

## The Effects of Pyruvate on Human Atrial-muscle Contractility Depressed by Methoxyflurane and by Pentobarbital

Gopal Krishna, M.D.,\* and Raymond R. Paradise, Ph.D.†

The effects of pyruvate on the force of contraction of isolated human atrial tissue depressed by methoxyflurane and by pentobarbital were studied. The tissue was suspended in Krebs-Ringer bicarbonate solution containing 20 mM glucose at 37 C and stimulated with supramaximal voltage at a rate of 70 pulses/min. Either methoxyflurane in a concentration of  $6.0 \pm 0.2$  mg/100 ml medium, representing 0.22 vol per cent or MAC 1.4, or pentobarbital, 7.5 mg/ml medium, depressed the force of contraction approximately 50 per cent. Pyruvate, 15 mM, elicited a marked positive inotropic effect in control atria. The response to pyruvate remained unimpaired in the presence of methoxyflurane, whereas pentobarbital markedly inhibited it. The mechanism of the negative inotropic effect of methoxyflurane in human atria is probably not an inhibition of energy production at sites below pyruvate in the Krebs cycle or electron transport system, but may involve a block in glycolysis, as previously demonstrated for rat atria. Pentobarbital, on the other hand, may interfere with energy production below pyruvate or with energy utilization. (Key words: Pyruvate; Methoxyflurane; Pentobarbital; Atria; Contractility.)

THE NEGATIVE INOTROPIC EFFECTS of the inhalation anesthetics and barbiturates are well documented.<sup>1-6</sup> In recent years, Ko and Para-

dise<sup>7-12</sup> have investigated the effects of halothane on carbohydrate metabolism in isolated rat atria and human atrial appendages. Addition of fructose, lactate, acetate or pyruvate to halothane-depressed rat atria partially restored the force of contraction. Recovery could not be achieved with addition of glucose. These results show that halothane causes a block of glycolysis above the phosphofructokinase step.<sup>7,9</sup>

Paradise and Ko also studied the effects of glucose and pyruvate on halothane-depressed strips of human atrial appendage. The results were similar to those in rat atrial preparations.<sup>9</sup> Other substrates were not studied because they are poorly utilized by the human heart.<sup>13</sup>

The purpose of the present study was to investigate whether the mechanisms of the negative inotropic actions of methoxyflurane and pentobarbital are similar to that of halothane. We investigated the inotropic effects of pyruvate on methoxyflurane-depressed and pentobarbital-depressed isolated human atrial muscle.

### Materials and Methods

Pieces of right atrial appendage were obtained from patients of all age groups undergoing cardiac surgery on cardiopulmonary bypass. The pieces were transported (10 minutes) to the laboratory in iced Krebs-Ringer solution aerated with 95 per cent O<sub>2</sub>-5 per cent CO<sub>2</sub>. The tissue was carefully dissected and muscle bundles 10-15 mm long were isolated. Each muscle bundle was suspended in a muscle chamber containing modified Krebs-Ringer solution at 37 C and pH 7.4. The composition (mM) of the medium was: NaCl 120; KCl 4.8; CaCl<sub>2</sub> 1.22; MgSO<sub>4</sub>·7 H<sub>2</sub>O 1.33; KH<sub>2</sub>PO<sub>4</sub> 1.2; NaHCO<sub>3</sub> 25.3; glucose 20. The

\* Instructor, Department of Anesthesiology.

† Associate Professor, Departments of Pharmacology and Anesthesiology.

Received from the Departments of Pharmacology and Anesthesiology, Indiana University School of Medicine, Indianapolis, Indiana 46202. Accepted for publication November 11, 1971. Supported in part by USPHS Grant HE 07718, in part with facilities provided by the Heart Research Center Grant H 6308 from the National Heart Institute USPHS, in part by General Research Support Grant PHS-S01-FR 5371 and in part by a grant from the Anesthesiology Department of Indiana University. Presented at the Annual Meeting of the American Society of Anesthesiologists, Inc., Atlanta, Georgia, October 1971.

solution was continuously aerated with 95 per cent  $O_2$  and 5 per cent  $CO_2$ . A constant resting tension of 750 mg was maintained. All muscles were stimulated at a rate of 70 pulses/min with 80 volts. In our system the average threshold was 20–30 volts. The force of contraction developed was recorded with a Stat-ham strain gauge on a pen recorder. A stabilization period of 60 minutes was allowed before readings were taken. The force of contraction at the end of the stabilization period was expressed as 100 per cent. Methoxyflurane and pentobarbital were introduced into the medium at zero time (following the one-hour stabilization period).

Methoxyflurane was introduced into the medium by means of a vaporizer connected to a solenoid valve. The periodic opening of the solenoid valve, and thus the anesthetic concentration delivered to the medium, was regulated by an electronic device. Adjustments were made during the first 10 min to achieve 50 per cent depression of the force of contraction. One-milliliter samples of the medium were taken every 10 min. Methoxyflurane was extracted by tetrachloroethylene from these samples and concentrations were measured by a gas chromatograph with a thermal conductivity detector. Fresh standards were prepared for each experiment. Pentobarbital was introduced into the medium by a syringe to achieve a concentration of 7.5 mg/100 ml in the medium.

## Results

### CONTROL

Following the one-hour stabilization period, the force of contraction gradually declined with time. In a control series of four experiments a 20 per cent decrease in force was observed in an 80-minute period (fig. 1).

### EFFECT OF METHOXYFLURANE ON CONTRACTILITY OF ATRIA

Mean concentrations of methoxyflurane necessary to depress the force of contraction by approximately 50 per cent were in the range of 5.7–6.4 mg/100 ml (11 experiments). Recovery from methoxyflurane depression was studied in four experiments. Methoxyflurane administration was discontinued after 40 min-

utes. Recovery to 80 per cent of the initial value was attained within 10 minutes (fig. 1). If the decay factor is taken into account, this recovery can be considered complete.

From our data, 33 single values, a mean of  $6.0 \pm 0.2$  mg/100 ml of methoxyflurane was necessary to depress the force of contraction 50 per cent.

### EFFECT OF PYRUVATE ON CONTROL ATRIA

Addition of 15 mM pyruvate at 80 minutes in four control experiments caused a marked increase in contractility from 78 to 198 per cent (fig. 2).

### EFFECT OF PYRUVATE ON METHOXYFLURANE-DEPRESSED ATRIA

The effect of pyruvate on methoxyflurane-depressed atrial muscle was studied in four experiments. Figure 3 illustrates that addition of 15 mM pyruvate 40 minutes after the start of methoxyflurane administration resulted in a prompt increase in the force of contraction (from 50 to 130 per cent). Discontinuation of methoxyflurane 30 minutes after the addition of pyruvate resulted in a further increase of 20 per cent in the force of contraction. This experiment clearly shows that the positive inotropic effect of pyruvate is not blocked by methoxyflurane.

### EFFECT OF PENTOBARBITAL ON CONTRACTILITY

Pentobarbital in a concentration of 7.5 mg/100 ml decreased the force of contraction approximately 50 per cent within 10 minutes. The force of contraction decreased further with time (26 per cent at 60 minutes). The recovery after washout of pentobarbital at 60 minutes (three times with medium) was considerably less than that after discontinuation of methoxyflurane (58 per cent vs. 80 per cent) (fig. 4).

### EFFECT OF PYRUVATE ON PENTOBARBITAL-DEPRESSED ATRIA

Figure 4 compares the effect of 15 mM pyruvate on atrial preparations depressed by pentobarbital for 30 minutes with its effect on methoxyflurane-depressed atria. Pyruvate caused only a small increase in the force of contraction of pentobarbital-depressed atria (from 33 to 55 per cent). Thirty minutes

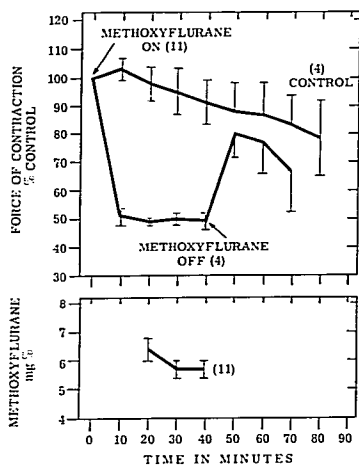


FIG. 1. Effect of methoxyflurane on the force of contraction of isolated human atrial muscle. Methoxyflurane was added at zero time (following 60 min of equilibration of the muscle in modified Krebs-Ringer bicarbonate medium containing 20 mM glucose). Methoxyflurane administration was stopped at 40 min. The lower part of the figure represents the concentrations of methoxyflurane in the medium as mg/100 ml medium. Vertical bars indicate  $\pm 1$  SE. Figures in parentheses represent numbers of experiments. Control represents force of contraction for an additional 80 min in the normal medium.

after washout, *i.e.*, at 90 minutes an additional 15 mM of pyruvate elicited a marked positive inotropic effect (from 62 to 130 per cent increase in force of contraction). This indicates that pentobarbital markedly inhibited the effect of pyruvate.

### Discussion

On the basis of studies in the perfused rat heart, Paradise and Bibbins<sup>1</sup> compared equidepressant concentrations of different inhalation anesthetics with concentrations necessary to produce a certain level of anesthesia. They

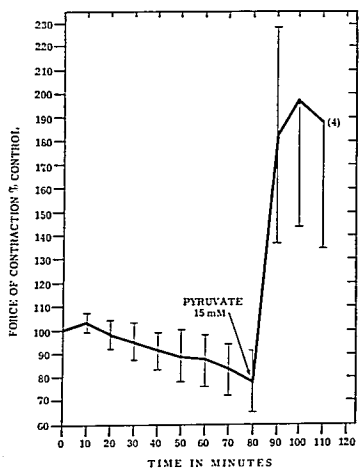


FIG. 2. Effect of pyruvate on the force of contraction of isolated human atrial muscle. Pyruvate was added at 80 min to four atrial muscle preparations in normal medium.

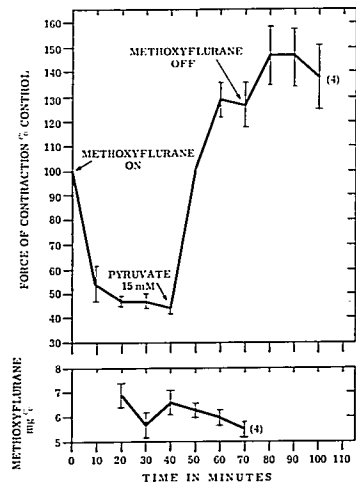


FIG. 3. Effect of pyruvate on methoxyflurane-depressed isolated human atrial muscle. Methoxyflurane was added at zero time, pyruvate at 40 min. Methoxyflurane was stopped at 70 min.

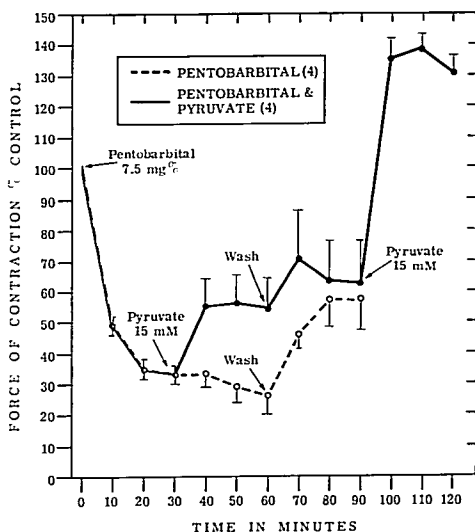


Fig. 4. Effect of pyruvate on pentobarbital-depressed isolated human atrial muscle. Pentobarbital was added at zero time to eight atrial muscle preparations. At 30 min pyruvate was added to four of these preparations. Wash represents return of the muscles to the normal Ringer's medium. Pyruvate was again added at 90 min to the four muscles previously exposed to it.

concluded that at particular levels of anesthesia halothane causes greater myocardial depression than methoxyflurane. Brown *et al.*<sup>4</sup> obtained similar results in studies on cats. Ko and Paradise,<sup>8</sup> in studies in human atrial appendages, found that 0.83 vol per cent halothane, or MAC 1.07, was needed to achieve 50 per cent depression of force of contraction. Our results indicate that  $6.0 \pm 0.20$  mg/100 ml methoxyflurane are necessary to depress the force of contraction 50 per cent in isolated human atrial muscle. On the basis of a computed saline solution/gas partition coefficient of 4.3 (assumed to be 5 per cent less than a reported water/gas partition coefficient of 4.5, as for halothane),<sup>14, 15</sup> our value of 6.0 mg/100 ml methoxyflurane in saline solution represents 1.67 torr, or MAC 1.4. Thus, it can be concluded that halothane produces greater myocardial depression than methoxyflurane in humans at concentrations equieffective for anesthesia.

The negative inotropic effects of halothane and methoxyflurane have been demonstrated by numerous authors. Paradise and Griffith<sup>16</sup>

showed that halothane and methoxyflurane concentrations sufficient to depress the force of contraction 50 per cent had no effect on the potassium content of the heart, suggesting that low concentrations of these anesthetics do not alter the Na-K pump. Ko and Paradise<sup>7, 9</sup> investigated the effects of substrates on halothane-depressed contractility in isolated rat atria. They found that pyruvate, lactate, acetate, and fructose caused partial recovery of halothane-depressed contractility, whereas glucose had no effect. In studies in human atrial appendage tissue they demonstrated that pyruvate, but not glucose, caused recovery of halothane-depressed contractility. On the basis of these findings, they suggested that halothane causes a block in glycolysis.<sup>7, 8</sup>

The effects of pyruvate on methoxyflurane-depressed contractility in human atrial muscle are strikingly similar to the effects of pyruvate on halothane-depressed human atrial tissue. It has been shown in rat studies<sup>17</sup> that the response of methoxyflurane-depressed atria and that of halothane-depressed atria to exogenous substrates are very similar.

The mechanism of the negative inotropic effect of barbiturates is not well defined. Two possible mechanisms have been suggested. It has been proposed that pentobarbital may increase the affinity of the cell membrane for calcium, thereby decreasing the release of calcium by the action potential.<sup>6, 18</sup> Other studies suggest that the depressive action of barbiturates may be due to alteration of oxidative metabolism.<sup>19</sup> Interference with oxidative metabolism has also been shown to occur in brain tissue.<sup>20, 21</sup>

In our study, 7.5 mg/100 ml pentobarbital depressed the force of contraction of human atrial muscle at 37 C 50 per cent within 10 minutes and 33 per cent at 30 minutes. This dose closely approximates the amount reported by Dressel *et al.*<sup>6</sup> to depress the force of contraction of cat papillary muscle at 37 C to a similar extent. The pyruvate response was much smaller in the presence of pentobarbital than in its absence.

Although our data do not provide definitive conclusions, the difference between the responses to pyruvate of pentobarbital-depressed atria and methoxyflurane-depressed atria is evident. Methoxyflurane, like halothane, did not block the pyruvate response, whereas pentobarbital markedly inhibited it. This is consistent with a block in glycolysis by methoxyflurane, whereas pentobarbital may act by interfering with energy production or utilization below pyruvate.

The authors thank Drs. H. B. Shumacker, H. King, and R. King for supplying the pieces of human atrial appendage used in this study.

### References

- Paradise RR, Bibbins F: Comparison of the effects of equieffective concentrations of anesthetics on the force of contraction of isolated perfused rat heart: Correlation with the equieffective anesthetizing partial pressures. *ANESTHESIOLOGY* 31:349-355, 1969
- Brown JM: Anesthesia and the contractile force of the heart: A review. *Anesth Analg (Cleve)* 39:487-498, 1960
- Shimosato S, Sugai N, Etsten BE: The effect of methoxyflurane on the inotropic state of myocardial muscle. *ANESTHESIOLOGY* 30: 506-512, 1969
- Brown BR Jr, Crout JR: A comparative study of the effects of five general anesthetics on myocardial contractility: I. Isometric contractions. *ANESTHESIOLOGY* 34:236-245, 1971
- Smith NT, Corbascio AN: Interaction between pentobarbital and decreased calcium ion on guinea pig atria. *Anesth Analg (Cleve)* 45: 576-581, 1966
- Dressel PE, Ellett RW, Schluter L: Effect of pentobarbital on isometric contractions and parameters of active state in cat papillary muscle. *Life Sci* 9:759-764, 1970
- Ko KC, Paradise RR: The effects of substrates on contractility of rat atria depressed with halothane. *ANESTHESIOLOGY* 31:532-539, 1969
- Ko KC, Paradise RR: The effects of substrates on halothane-depressed isolated human atria. *ANESTHESIOLOGY* 33:508-514, 1970
- Paradise RR, Ko KC: The effect of fructose on halothane-depressed rat atria. *ANESTHESIOLOGY* 32:124-129, 1970
- Ko KC, Paradise RR: Contractile depression of rat atria by halothane in the absence of glucose. *ANESTHESIOLOGY* 34:152-156, 1971
- Ko KC, Paradise RR: The effects of substrates on contractility of isolated rat atria depressed by hydrochloric acid. *Proc Soc Exp Biol Med* 136:178-182, 1971
- Ko KC, Paradise RR: The effects of substrates on rat atria depressed with bicarbonate-free medium, citrate, or low calcium. *Proc Soc Exp Biol Med* 134:469-476, 1970
- Ko KC, Paradise RR: The effects of substrates on contractility of isolated human atria. *Proc Soc Exp Biol Med* 134:386-389, 1970
- Larson CP Jr, Eger EI II, Severinghaus JW: The solubility of halothane in blood and tissue homogenates. *ANESTHESIOLOGY* 23: 349-355, 1962
- Eger EI II, Shargel R: The solubility of methoxyflurane in human blood and tissue homogenates. *ANESTHESIOLOGY* 24:625-627, 1963
- Paradise RR, Griffith LK: Influence of halothane, chloroform and methoxyflurane on potassium content of rat atria. *ANESTHESIOLOGY* 26:195-198, 1965
- Ko KC, Paradise RR: The mechanism of the negative inotropic effect of methoxyflurane on isolated atria. *ANESTHESIOLOGY* 36: 64-68, 1972
- Daniel EE, Johnston PK, Foulks JG: The mechanism of the effects of sodium pentobarbital and norepinephrine on isolated cardiac muscle. *Arch Int Pharmacodyn* 138: 276-301, 1962
- Harvey SC: Radiophosphorus metabolism of the guinea pig heart and the actions of digitoxin and pentobarbital. *Am J Physiol* 183: 559-564, 1955
- Mellwain H: The effect of depressants on the metabolism of stimulated cerebral tissues. *Biochem J* 53:403-412, 1953
- Cohen MM, Heald PJ: The effects of phenobarbital upon phosphate metabolism of cerebral cortex *in vitro*. *J Pharmacol Exp Ther* 129:361-367, 1960