothane on cerebral metabolism have demonstrated that there are minimal changes or none in the rate of cerebral

oxygen consumption.[11] Using various substrates, halothane was found to uncouple oxidative phosphorylation in rat liver mitochondria.[15] However, the concentrations of halothane used were extremely high (7–14 mM), of the order of 30 times the amount required to depress the force of contraction of the beating rat heart by 50 per cent.[4]

Hoech et al.[16] reported the effects of halothane with respect to the oxygen consumption of rat brain, liver and heart and anaerobic glycolysis of rat brain. They concluded that anesthetic concentrations of halothane (1 per cent) caused a significant decrease in oxygen consumption of unstimulated rat brain slices and that 2 per cent halothane decreased the oxygen consumption of both heart and liver slices. They also found 5 per cent halothane to be without effect on anaerobic glycolysis of rat brain. However, these studies were done in homogenates in which the effects of halothane may be very different from those in intact tissue. A recent report suggests that halothane reduces ATPase activity of both myocardial and skeletal muscle myofibrillar preparations.[17] The hypothesis was offered that 1) there is a close association between the myocardial tension developed and enzymatic hydrolysis of ATP and 2) halothane exerts its cardiac effects by inhibiting this enzymatic hydrolytic process. However, the concentrations of halothane in the medium in contact with the myofibrillar preparations were exceedingly high (lowest concentration reported was 20 mM). Depression of the isolated perfused rat heart by 50 per cent at 29 C requires about 0.33 mM.[4]

The direct action of halothane on isometric and isotonic contractions of cat papillary muscles has recently been studied.[12] The negative inotropism induced by halothane was thought to be the result of a dose-dependent depression of the intensity of energy conversion by the contractile element of the heart. Evidence presented by Goldberg and Ullrick[6] and Goldberg and Phear[18] concerning the influence of halothane on the mechanical properties of rat trabeculae carnae preparations points to an indirect effect of halothane on the contractile mechanism. Such an action could be elicited by inhibition of substrate uptake or utilization.
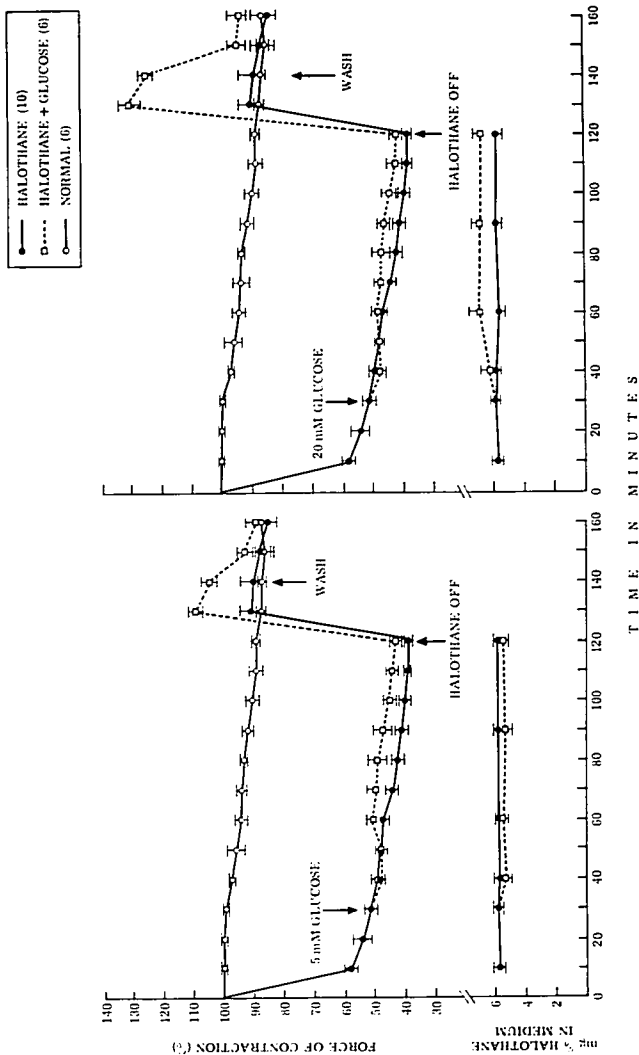
Fig. 1. Effects of 5 and 20 mM additional glucose on halothane-depressed atria.
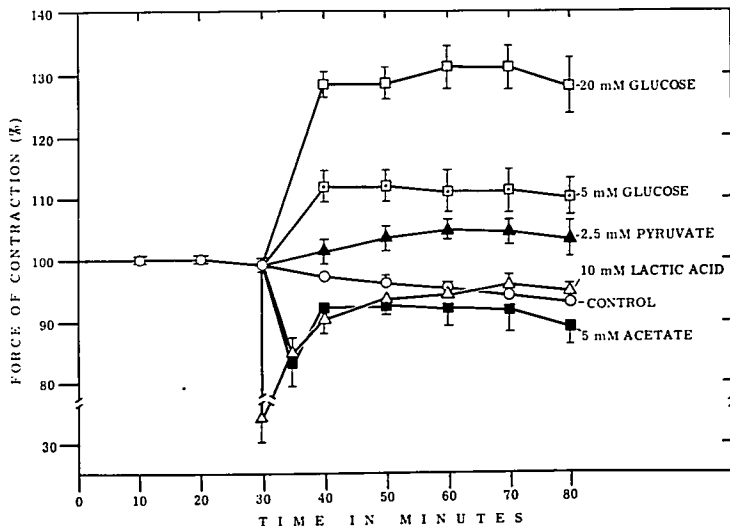
FIG. 5. Effects of substrates on normal atria. Each curve represents six experiments.

The utilization of substrates has been widely studied in cardiac tissue slices,[19-22] isolated hearts,[23-26] and heart in situ,[27-29] and it has been demonstrated that glucose, pyruvate, lactate and acetate can be oxidized by the myocardium. Results with rat atria,[30] rat ventricle strips,[10] and rabbit atria[31] suggest that either the uptake of glucose or the operation of the glycolytic pathway is important for a fraction of the contractile activity of the heart, inasmuch as pyruvate, acetate and lactate are only partially effective in restoring the developed tension in the absence of glucose or during block of glycolysis with enzyme inhibitors. The data in figure 5, in which glucose, but not pyruvate, acetate or lactate, had an appreciable positive inotropic effect on the normal atria, also suggest a role of glucose in contractility not shared by the other substrates.

Our results, in which glucose was ineffective, whereas pyruvate, acetate and lactate were partially effective, in restoring the force of contraction of atria depressed with halothane, are thus consistent with the hypothesis that halothane blocks the uptake or utilization of glucose via the glycolytic pathway. This blockade must occur prior to the conversion of pyruvate to acetyl CoA, and is at least partly responsible for the negative inotropic action of halothane in isolated rat atria dependent on glucose for their energy supply.

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