The Effects of Methoxyflurane on Myocardial Contractility and Reactivity

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Methoxyflurane reduced the total developed tensions of isolated guinea pig atria and isolated cat papillary muscle but did not change the rate of spontaneous beating of isolated atria. The staircase phenomenon and postextrasystolic potentiation appeared not to be affected when the increases in developed tension were expressed as percentages of the preceding values. These effects are similar to those reported for halothane. Unlike halothane, methoxyflurane did not produce significant changes in the increases of developed tension induced in papillary muscle by high-voltage electrical stimulation or tyramine or in the effective refractory period of papillary muscle.

(Key words: Methoxyflurane; Myocardial contractility; Catecholamines; Staircase phenomenon; Myocardial excitability; Effective refractory period; Postextrasystolic potentiation.)

Several authors have reported that methoxyflurane exerts a negative inotropic effect upon the intact heart, and recently this finding has been confirmed by study of the force-velocity relationship in the intact canine left ventricle. Changes in the inotropic state of the myocardium resulting from a direct depressant effect of methoxyflurane may be complicated in vivó by nervous, humoral and metabolic factors, however. The present study was designed to determine the direct effect of methoxyflurane on isolated heart preparations. The guinea pig atrial preparation is useful for identification of pharmacologic effects on cardiac rhythmicity and active tension of contrac-

tions, and the isolated cat papillary muscle is suitable for study of the relationship between rhythm and contractile force, the threshold to electrical stimulation, and the duration of the refractory period.

Since a positive staircase phenomenon is a good index of the functional state of the ventricular myocardium and could be considered an intrinsic property of the heart, allowing rapid adaptation of contractility with changes in rate, we also studied the effect of methoxyflurane on this phenomenon in electrically driven isolated papillary muscle. The mechanism of postextrasystolic potentiation is unknown, although it has been used experimentally in the temporary treatment of acute cardiac failure. These facts and the lack of information about the effect of methoxyflurane on postextrasystolic potentiation prompted a study of this problem in preparations of papillary muscle.

Since methoxyflurane does not release catecholamines during anesthesia and apparently does not sensitize the heart to these substances, we studied the responses of papillary muscle to catecholamines released either by high-voltage stimulation (electrorelease) or by addition of tyramine to the papillary muscle (chemical release) in the presence of methoxyflurane.

Material and Methods

The cat papillary muscle was prepared according to the method of Cattell and Gold, with modifications reported previously. The muscle was kept in a bath containing Locke-Ringer's solution (composition: NaCl 119.6 mM; KCl 5.6 mM; CaCl₂ 2.2 mM; MgCl₂ 2.1 mM; glucose 10 mM; NaHCO₃ 25 mM) gassed with 95 per cent O₂ and 5 per cent CO₂. The solution was maintained at 37 °C at equilib-
METHOXYFLURANE AND MYOCARDIAL ACTIVITY

TABLE 1. Effects of Methoxyflurane on Tension Developed by Spontaneously Beating Isolated Guinea Pig Atria and Electrically Driven Isolated Cat Papillary Muscle (Mean ± SE)

<table>
<thead>
<tr>
<th>Methoxyflurane Concentration</th>
<th>Guinea Pig Atria</th>
<th>Cat Papillary Muscle</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number of Preparations Tested</td>
<td>Changes in Developed Tension</td>
</tr>
<tr>
<td></td>
<td>mg</td>
<td>Per Cent</td>
</tr>
<tr>
<td>0.15 per cent</td>
<td>9</td>
<td>-134 ± 45</td>
</tr>
<tr>
<td>0.25 per cent</td>
<td>10</td>
<td>-432 ± 23</td>
</tr>
<tr>
<td>0.45 per cent</td>
<td>9</td>
<td>-474 ± 133</td>
</tr>
</tbody>
</table>

rium pH was 7.4. Muscles 7 to 10 mm long, with diameters ranging from 0.7 to 1.2 mm, were selected. The diastolic (resting) tension was adjusted somewhat below that initially determined in each muscle to be associated with near-maximum development of tension, between 0.8 and 1.5 g depending on the cross-sectional area of the muscle. The muscles were stimulated at the base by pointed electrodes immersed in the Locke–Ringer’s solution. The electrodes connected to a model 951 American Electronics stimulator produced square-wave pulses 2 msec in duration with voltages slightly above threshold. With this current, release of catecholamines by electrical stimulation was of minor importance.17,20 The tension developed by papillary muscle during isometric contraction was registered by means of a Grass FT03 force transducer coupled to a Grass model 3D polygraph. The absolute tensions developed (mg) and the percentage tension changes are presented in tables 1 to 5. The experiments were performed after stabilization periods lasting 40 to 60 minutes, depending on the individual muscle preparations.

In most of these experiments the papillary muscles were exposed to methoxyflurane bubbled into the bath from a Takaoka Universal Vaporizer inserted between the oxygen–CO₂ cylinder and the isolated heart preparation.21 The vaporizer was adjusted to deliver concentrations of 0.25 and 0.45 per cent, but the actual concentrations in the bath were not measured.

The muscle preparations were exposed to a methoxyflurane concentration of 0.25 per cent for a little more than ten minutes, until the developed tensions had remained stable for ten minutes. After exposure to methoxyflurane, the muscles were allowed to recover for 30 to 45 minutes, after which methoxyflurane in a concentration of 0.45 per cent was bubbled through the bath. At the ends of both recovery periods the tensions developed by papillary muscles were similar to the initial value. In most muscle preparations the studies were repeated after the recovery periods, and did not produce values significantly different from control values. Furthermore, control studies of some muscle preparations were done at the same time as the experimental runs, but in the absence of anesthetic, and no significant differences were observed.

A methoxyflurane concentration of 0.15 per cent had only a very slight effect on the tension developed by papillary muscle but had a consistent effect on isolated guinea pig atria. Therefore, this concentration was not studied systematically in the papillary muscle preparation.

Electrical excitability was determined by measuring threshold voltages. The refractory period was determined at the rate of 60/min, using two Tektronix stimulators coupled to give a premature (msec) electrical stimulus after the regular pulse (paired pulse). The same stimulators were used to study postextrasystolic potentiation. The staircase phenomenon was studied by increasing the rate of stimulation from 30 to 60 and from 30 to 120/min. The effect of high-voltage electrical stimulation on tension developed was studied by increasing the stimulation voltage to ten times the threshold value both during the con-
isolated atria

0.15%

Tension (gm)

2
1
0

30'  20'

Papillary Muscle

0.25%

Tension (gm)

2
1
0

20'  40'

20'  0.45%  60'

I--Methox--I

I--Methox--I

30 sec.

Fig. 1. Effects of methoxyflurane on the developed tensions of isolated guinea pig atria and isolated cat papillary muscle. Percentages are methoxyflurane concentrations.

trol period and during exposure to methoxyflurane; the rate and duration of square-wave pulses were kept constant during the period of high-voltage stimulation. The interaction of methoxyflurane with the increase in tension produced by tyramine was compared with the effect of tyramine in the absence of methoxyflurane. For this study tyramine was added in two cumulative doses to achieve concentrations of 0.15 and 0.30 μg/ml in the bath. After the addition of each dose the highest tension developed was measured.

Atria from guinea pigs, each killed by a blow on the head, were quickly dissected and suspended in an organ bath containing Locke-Ringer's solution at 29 C, oxygenated with a mixture of 95 per cent O₂ and 5 per cent CO₂ as described previously.¹⁹

After about 40 minutes a steady rate of beating was attained. Spontaneous rate, rhythm,
TABLE 2. Effect of Methoxyflurane on the Tension Developed by Isolated Cat Papillary Muscle during “Staircase” Stimulation (Mean ± SE)

<table>
<thead>
<tr>
<th>Methoxyflurane Concentration</th>
<th>Number of Preparations Tested</th>
<th>20 Beats/Min mg</th>
<th>60 Beats/Min mg</th>
<th>120 Beats/Min mg</th>
<th>mg</th>
<th>Per Cent*</th>
<th>mg</th>
<th>Per Cent*</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>9</td>
<td>1,046 ± 166</td>
<td>1,059 ± 168</td>
<td>1,200 ± 173</td>
<td>10.2 ± 6.3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.25 per cent</td>
<td>9</td>
<td>745 ± 119</td>
<td>782 ± 124</td>
<td>864 ± 139</td>
<td>19.0 ± 7.7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.45 per cent</td>
<td>9</td>
<td>461 ± 86</td>
<td>476 ± 91</td>
<td>509 ± 93</td>
<td>12.3 ± 5.5</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Per cent change in relation to the tension developed by stimulation at 30 beats/min.

Results

In nine experiments on isolated guinea pig atria exposed to methoxyflurane in concentrations of 0.15, 0.25, or 0.45 per cent, the rates of spontaneous beating were kept in the range of spontaneous variation. The data summarized in Table 1 show that methoxyflurane decreased the tensions developed by both isolated guinea pig atria and isolated papillary muscle. According to these data papillary muscle seems less sensitive to the depressant effect of methoxyflurane than isolated guinea

![Graph](image-url)
TABLE 3. Effect of Methoxyflurane, 0.45 per cent, on Postextrasystolic Potentiation in the Isolated Cat Papillary Muscle (13 Preparations) (Mean ± SE)

<table>
<thead>
<tr>
<th>Interval*</th>
<th>Pretreatment</th>
<th>Methoxyflurane</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Developed Tension (mg)</td>
<td>Increase (Per Cent)</td>
</tr>
<tr>
<td>Control†</td>
<td>973 ± 36</td>
<td>—</td>
</tr>
<tr>
<td>200 msec</td>
<td>2,138 ± 202</td>
<td>118 ± 17</td>
</tr>
<tr>
<td>250 msec</td>
<td>1,873 ± 159</td>
<td>100 ± 9</td>
</tr>
<tr>
<td>300 msec</td>
<td>1,704 ± 125</td>
<td>76 ± 7</td>
</tr>
<tr>
<td>350 msec</td>
<td>1,525 ± 144</td>
<td>57 ± 5</td>
</tr>
<tr>
<td>400 msec</td>
<td>1,405 ± 136</td>
<td>44 ± 3</td>
</tr>
</tbody>
</table>

* Interval between the premature stimulus and the regular preceding one (paired stimulation).
† Tension developed during regular stimulation.

pig atria. Examples of the effects of methoxyflurane on the tensions developed by both isolated heart preparations appear in figure 1.

STAIRCASE PHENOMENON

Papillary muscle preparations were stimulated rhythmically at a rate of 30 beats/min and then the frequency was increased to 60 beats/min. After about five minutes at 30 beats/min, the rate was increased to 120 beats/min (fig. 2). The results of these experiments (table 2) show that although methoxyflurane reduced the tension developed by each stimulation rate, the percentage increase elicited by the staircase phenomenon during exposure to methoxyflurane were not significantly different from controls.

POSTEXTRASYSTOLIC POTENTIATION

Postextrasystolic potentiation was studied at a rate of 60 beats/min by adding a premature stimulus after every regular beat (paired pulse). Since this potentiation is greater when the extra stimulus is close to the refractory period, the extra beats were set at intervals of 400, 350, 300, 250, and 200 msec after the regular contractions (fig. 3). The data (table 3) show that although methoxyflurane reduced the tensions developed during both regular and paired stimulation, the percentage increases during postextrasystolic potentiation were not affected significantly at the various intervals studied.

HIGH-VOLTAGE STIMULATION

In the papillary muscle preparation increasing the stimulating voltage to ten times the threshold value increases the developed tension.16,17 The data (table 4) show that the absolute tension developed (mg) by high-voltage stimulation during exposure to methoxyflurane was significantly lower than that observed during high-voltage stimulation in the absence of the anesthetic. However, the percentage increase seems to be higher in the presence of the greater concentration of me-

TABLE 4. Effect of Methoxyflurane on the Isotropic Effect of High-Voltage Stimulation of Isolated Cat Papillary Muscle (Mean ± SE)

<table>
<thead>
<tr>
<th>Methoxyflurane Concentration</th>
<th>Number of Preparations Tested</th>
<th>Developed tension</th>
<th>Change (Per Cent)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Control (mg)</td>
<td>High-Voltage* (mg)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1,015 ± 158</td>
<td>1,580 ± 242</td>
</tr>
<tr>
<td>0.25 per cent</td>
<td>9</td>
<td>736 ± 117</td>
<td>1,293 ± 223</td>
</tr>
<tr>
<td>0.45 per cent</td>
<td>9</td>
<td>453 ± 87</td>
<td>942 ± 197</td>
</tr>
</tbody>
</table>

* Ten times the threshold stimulus.
methoxyflurane than without methoxyflurane, but the difference was not significant \((P < 0.10)\).

**Effect of Tyramine**

The data in table 5 show that the absolute tensions developed (mg) by tyramine during exposure to methoxyflurane were significantly lower \((P < 0.02\) and \(< 0.005\) for doses of 0.15 and 0.30 \(\mu g/ml\), respectively) than control values. The relative increases (percentages) were not significantly different in the two conditions.

**Refractory Period**

The effective refractory period was determined by means of paired-pulse stimulation, reducing gradually the interval between the regular stimulus and the premature interpolated stimulus.

In 13 muscles the average control effective refractory period did not change significantly during methoxyflurane \((190 \pm 3\) msec vs. \(184 \pm 4\) msec; \(P > 0.2\)).

**Threshold of Electrical Stimulation**

In 13 muscles methoxyflurane in concentrations of 0.25 and 0.45 per cent (see Methods) did not change the threshold to electrical stimulation significantly. The control threshold stimulus was \(3.5 \pm 0.4\) volts; during exposure to methoxyflurane, 0.45 per cent, it was \(4.2 \pm 0.7\) volts.

The mean of the differences observed in all experiments was \(0.8 (\pm 0.4\) S.E.) volts \((P = 0.05)\).

**Discussion**

Methoxyflurane did not induce significant changes in the rate of spontaneous beating of isolated guinea pig atria at 29 C. We have not found in the literature any information about the effect of methoxyflurane on heart rate during hypothermia.

In spontaneously beating guinea pig atria methoxyflurane decreased the developed tension, a change which cannot be attributed to changes in frequency.

Isolated cat papillary muscle seems less sensitive to the depressant effect of methoxyflurane than isolated guinea pig atria. In a previous paper \(^{28}\) we reported a similar difference between the sensitivities of the two preparations to the depressant effect of halothane. It is possible that species differences, different experimental temperatures, or greater diffusion of the anesthetic through the thin wall of atrial myocardium compared with the ventricular papillary muscle could explain this difference in sensitivity.

Methoxyflurane did not change significantly the relative increases in developed tension (per cent) during the staircase phenomenon. It is pertinent to point out that Vazquez et al.\(^{29}\) observed that when the developed tension in the papillary muscle was spontaneously reduced to half the initial value after electrically induced beating for 24 hours no staircase phenomenon could be demonstrated, i.e., the tension did not change when frequency was increased. This means that the mechanism of reduction of the developed tension by methoxyflurane differs from the mechanism of the reduction observed after 24 hours of beating.

The effects of methoxyflurane on staircase and postextrasystolic potentiation phenomena were similar to those we found with halothane.\(^{25}\) It is important to point this out, since postextrasystolic potentiation has been used.
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Table 5. Effect of Methoxyflurane on the Positive Inotropic Effect of Tyramine in the Isolated Cat Papillary Muscle (12 Preparations) (Mean ± SE)

<table>
<thead>
<tr>
<th>Initial Tension</th>
<th>Tension Developed during Tyramine*</th>
</tr>
</thead>
<tbody>
<tr>
<td>(mg)</td>
<td>0.15 µg/ml</td>
</tr>
<tr>
<td></td>
<td>(mg)</td>
</tr>
<tr>
<td>Control</td>
<td>794 ± 104</td>
</tr>
<tr>
<td>Methoxyflurane,</td>
<td>487 ± 65</td>
</tr>
<tr>
<td>0.45 per cent</td>
<td></td>
</tr>
</tbody>
</table>

* Tyramine HCl added in two successive doses of 0.15 µg·ml each.

Experimentally to treat acute cardiac failure. The effect of methoxyflurane on postextrasystolic potentiation cannot be a mere consequence of the decrease of developed tension, since quinidine in a low dose, which does not change developed tension significantly, also reduced postextrasystolic potentiation. Furthermore, the effect of methoxyflurane cannot be attributed to modification of the effective refractory period, because it did not change significantly and because the results were similar after several fixed intervals.

The negative inotropic effect of methoxyflurane may be related to decreases in both intensity and duration of the active state, as has been suggested by Shimosato et al. Further studies of the effects of methoxyflurane on the active state during staircase and postextrasystolic potentiation phenomena are needed for the interpretation of our results.

Methoxyflurane did not change significantly the increase in tension induced by high-voltage stimulation in papillary muscle, in spite of the fact that it reduced developed tension to less than half. It is well known that the positive inotropic effect of high-voltage stimulation is partially due to the electrorelease of catecholamines at the adrenergic nerve endings. In some ways this finding is in agreement with results obtained by Shimosato et al. in the intact canine left ventricle, in which methoxyflurane decreased maximum force (F₀) with very little effect on maximum velocity of shortening (Vₘₐₓ) and superimposed cardiac sympathetic nerve stimulation increased maximum force towards control levels without changing Vₘₐₓ. This finding suggests responsiveness of beta-adrenergic receptors in the myocardium during methoxyflurane anesthesia.

The increase in tension induced in papillary muscle by tyramine was not reduced significantly by methoxyflurane. This fact would indicate that neither catecholamine stores nor sensitivity of adrenergic receptors are affected by the anesthetic. This effect is consistent with the findings here reported, showing that the inotropic effect of electrorelease by high-voltage stimulation is not blocked by methoxyflurane. In contradistinction to this we have reported that in the presence of halothane release of catecholamines by either procedure induced in papillary muscle a significantly higher percentage increase in tension than that seen in the control period. This difference could be related to the opposite effects of the two anesthetics on the duration of the active state as reflected by time to peak force, TTFₘₐₓ.

The lack of effect of methoxyflurane on the effective refractory period in papillary muscle cannot be extended to the atria. Methoxyflurane differs from halothane, which lengthened the effective refractory period in papillary muscle.

The authors thank Abbott Laboratories for supplying methoxyflurane (Penthrane).

References


Obstetrics and Pediatrics

MATERNAL HYPERCARBIA The effects of maternal hypercarbia on the clinical condition and biochemical status of the fetus were studied in 45 pregnant women at term. Maternal hypercarbia increased umbilical vein oxygen tension but did not significantly improve oxygen saturation. Higher one-minute Apgar scores and shorter times to sustained respiration were observed with higher carbon dioxide levels and could be explained by the stimulatory effect of carbon dioxide on initiation of respiration in the neonate. (Ivanovic, A. D., Elam, J. O., and Huffman, J.: Effect of Maternal Hypercarbia on the Newborn Infant, Amer. J. Obstet. Gynec. 107: 939 (Sept.) 1970.)