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

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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 ± 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. In recent years, Ko and 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. Other substrates were not studied because they are poorly utilized by the human heart.

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 O₂-5 per cent CO₂. 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; CaCl₂ 1.22; MgSO₄·7 H₂O 1.33; KH₂PO₄ 1.2; NaHCO₃ 25.3; glucose 20. The
solution was continuously aerated with 95 per
cent O₂ and 5 per cent CO₂. A constant rest-
ing 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 con-
traction developed was recorded with a Stat-
ham strain gauge on a pen recorder. A sta-
bilization period of 60 minutes was allowed
before readings were taken. The force of con-
traction at the end of the stabilization period
was expressed as 100 per cent. Methoxyflu-
rane and pentobarbital were introduced into
the medium at zero time (following the one-
hour stabilization period).

Methoxyflurane was introduced into the me-
dium by means of a vaporizer connected to a
solvent valve. The periodic opening of the
solvent valve, and thus the anesthetic concen-
tration delivered to the medium, was regu-
lated by an electronic device. Adjustments
were made during the first 10 min to achieve
50 per cent depression of the force of con-
traction. 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 conduc-
tivity detector. Fresh standards were prepared
for each experiment. Pentobarbital was intro-
duced into the medium by a syringe to achieve
a concentration of 7.5 mg/100 ml in the me-
dium.

Results

Control

Following the one-hour stabilization period,
the force of contraction gradually declined
with time. In a control series of four exper-
iments 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 nec-
essary 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). Re-
covery 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 ± 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 ex-
periments. 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 addi-
tion of pyruvate resulted in a further increase
of 20 per cent in the force of contraction.
This experiment clearly shows that the posi-
tive 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 ap-
proximately 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 con-
siderably 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
causèd 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 ±1 SE. Figures in parentheses represent numbers of experiments. Control represents force of contraction for an additional 60 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 compared equivalent concentrations of different inhalation anesthetics with concentrations necessary to produce a certain level of anesthesia. They
concluded that at particular levels of anesthesia halothane causes greater myocardial depression than methoxyflurane. Brown et al. obtained similar results in studies on cats. Ko and Paradise, 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 ± 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), 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 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 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.

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 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. Other studies suggest that the depressive action of barbiturates may be due to alteration of oxidative metabolism. Interference with oxidative metabolism has also been shown to occur in brain tissue.

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. 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

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