The Effect of Halothane on the Contractility of Atria from Starved Rats

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The purpose of this experiment was to determine whether halothane interferes with the utilization of lipid as a source of fuel for the contractile process by the isolated rat atria. Rats were starved 24 hours in order to increase the lipid content of the heart. Atria from starved rats were better able to maintain their contractility in the absence of exogenous substrate and also were more resistant to depression by halothane than atria from fed rats. Approximately 11 mg/100 ml (1.41 vol per cent) halothane were necessary to achieve 50 per cent depression of the force of contraction of atria from starved rats whether glucose was present in the bathing medium or not. To achieve the same degree of depression in the presence or absence of glucose in atria from fed rats required about 6.5 mg/100 ml (0.83 vol per cent). Atria from starved rats behaved like atria from fed rats in that the depressant action of halothane was independent of external glucose. The site of action of halothane in either case cannot be related to the uptake or phosphorylation of glucose, but may involve the glucose phosphate isomerase step, as previously suggested. The greater halothane requirement for atria from starved rats suggests that endogenous lipid accumulates during starvation and is used as an energy source for the contractile process in the face of a halothane-induced block in glycolysis. Other possible reasons for the resistance of atria from starved rats to halothane's depressant action are discussed. (Key words: Halothane; Contractility; Heart; Starvation; Atria.)

IN AN EFFORT to determine the mechanism of the depressant action of inhalation anesthetics on cardiac contractility, we have carried out investigations in isolated rat and human heart preparations.[1-9] Depression of contractility by halothane was overcome by the metabolizable substrates, pyruvate, lactate, acetate, and fructose, but not by additional glucose in rat atria.[4, 6] Since fructose is apparently metabolizable via the phosphofructokinase step, this implicated glucose uptake, phosphorylation or isomerization of glucose-6-phosphate to fructose-6-phosphate as the site of halothane blockade.[6, 10] Recent experiments in which halothane had its usual depressant action in the absence of exogenously supplied glucose ruled out glucose uptake or phosphorylation as the site of blockade and suggested the glucose phosphate isomerase step (conversion of glucose-6-phosphate to fructose-6-phosphate) as the critical site, assuming a single site were involved.[7]

A halothane-induced block in glycolysis also occurs in isolated human atrial appendages, as evidenced by the marked positive inotropic action of pyruvate, but not additional glucose, in halothane-depressed atria.[5] Fifty per cent depression of contractility required only 4.5 mg/100 ml, corresponding to 0.83 volumes per cent (very close to the MAC value of 0.77 volumes per cent). At this concentration of halothane, one would not expect to see such marked impairment of cardiac function *in vivo*. Since the human heart is known to utilize fatty acids and other substrates besides glucose, the possibility that the heart *in vivo* can compensate for a block in glycolysis by using fatty acids was presented.[8] This suggestion presupposes, of course, that the utilization of fatty acids by the heart is unimpaired by halothane.

The purpose of the present investigation was to determine whether halothane appreciably impairs the utilization of fatty acids by the heart. We felt it would be of interest to add fatty acids to the halothane-depressed isolated

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Received from the Departments of Pharmacology and Anesthesiology, Indiana University School of Medicine, Indianapolis, Indiana 46202. Accepted for publication February 22, 1971. Supported in part by USPHS Grants HE 07718 and RR 00162, in part with facilities provided by the Heart Research Center Grant H 6308 from the National Heart Institute, USPHS, and in part by General Research Support Grant PHS-S01-FR 5371.

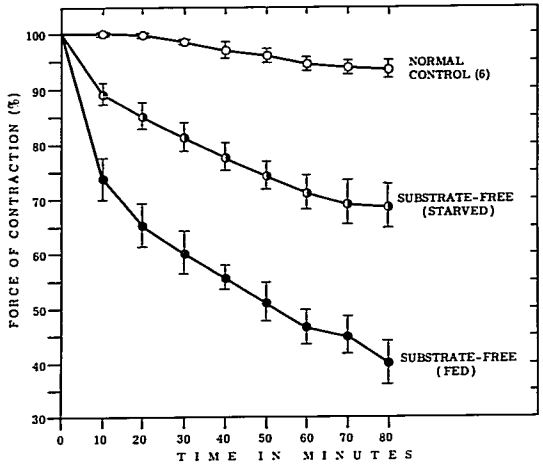


FIG. 1. Contractility of substrate-depleted atria from seven normal (fed) and four starved rats. Zero time represents one hour in the normal medium containing 5.5 mM glucose. Normal control values from "fed" atria represent 80 additional min in this medium. At zero time the medium was changed to substrate-free, *i.e.*, free of glucose in the other experiments. Vertical bars represent ± one standard error of the mean.

atria and determine whether the force of contraction could be increased, an approach found useful in our previous experiments with other substrates. Fatty acids, however, are insoluble in the saline medium bathing the atria, so they must be coupled to albumin or other protein to remain in solution. The presence of protein, however, prevents the bubbling process for admission of oxygen, since foam results. In addition, it is impossible to be sure which fatty acid should be added, as several are used by the heart *in vivo*. For these reasons we decided to increase the endogenous supply of lipid in the heart prior to sacrifice by the simple and well-known process of starvation.[11] Then we compared the rates of decline of contractility in atria from fed and starved rats bathed in substrate-free medium. The slower rate of decline in atria from starved rats suggested an increased supply and utilization of endogenous substrate, in support of the concept that starvation increases the lipid content of the heart. The degrees of sensitivity of atria from fed and starved rats were next tested. Almost twice the concentration of halothane was needed to depress the atria from starved rats (11 mg/100 ml *vs.* 6.5 mg/100 ml for atria from fed rats). These experiments provide evidence for the view that halothane has little or no effect on the utilization of endogenous lipid for contractility by rat atria.

Methods

Male Sprague-Dawley rats weighing 180 to 200 g with *ad lib.* access to food and water were used for the study. Atria were removed from decapitated rats and suspended in a modified Krebs-Ringer bicarbonate glucose medium of the following composition (mM)[4, 6]: NaCl 120; KCl 4.8; $CaCl_2$ 1.22; $MgSO_4 \cdot 7\,H_2O$ 1.33; KH_2PO_4 1.2; $NaHCO_3$ 25.3; glucose 5.55. The medium was gassed with 95 per cent O_2–5 per cent CO_2 at *p*H 7.4 and 30 C. A constant tension of 750 mg was maintained throughout the experiments. The developed tension was recorded with a Statham strain gauge, and the atria were electrically stimulated at a rate of 200 pulses/min. An equilibration period of one hour was allowed before readings were taken. The experimental values of contractility (peak tension) were compared with those of control records obtained at zero time (following equilibration) and expressed as per cent change in developed tension.

HALOTHANE EXPERIMENTS

Halothane was administered to the medium by means of the anesthetistat previously described by Paradise and Griffith.[2, 3] Halothane

concentrations in the medium were determined at 5- to 10-min intervals with a gas chromatograph throughout the experimental period.[2]

Substrate Experiments

The normal medium was changed to substrate-free medium (*i.e.*, free of glucose) following the one-hour equilibration period.

Starvation Experiments

Rats were starved for 24 hours prior to the time they were killed. The atria from the starved rats were allowed to beat in the normal Krebs-Ringer bicarbonate glucose medium for a 60-min equilibration period.

Results

Contractility of Substrate-Depleted Atria from Fed and Starved Rats

Experiments were performed to ascertain the importance of endogenous substrate for the force of contraction of atria from starved rats by comparing the rate of contractile depression of these atria in substrate-free medium with that of atria from fed rats.

Figure 1 shows the effects of omitting exogenous glucose from the medium (substrate-free) on the tension developed by atria from fed and starved rats following one-hour equilibration periods in normal Krebs-Ringer bicarbonate glucose medium. It is evident from the figure that atria from starved rats suspended in substrate-free medium show a significantly smaller reduction in contractility than do those from fed rats. This implies a greater availability of endogenous substrate for the contractile process in atria from starved rats.

Effect of Halothane on Contractility of Atria from Fed and Starved Rats, in the Presence and Absence of Glucose

Atria from fed or starved rats were equilibrated for one hour in normal Krebs-Ringer bicarbonate glucose medium. Halothane administration was then started (zero time in fig. 2). During the first 5 to 10 min the anesthetistat was adjusted to deliver enough halothane to achieve 50 per cent depression of contractility. Following this period no further adjustments were made. For this degree of depression 11 mg/100 ml halothane were

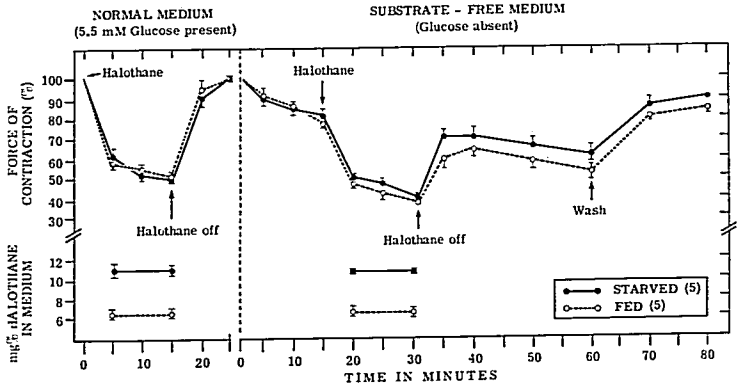
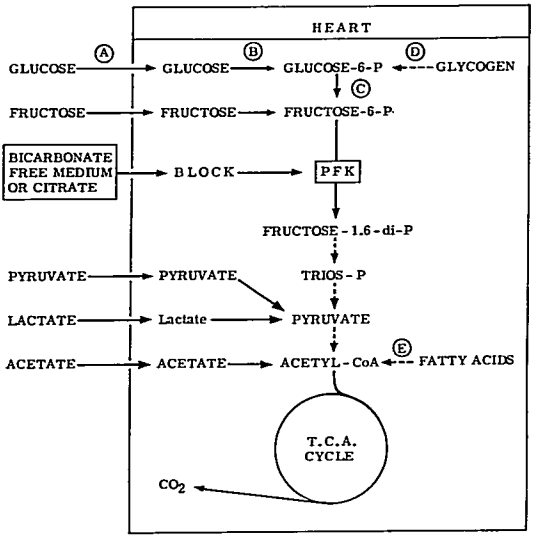


Fig. 2. Effects of halothane on contractility of rat atria from fed and starved rats in the presence and in the absence of glucose. Halothane was added at zero time (following a one-hour equilibration period) to five atria in the presence of 5.5 mM glucose. The tube delivering halothane was removed from the bathing medium after 15 min and the atria were allowed to recover for an additional 15 min. The medium was then changed to substrate-free, *i.e.*, free of glucose. Fifteen minutes later the halothane tube was readmitted to medium and halothane administration continued for 15 min. Halothane administration was then stopped and the atria allowed to recover for 30 min. The medium was then changed to the normal medium containing 5.5 mM glucose (wash).



PFK : PHOSPHOFRUCTOKINASE

FIG. 3. Schematic representation of glycolysis, showing the points at which various substrates enter the scheme. In previous studies[4,6] with glucose-containing medium, acetate, lactate, pyruvate, and fructose were shown to be effective in overcoming halothane-induced cardiac depression, while additional glucose was ineffective. Fructose is apparently metabolized via phosphofructokinase, since it is ineffective in the presence of bicarbonate-free medium or citrate, inhibitors of this enzyme.[6,10] The site of action of halothane thus is localized to site *A*, *B* or *C*. Sites *A* and *B* are ruled out, since halothane decreases contractility in the absence of exogenous glucose.[7] This report proposes that site *E*, metabolism of fatty acids to acetyl CoA, is insensitive to halothane, or at least less sensitive than an early site in glycolysis.

needed in atria from starved rats, but only 6.5 mg/100 ml in atria from fed rats.

Following 15 min of halothane administration, the tube delivering halothane was removed from the bathing medium and the depressed contractility was completely restored to the control level within 10 min (fig. 2). The anesthetistat, however, was continuously operating outside the tissue bath without any alteration of its regulation, in order to deliver the same concentration of halothane for the next step of the experiment. The medium was then changed to one free of glucose. Fifteen minutes later the tube delivering halothane was reinserted into the tissue bath. The degrees of depression achieved again in atria from fed and starved rats were similar to the initially-achieved degrees of depression despite marked changes in the halothane concentrations (10.8 mg/100 ml in "starved" *vs.* 6.5 mg/100 ml in "fed" atria). After the end of halothane administration the depressed contractility was again restored to the expected level, and it was further restored to the control level of normal atria after the bath was changed to normal glucose medium.

Discussion

That more halothane is needed to depress atria from starved than fed rats may be explained by the following possibilities:

1) The absolute forces of contraction at zero time (following the one-hour equilibration period) were different in the two sets of atria. This could not be demonstrated in our studies, and was certainly not true in the paired studies of Cimeno *et al.*[12]

2) Starvation affects the site involved in halothane's depressant action in such a way as to make it less sensitive to the anesthetic. There is no evidence at present to support or deny such a mechanism.

3) Glycogen was present in higher concentration in atria from starved rats and thus was able to overcome the early block in glycolysis produced by halothane. Glycogen is indeed present in higher concentration in hearts from starved rats.[11-13] However, after a one-hour equilibration period in normal Krebs-Ringer

bicarbonate medium containing 5.5 mM glucose (the same as our medium), the glycogen content of atria from starved rats fell to the same value as that in atria from fed rats.[12] Thus, at the time of halothane administration in our experiments there would have been no difference between glycogen concentrations in "fed" and "starved" atria. Furthermore, even if there were a difference it is doubtful that excess glycogen could be used to overcome the halothane block, since this block has been demonstrated to be insurmountable.[4, 14] Neither 5 nor 20 mM glucose were able to overcome this block, although in control atria 20 mM glucose had a greater positive inotropic action than 5 mM.

4) Endogenous substrates, less sensitive or insensitive to halothane, accumulated in the atria during the starvation process and served as part of the energy supply during halothane's action. Figure 1 suggests that endogenous substrate accumulates during starvation and can be used as a source of fuel for the contractile process. Lipid, as well as glycogen, accumulates in the heart during starvation and is utilized *in vitro*.[11]

Thus, the most likely explanation for the greater halothane requirement to achieve 50 per cent depression of the force of contraction in atria from starved *vs.* fed rats is the greater accumulation of endogenous lipid during the starvation process. This lipid is utilized for maintenance of the force of contraction in the presence of halothane. Site *E* in figure 3 is not affected by halothane or at least is less sensitive than an early site in glycolysis. This supports the concept that *in vivo* the force of contraction is not markedly altered at concentrations of halothane which *in vitro*, with glucose as substrate, have marked depressant effects, because the heart is able to utilize circulating or endogenous lipid *in vivo* as a source of fuel for the contractile process.[8] This, then, implies that halothane's negative inotropic effect will be a function of the nutritional status of the heart. Perhaps in hearts utilizing mainly lipid, as in starved or diabetic individuals,[11] the effects of halothane may be expected to be less depressant than the effects in the hearts of "fed" individuals, which use glucose.

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Erratum

An error appeared in the article, "The Effect of Levodopa on the Norepinephrine Stores in Rat Heart" (ANESTHESIOLOGY 34:4–8, 1971) by Philip L. Liu, Laurence J. Krenis, and S. H. Ngai. On page 6, the legends to figures 1 and 2 are reversed.