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The Mechanism of the Negative Inotropic Effect of Methoxyflurane on Isolated Rat Atria

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The force of contraction of isolated rat atria suspended in Krebs-Ringer bicarbonate medium containing 5.5 mM glucose was depressed 55 per cent by approximately 9 mg/100 ml of methoxyflurane in the medium bathing the atria. This corresponds to a partial pressure of 1.84 torr (0.24 vol per cent). Addition of fructose (30 mM) or pyruvate (5 mM) to atria depressed by methoxyflurane resulted in a marked positive inotropic action. Additional glucose (20 mM) was relatively ineffective in atria similarly treated. These results suggest an interference with glucose utilization at an early step in glycolysis above the phosphofructokinase step. Methoxyflurane had its usual depressant action in the absence of exogenous glucose (substrate-free medium). Thus, glucose uptake and phosphorylation were ruled out as the site of action of methoxyflurane. These results suggest that methoxyflurane's depressant action results from a block at the glucose phosphate isomerase step, the step responsible for converting glucose-6-phosphate to fructose-6-phosphate. Multiple sites of action are not ruled out by these results. (Key words: Methoxyflurane; Heart; Contractility; Glucose; Pyruvate; Fructose metabolism.)

THE NEGATIVE INOTROPIC EFFECT of low partial pressures of halothane (approximately 6 torr, or 0.8 vol per cent) has been attributed to a block in glycolysis. If only a single step is involved, it is probably the glucose phosphate isomerase step. Elucidation of this mechanism was accomplished by the technique of addition of substrate to halothane-depressed rat atria. Pyruvate, lactate, acetate, and fructose were effective in reversing halothane depression, whereas glucose was not.[1, 2] Since fructose is apparently metabolized via the phosphofructokinase (PFK)[2, 3] step, the blockade had to be above this step in the glycolytic cycle. Since halothane also depressed the force of contraction in the absence of exogenous substrate, *i.e.*, with no glucose present in the bathing medium, the site of action could not be glucose uptake or phosphorylation of glucose to glucose-6-phosphate.[4] Conversion of glucose-6-phosphate to fructose-6-phosphate by glucose phosphate isomerase was then implicated as the site of inhibition by halothane, the glucose-6-phosphate coming from endogenous glycogen. There is evidence to suggest that endogenous glycogen is present in hearts *in vitro* and is used to support contractility in the absence of exogenously-supplied substrate.[5–7]

The purpose of the present investigation has been to determine whether methoxyflurane also depresses the force of contraction of isolated rat atria by a block in the glycolytic cycle. The techniques used were similar to those reported above for halothane. The data suggest a mechanism similar to that seen with halothane.

Methods

Male Sprague-Dawley rats, weighing 180 to 200 g, which had access to food and water *ad lib.*, were studied. Atria were removed from decapitated rats and suspended in a modified Krebs-Ringer bicarbonate–glucose medium of the following composition (mM)[1]: NaCl 120; KCl 4.8; $CaCl_2$ 1.22; $MgSO_4 \cdot 7H_2O$ 1.33; KH_2PO_4 1.2; $NaHCO_3$ 25.3; glucose 5.55. The medium was gassed with 95 per cent O_2 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 experi-

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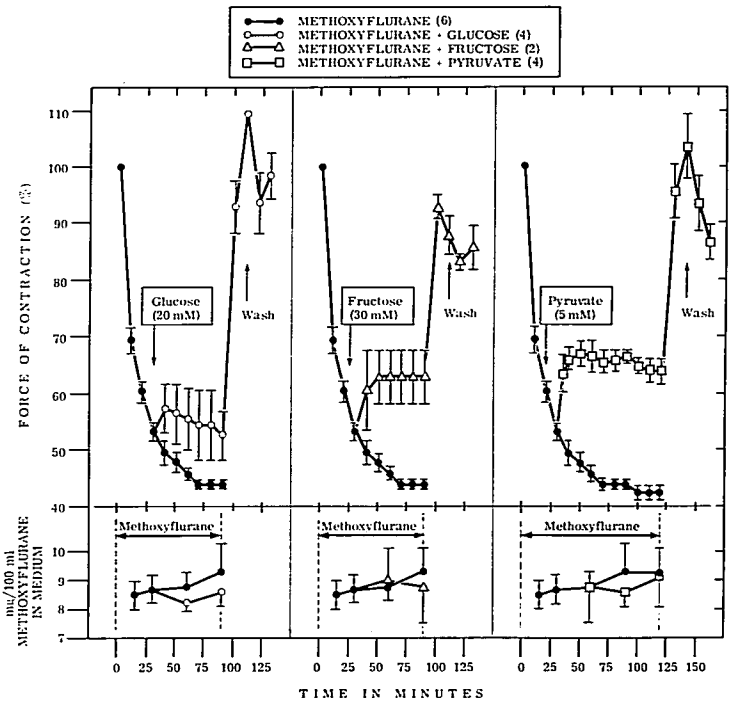

FIG. 1. Effects of substrates on methoxyflurane-depressed rat atria. Glucose (20 mM), fructose (30 mM), or sodium pyruvate (5 mM) was added 30 min after the start of methoxyflurane administration. Methoxyflurane was added at zero time (*i.e.*, following a 60-min equilibration period in modified Krebs-(Ringer bicarbonate–glucose medium). Vertical bars represent ± SE. Figures in parentheses represent numbers of experiments.

ments. 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 an hour was allowed before readings were taken. 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. Methoxyflurane was administered to the medium by means of the anesthetistat previously described by Paradise and Griffith.[8, 9] The methoxyflurane concentration in the medium was determined at 5 to 10 min by gas chromatography.[10]

In the experiments with substrate-free medium, the medium was changed to substrate free (*i.e.*, free of glucose) 30 min after the start of the experimental period. The techniques involved in administering methoxyflurane to the same atria in the presence and in the absence of glucose were the same as those described previously for halothane.[4]

The concentration of methoxyflurane in the bathing medium needed to depress the atrial force of contraction approximately 55 per cent

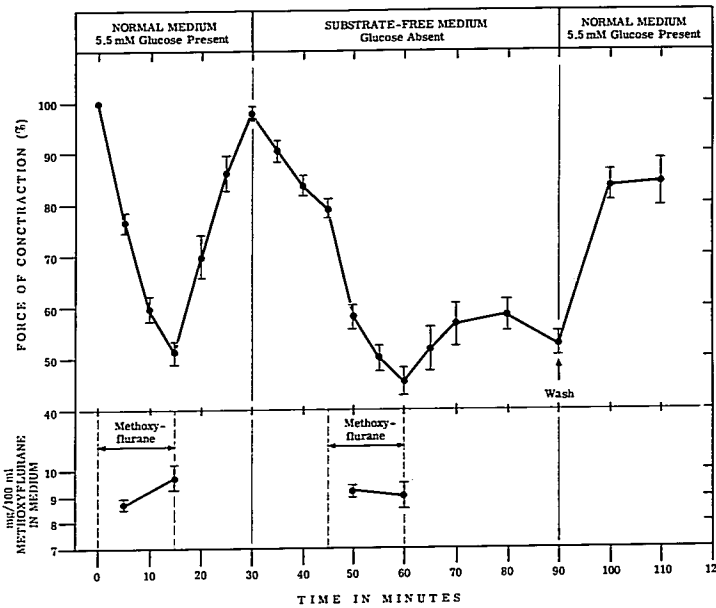

FIG. 2. Effects of methoxyflurane on contractility of rat atria in the presence and in the absence of glucose (four experiments). Methoxyflurane was added at zero time (following a one-hour equilibration period) to five atrial preparations in the presence of 5.5 mM glucose. The tube delivering methoxyflurane 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 methoxyflurane tube was readmitted to the medium and methoxyflurane administration was continued for 15 min. Methoxyflurane administration was then stopped and the atria were allowed to recover for 30 min. The medium was then changed to the normal medium containing 5.5 mM glucose (wash). Verticle bars represent ± SE.

was about 9 mg/100 ml. A water–gas partition coefficient of 5.9 for methoxyflurane at 30 C was calculated from vapor pressure and solubility data in the paper by Cherkin and Catchpool.[11] The method of making this calculation has been described.[10] The saline–gas partition coefficient was assumed to be 5 per cent less than the corresponding water–gas value. Larson *et al.*[12] found the saline–gas partition coefficient for halothane to be 5 per cent less than the water–gas partition coefficient at 37 C. Using a calculated saline–gas partition coefficient of 5.6, a concentration of 9 mg/100 ml methoxyflurane in saline medium represents a partial pressure of 1.84 torr (0.24 vol per cent).

Results

Effect of Methoxyflurane on Atrial Contractility

The behavior of atria in the presence of methoxyflurane was determined to provide data with which the responses to substrates of the depressed atria could be compared. Methoxyflurane administration was begun at zero time (following a one-hour equilibration period). During the first 30 min the anesthetistat was adjusted to deliver enough meth-

oxyflurane to achieve approximately 50 per cent depression of contractility.

Following this period no further adjustments were made. Relatively stable concentrations of methoxyflurane were present in the bathing medium during the following 90 min (8.5 to 9.3 mg/100 ml mean values). The force of contraction declined slightly to about 43 per cent of the control value (fig. 1). After administration of methoxyflurane was stopped, recovery was complete.

Effects of Substrates on Atria Depressed by Methoxyflurane

Glucose (20 mM), fructose (30 mM), or sodium pyruvate (5 mM) was added to the bathing medium 30 min after the start of methoxyflurane administration (fig. 1). Despite the continued administration of methoxyflurane to maintain levels in the medium similar to those in the four control experiments, the addition of fructose or pyruvate resulted in a marked increase in the force of contraction, while glucose had only a slight effect. The glucose effect was not significant ($P > 0.05$). The fructose and pyruvate effects were highly significant ($P < 0.001$).

Effects of Methoxyflurane on Contractility of Atria in the Presence and in the Absence of Glucose

Figure 2 indicates that methoxyflurane (approximately 9 mg/100 ml) depressed the force of contraction to about the same extent whether glucose was present (normal medium) or absent (substrate-free medium). Thus, the negative inotropic effect of methoxyflurane is not dependent on the presence of glucose in the medium.

Discussion

Glucose (20 mM), fructose (30 mM), or pyruvate (5 mM) can be used as a source of energy to support contractility, as evidenced by their positive inotropic effects when added to isolated rat atrial preparations incubated in substrate-free medium.[1,2] Of the three substrates, glucose is the most effective. That fructose and pyruvate were much more effective than glucose in overcoming the negative inotropic action of methoxyflurane strongly suggests that the utilization of glucose is interfered with by this anesthetic. The site of interference must be at an early step in glycolysis above the phosphofructokinase step, since fructose is apparently metabolized via this step.[2,3] The possible sites (fig. 3) are: A) glucose uptake into the heart cells; B) phosphorylation of glucose to glucose-6-phosphate by hexokinase; C) conversion of glucose-6-phosphate to fructose-6-phosphate by glucose phosphate isomerase. The first two of these steps are not indicated, since methoxyflurane exerts its usual depressant effect in the absence of exogenously supplied glucose. Thus, there is no glucose to be taken up or to be phosphorylated. The third site is possible and likely, since glucose-6-phosphate can be supplied from glycogen (site *D* in fig. 3). There is a growing body of evidence to suggest that endogenous glycogen is an important source of fuel to support the contractility of hearts deprived of exogenous substrates.[5-7]

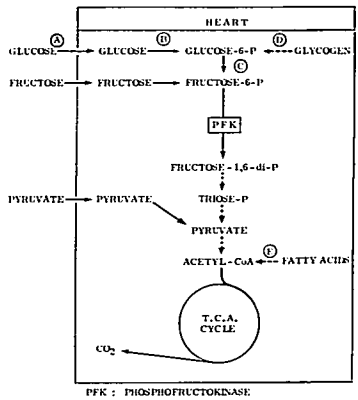

Fig. 3. Schematic representation of glycolysis, showing the points at which various substrates enter the scheme. In this study pyruvate and fructose were effective in overcoming methoxyflurane-induced cardiac depression, while additional glucose was ineffective. Fructose apparently is metabolized via phosphofructokinase, since it is ineffective in the presence of bicarbonate-free medium or citrate, both inhibitors of this enzyme.[2,3] Thus, the site of action of methoxyflurane is localized to steps *A*, *B* and *C*. This report rules out steps *A* and *B*, since methoxyflurane decreases contractility in the absence of exogenous glucose.

Thus, if a single site of action is involved in the depressant action of methoxyflurane on the heart, that site would appear to be the glucose phosphate isomerase step. By a similar line of reasoning, this is also the step implicated for halothane.[1, 2, 4]

References

1. Ko K-C, Paradise RR: The effects of substrates on contractility of rat atria depressed with halothane. ANESTHESIOLOGY 31:532–539, 1969
2. Paradise RR, Ko K-C: The effect of fructose on halothane-depressed rat atria. ANESTHESIOLOGY 32:124–129, 1970
3. Ko K-C, 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
4. Ko K-C, Paradise RR: Contractile depression of rat atria by halothane in the absence of glucose. ANESTHESIOLOGY 34:152–156, 1971
5. Evans G: The glycogen content of the rat heart. J. Physiol 83:468–480, 1934
6. Neely JR, Whitfield CF, Morgan HE: Regulation of glycogenolysis in hearts: Effects of pressure development, glucose, and FFA. Amer J Physiol 219:1083–1088, 1970
7. Ko K-C, Paradise RR: Nature of endogenous substrates used to support contractility of isolated rat atria. Proc Soc Exp Biol Med 137:928–931, 1971
8. Paradise RR, Griffith LK: Electrolyte content of perfused rat ventricles exposed to halothane or anoxia. J Pharmacol Exp Ther 154:281–288, 1966
9. Paradise RR, Griffith LK: Control of concentration of volatile agents in open *in vitro* systems. ANESTHESIOLOGY 27:687–688, 1966
10. Paradise RR, Bibbins F: Comparison of the effects of equieffective concentrations of anesthetics on the force of contraction of isolated perfused rat hearts: Correlation with the equieffective anesthetizing partial pressures. ANESTHESIOLOGY 31:349–355, 1969
11. Cherkin A, Catchpool JF: Temperature dependence of anesthesia in goldfish. Science 144:1460–1462, 1964
12. Larson CP, Eger EI II, Severinghaus JW: Solubility of halothane in blood and tissue homogenates. ANESTHESIOLOGY 23:349–355, 1962
13. Ko K-C, 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

Respiration

FAMILIAL DYSAUTONOMIA AND RESPIRATORY CONTROL The authors describe a detailed study of a patient with acquired dysautonomia (*i.e.*, inappropriate response of autonomic function) who had abnormal circulatory control mechanisms with orthostatic hypotension (*i.e.*, on tilting to head-up position, arterial blood pressure fell from 102/60 to 70/35 torr without a change in heart rate; there was no blood-pressure response to cold stimulation nor a post-Valsalva blood-pressure overshoot). Exposure to a mixture of 10 per cent oxygen and 90 per cent nitrogen was not associated with a change of ventilation despite a decrease in Pa_{O_2} from 64 to 30 torr. This response was similar to that observed in the experimental animal with a denervated carotid body or in man following bilateral block of the ninth and tenth cranial nerves. The positive interaction between elevated P_{CO_2} and lowered P_{O_2} was absent; *i.e.*, when the patient was challenged with 5 per cent CO_2 in 95 per cent oxygen there was an increase in ventilation (less than in normal man) which was as large as the response to 5 per cent CO_2 with 15 per cent oxygen. Administration of atropine intravenously in increments of 0.4 mg to a total dose of 2.0 mg did not increase heart rate, although a characteristic increase followed the infusion of isoproterenol. The lack of response to hypoxia demonstrates absent peripheral chemoreceptor activity, while the weak response to CO_2 suggests some central chemoreceptor involvement. (*Eisele, J. H., and others: Abnormal Respiratory Control in Acquired Dysautonomia, New Eng. J. Med. 285: 366, 1971.*)