

## The Effects of Substrates on Halothane-depressed Isolated Human Atria

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Isolated right atrial appendages from 7-10-year-old patients undergoing cardiac surgery were incubated in Krebs-Ringer bicarbonate medium at pH 7.4 and 37 C. Glucose and pyruvate at concentrations of 30 mM were better energy sources for contractility than similar concentrations of lactate or fructose. Contractile depression of atria in media containing 30 mM glucose to 50 per cent of control values required approximately 4.5 mg/100 ml halothane in the media. This represents a partial pressure of 6.3 mm Hg or 0.83 volumes per cent halothane in the air in contact with the medium under our steady-state conditions.

Addition of 30 mM pyruvate to halothane-depressed atria resulted in a prompt increase in the force of contraction to well above control levels. Addition of glucose at the same concentration had no effect on the halothane-depressed atria. These results in the human are consistent with the authors' previous findings in the rat, which indicated that halothane may inhibit the uptake or some early step in the utilization of glucose by the myocardium. (Key words: Halothane; Heart; Substrates.)

SINCE THE INTRODUCTION of halothane as an anesthetic agent,<sup>1</sup> its cardiac effects have attracted much attention. We have carried out many investigations dealing with the mechanisms of the depressant action of inhalation anesthetics on cardiac function in the rat heart,<sup>2-7</sup> and have demonstrated the following: 1) The contractility of isolated rat atria suspended in Krebs-Ringer bicarbonate glucose (5.5 mM) medium at 30 C was depressed 50 per cent by approximately 6 mg/100 ml halo-

thane.<sup>5</sup> 2) Anoxia and halothane produce similar decreases in contractility and potassium content in the perfused rat heart. That anoxia produced an increase in coronary flow rate not seen with halothane and produced irreversible damage to the contractile mechanism, again not seen with halothane, suggested that different biochemical changes in myocardial cells were occurring with the two variables.<sup>3</sup> 3) Partial recovery of the contractility of rat atria depressed by halothane was achieved with the metabolizable substrates pyruvate, lactate, acetate and fructose, but not with additional glucose.<sup>5,7</sup> Therefore, we suggested the hypothesis that the depressant action of halothane on myocardial contractility might be due to the inhibition of uptake or utilization of glucose above the phosphofructokinase step in the glycolytic pathway of the heart.

Although it is difficult to do quantitative experiments of this nature using human tissue, due to the difficulty of obtaining samples and the heterogeneity of the population, it is important to confirm in man at least the basic premises of animal investigations. If these premises can be found to hold true in man this justifies the further use of the animal as a model for man. With this in mind, we first determined which substrates could best serve as fuel for the contractile process in isolated human atrial appendages from 7-10-year-old patients undergoing cardiac surgery. We found glucose and pyruvate to be much better utilized than lactate, acetate or fructose. The optimal concentration of glucose was 30 mM.<sup>8</sup> Next, we depressed the contractility of atria bathed in media containing 30 mM glucose by 50 per cent with halothane. Addition of 30 mM pyruvate produced a marked positive inotropic effect. Additional glucose (30 mM), however, was without effect. These results are consistent with our previous findings in the

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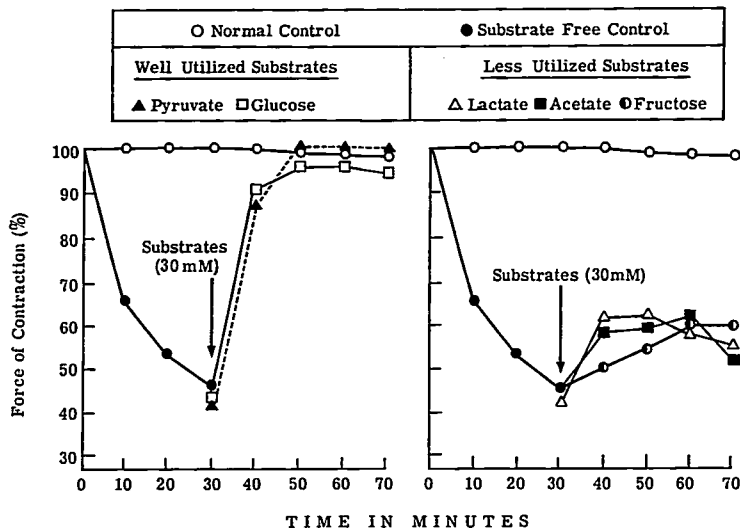


FIG. 1. Effects of substrates (30 mM) on contractility of human atria exposed to substrate-free medium for 30 min. Values shown for each substrate represent the average of two experiments, except for fructose, with which a single experiment was performed. Zero time in this and subsequent figures represents a 60-min equilibration period in medium containing 30 mM glucose.

rat and suggested a block by halothane of glucose uptake or metabolism in human atrial tissue.

### Methods

Right atrial appendages were obtained from patients undergoing corrective cardiac surgery for atrial septal defect, pulmonary stenosis, or aortic stenosis. The atria were obtained from both male and female patients, 7-10 years old. During cardiopulmonary bypass procedures, a portion of the right atrial appendage was excised and immediately placed in a modified Krebs-Ringer bicarbonate glucose medium of the following composition (mM): NaCl, 120; KCl, 4.8; CaCl<sub>2</sub>, 2.44; MgSO<sub>4</sub>·7H<sub>2</sub>O, 1.33; KH<sub>2</sub>PO<sub>4</sub>, 1.2; NaHCO<sub>3</sub>, 25.3; glucose, 30 at pH 7.4. The medium was aerated with 95 per cent O<sub>2</sub> and 5 per cent CO<sub>2</sub> and placed in an ice bath. The atria were transported in this iced medium from the operating room to the

laboratory (about 5 min). The tissue was then trimmed, cut into 10-15-mm lengths and placed in the medium at 37 C.

The experimental apparatus and procedures for recording the developed tensions of the atria were those previously used for rat atria.<sup>5,7</sup> A constant resting tension of 750 mg was maintained throughout the experimental period. The developed tension was recorded with a Statham strain gauge, and the atria were stimulated electrically at a rate of 70 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 added to the medium by means of an anesthetist previously described

by Paradise and Griffith.<sup>3</sup> Halothane concentration in the medium was determined at 15- to 30-min intervals with a gas chromatograph throughout the experimental period.<sup>4</sup>

In the experiments with substrate-free medium (fig. 1), the medium was changed to substrate-free (*i.e.*, free of glucose) following the one-hour equilibration period.

### Results

#### ABILITIES OF SUBSTRATES TO INCREASE THE CONTRACTILITY OF SUBSTRATE-DEPLETED HUMAN ATRIA

The effects of substrates on the functional properties of isolated human atria had to be established to study their actions on the halothane-depressed atria. Experiments were performed using substrate-free medium to determine the abilities of individual substrates to increase the contractility of human atria in the absence of exogenous substrate, to provide control data with which the responses to substrates might be compared. In order to observe the relationship between substrate utilization and contractile behavior of human atria, one can introduce the metabolizable substrate after the force of contraction has declined due to prolonged activity in substrate-free medium, and determine the effects of individual substrates on mechanical performance. We carried out such experiments with glucose, pyruvate, lactate, acetate and fructose. These results are summarized in figure 1. Developed tension of the human atria progressively decreased in the substrate-free medium after the one-hour equilibration period with Krebs-Ringer bicarbonate glucose (30 mM) medium.

After the atria had been in the substrate-free medium for 30 min, the substrate being tested was added at a concentration of 30 mM. It is evident from figure 1 that the addition of glucose or pyruvate promptly restored the depressed contractility of isolated human atria to the control level. However, lactate, acetate and fructose only partially restored the contractility. Therefore, pyruvate and glucose were chosen for the study of halothane-depressed human heart.

#### EFFECT OF HALOTHANE ON CONTRACTILITY OF ISOLATED HUMAN ATRIA

The behavior of human atria in the presence of halothane was determined in order to provide data with which the responses of the depressed atria to substrates 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. Following this period no further adjustments were made. Relatively stable concentrations of halothane were present in the bathing medium during the following 90 min, although the force of contraction declined during the second hour of halothane administration. After administration of halothane was stopped, recovery was not complete (fig. 2). This probably was due to progressive deterioration with time of relatively thick pieces of atria from sick patients, which in the isolated tissue bath rely on diffusion for nourishment and oxygen.<sup>9</sup> Apparently the atria can tolerate the first post-equilibration hour, as evidenced by the constant force of contraction of control hearts in figure 1 and by the complete recovery of contractility of substrate-depleted hearts produced by addition of glucose. The second post-equilibration hour is not well tolerated, however, as shown in figure 2 by the decreased force of contraction despite a reasonably uniform halothane concentration and as evidenced by the incomplete recovery of the force after halothane was discontinued.

#### EFFECTS OF SUBSTRATES ON HALOTHANE-DEPRESSED HUMAN ATRIA

Sodium pyruvate (30 mM) 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 halothane control, the addition of pyruvate resulted in a rapid and marked increase in the force of contraction. However, addition of glucose had no effect on the halothane-depressed human atria. The pyruvate response

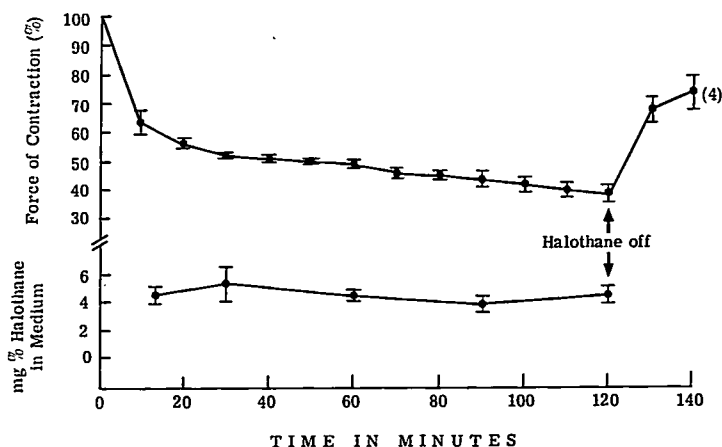


FIG. 2. Effect of halothane on contractility of human atria exposed to 30 mM glucose. Vertical bars indicate plus or minus one standard error.

was quite variable. For example, at the 60 min time period the forces of contraction in the different atrial appendages were 145, 183, 169 and 108 per cent. The reason for this variation is not clear.

### Discussion

We previously reported that partial recovery of the force of contraction in rat atria depressed with halothane was achieved by adding the metabolizable substrates pyruvate, lactate, acetate, and fructose, but not with additional glucose. With these results, we proposed the hypothesis that halothane may inhibit the uptake or utilization of glucose in the myocardium.

In the present investigation, we determined the abilities of substrates to increase the contractility of substrate-depleted isolated human atria, in order to investigate the effects of substrates on the functional properties of the halothane-depressed human heart.

The data (fig. 1) show that glucose and pyruvate were more effective than lactate,

acetate and fructose as energy sources for the contractile process in isolated human atria. The marked positive inotropic action of pyruvate (30 mM) on the substrate-depleted human atria is in sharp contrast to its effects on rat atria. In rat atria the maximally effective concentration is about 2.5 mM, and it is only partially effective in restoring the contractility of substrate-depleted or 2-deoxy-glucose-treated rat atria.<sup>10</sup> The apparently greater affinity of the rat heart for glucose than for pyruvate has been attributed to an extra effect of glucose on the contractile process due to early steps in its metabolism in addition to its ability to generate ATP.<sup>10, 11</sup> Under the conditions of the present human atrial experiment it appears that the early steps in glucose metabolism may not be important for a fraction of the contractile activity since pyruvate is as effective as glucose.

The better utilization of pyruvate than lactate suggests further experiments to determine whether the lactate in lactated Ringer's solution should be replaced by pyruvate.

Fructose has been shown to serve as an excellent substrate for the maintenance of contractility by the isolated rat atria when used at a concentration of 30 mM.<sup>7</sup> Opie *et al.*<sup>12</sup> found 5 mM fructose to be taken up and metabolized to CO<sub>2</sub> less than a fifth as rapidly as glucose at 5 mM. Gimeno *et al.*<sup>11</sup> demonstrated that fructose, at concentrations of 5.5 or 11 mM, is utilized for contractility of rat atria, but not nearly as effectively as the corresponding concentrations of glucose. The uptake of fructose or its conversion to fructose-6-phosphate may be rate-limiting. The data in the present study indicate that a similar situation may exist in the human atria, since fructose is less effective than glucose at similar concentrations.

Therefore, pyruvate and glucose were chosen for testing the halothane-depressed human atria. Pyruvate was markedly effective in increasing the contractility of isolated human atria depressed by halothane, while additional glucose was without effect. In one experiment an attempt was made to produce 50 per cent depression of the atria with pyruvate instead of glucose as the energy-supplying substrate. It was not possible to vaporize enough halothane with the anesthetist to produce this degree of depression, so liquid halothane was added to the bath with a syringe. Although the concentration of halothane was not measured it was estimated to be roughly 50 times the concentration required to depress the heart to the same extent when glucose was the substrate. These results in the human are consistent with the hypothesis, developed for the rat atria, that halothane blocks the uptake or some early step in the metabolism of glucose.<sup>7</sup>

Depression of human atrial appendages by 50 per cent required a mean concentration of 4.5 mg/100 ml halothane in the bathing medium. To achieve this a concentration of 6.4 mg/100 ml would be needed in the gas phase in equilibrium with the medium since the saline solution-to-gas partition coefficient is 0.70 at 37 C.<sup>13</sup> Using the gas law  $PV = nRT$ , the partial pressure of halothane was calculated to be 6.3 mm Hg, corresponding to 0.83 volumes per cent. This is very close to the minimum alveolar concentration of 0.77 volumes per cent (MAC-1) needed in man to

prevent muscular response to incision of the skin in 50 per cent of the subjects.<sup>14</sup> Clinical experience certainly would not indicate a 50 per cent interference with cardiac function at or near MAC-1. Therefore, we need to consider the possible reasons for the apparent discrepancies between the effects of halothane on the heart *in vivo* and *in vitro*.

First, cardiac function *in vivo* is determined by the mechanical activity of ventricular muscle, and we studied atrial tissue *in vitro*. This probably is not the reason for the discrepancy, since the forces of contraction of isolated rat atria and intact rat ventricles were depressed equally by similar concentrations of halothane.<sup>3, 5-7</sup>

Second, neuronal and neurohumoral compensatory mechanisms may be operating *in vivo* to overcome some of the effects of halothane.

Finally, and perhaps most important, a growing body of evidence indicates that fatty acids are an important fuel for animal and human hearts.<sup>15-22</sup> If the metabolism of fatty acids were not inhibited by low partial pressures of halothane, the heart could be supplied with the necessary fuel to maintain its contractility in the face of a blockade of glucose uptake or metabolism by halothane. Studies dealing with the effects of halothane on fatty acid utilization are needed to clarify this point.

These studies may be providing us with a clue to the mechanism of action of anesthetics in the brain. Since the brain is known to have a respiratory quotient very close to 1.00, and to use glucose as its main, if not its exclusive, source of fuel,<sup>23</sup> an agent interfering with glucose uptake or metabolism would be expected to produce marked effects on brain function, which might well result in anesthesia. Clinically, a single partial pressure of anesthetic does not produce uniform depression of the brain. This may reflect different sensitivities of the various parts of the brain to the anesthetic or differences in the abilities of the various parts to use fuels other than glucose once deprived of this fuel by the anesthetic. Also, the fact that anesthesia can be produced at concentrations of halothane that have little or no effect on cardiac function *in vivo* would be

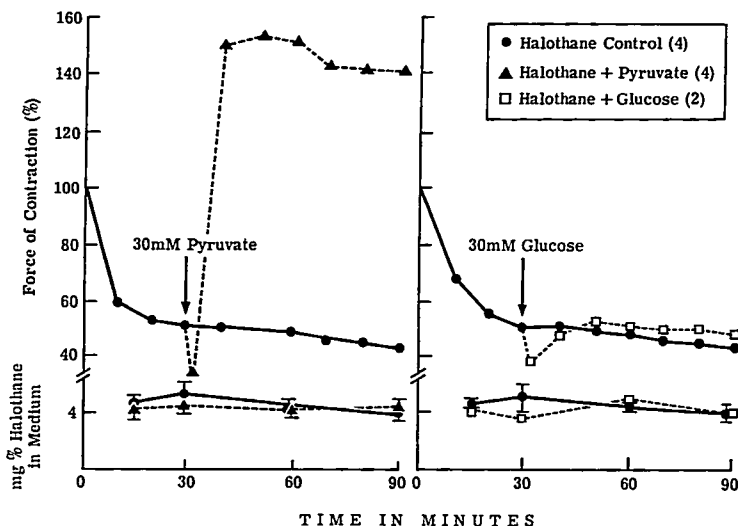


FIG. 3. Effect of 30 mM pyruvate or glucose on human atria depressed by halothane.

consistent with the view that certain parts of the brain are very sensitive to glucose deprivation, whereas the heart can use fatty acids and other substrates for its energy supply. Arguing against this concept is the work of Hoech *et al.*<sup>24</sup> They found anaerobic glycolysis of rat brain to be unaffected by 5 per cent halothane. Their experiments were done on homogenates of brain, however. It is possible that the effect of halothane on glucose metabolism of brain requires structural and functional integrity of the cells. Recently, evidence for ketoacid utilization by the brain during prolonged starvation has been presented by Cahill *et al.*<sup>25</sup> Whether the brain could adapt to inhibition of glucose metabolism and use ketoacids during short-term exposures to halothane is a subject for further investigation.

Another interesting fact that emerged from this study was that the calculated volumes per cent of halothane to produce 50 per cent depression of contractility in the rat atria at 30 C (0.77)<sup>5,7</sup> and in human atria at 37 C (0.83)

are similar. Unless a species difference is operating, this implies that the effects of halothane on the heart are not temperature-dependent. This would be in contrast to the findings of Eger *et al.* in the dog<sup>26</sup> and Cherkin and Catchpool in the goldfish<sup>27</sup>: in these experiments MAC fell by half as the temperature was reduced 10 C. Further studies along these lines will be carried out in the rat to rule out the effects of species in these findings.

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### Drugs

**DISPOSITION OF BARBITURATES** Pentobarbital, 30 mg/kg. and barbital, 225 mg/kg. were given intravenously to two groups of pregnant dogs. Barbiturate levels were measured in maternal blood, fetal blood, and amniotic fluid. Pentobarbital, a highly lipid-soluble drug, appeared in the fetal blood within five minutes of injection, and reached equilibrium in 15 minutes. Barbital, less lipid-soluble, also crossed the placenta and reached equilibrium between mother and fetus, although the time relationships were not determined. Both drugs entered the amniotic fluid at rapid rates; a greater fraction of the administered dose of barbital appeared in the amniotic fluid. This might be explained on the basis of less maternal tissue distribution of barbital, or its greater water solubility. (*Carrier, C., and others: Disposition of Barbiturates in Maternal Blood, Fetal Blood, and Amniotic Fluid, Amcr. J. Obstet. Gynec.* 105: 1069 (Dec.) 1969.)