EFFECT OF CYCLOPROPANE ON CONTRACTILE PERFORMANCE OF CARDIAC MUSCLE WITH EXPERIMENTAL CHRONIC HEART FAILURE

Y. HASHIMOTO, O. KEMMOTSU AND S. SHIMOSATO

SUMMARY

The effect of cyclopropane on contractile performance of papillary muscles obtained from cats with congestive heart failure induced by pulmonary artery constriction was studied and compared with non-operated normal animals. A significant depression of contractile performance in the papillary muscles of cats with congestive heart failure was observed along with elevated right ventricular pressure and reduced cardiac index. In both groups of muscles, cyclopropane caused a shift of the force-velocity curve to the left and downward. At an equipotent anaesthetic concentration (MAC) of cyclopropane, reduction in contractile performance was similar in both muscles. When changes in velocity of shortening and maximal isometric force in muscles with congestive heart failure were compared with the control values of normal muscles at MAC, the depression was 49% and 44% respectively. However, the effect of cyclopropane on the diseased muscle was significantly less than that of either isoflurane or halothane.

The contractile performance of the failing heart has attracted the interest of investigators of both the intact heart and isolated heart muscle preparations. Spann et al. (1967) showed clearly that the basic contractile state of cardiac muscle was severely depressed in cats with experimentally produced congestive heart failure.

In our previous study (Shimosato, Shanks and Etsten, 1970), we reported that cyclopropane (C₃H₆) anaesthesia did not alter the contractile performance of the myocardium as determined by the force-velocity relationship in the intact normal dog. In contrast, Brown and Crout (1971) observed that C₃H₆ induced a negative inotropic response in isolated cat papillary muscle contracting isometrically. In the present study, congestive heart failure (CHF) was produced experimentally by pulmonary artery constriction in cats. The inotropic influence of C₃H₆ on isolated papillary muscles obtained from these cats was compared with that of normal cats. It has been shown, both in vitro and in the intact organism (Bing et al., 1971a; Goldberg, Sohn and Phear, 1972), that contractile performance of the heart muscle is depressed as the Po₂ is lowered. Changes in contractile performance with C₃H₆ administration might be partially due to the effect of lowered Po₂, especially for failing heart muscles. Therefore we administered nitrogen (N₂) in the same concentration as C₃H₆ to determine the effect of the decrease in Po₂ on the muscle, independent of C₃H₆.

MATERIALS AND METHODS

Adult cats of either sex, weighing 1.5-4.4 kg, were premedicated with sodium pentobarbitone (10 mg/kg body weight) injected into the peritoneum. Anaesthesia was induced with halothane (0.5-1.5%) in nitrous oxide-oxygen (50:50 vol.%), using a specially designed face mask. Endotracheal intubation was performed and respiration was controlled manually with an Ayre's T-piece. The left side of the chest was opened at the fifth intercostal space in order to achieve pulmonary artery constriction. The pericardium was opened, and a circular clip (3.0 mm i.d.) was placed around the proximal pulmonary artery under sterile conditions. Penicillin (500,000 units) and streptomycin (0.5 g) were given i.m. daily for the first 3 postoperative days. Not less than 4 weeks (28-34 days) later, the cats were anaesthetized with sodium pentobarbitone (30 mg/kg) and allowed to breathe spontaneously. Non-operated normal cats served as controls.

An 18-gauge catheter was inserted into the right ventricle and superior vena cava, via the right external jugular vein, and another catheter was placed in the ascending aorta via the right carotid artery. Aortic, right ventricular and central venous press...
sures were measured with a Statham model P23Db pressure transducer and were recorded on a Sanborn series 560 optical recorder. Heart rate was obtained from the e.c.g. (lead II) recordings. Cardiac output was measured in duplicate by the dye-dilution technique using indocyanine green.

After these measurements, the hearts were removed rapidly through a midline thoracotomy and immersed in oxygenated Krebs-Henseleit solution with 100 mg/100 ml of dextrose. One or two right ventricular papillary muscles were excised, mounted between two spring clips, and transferred immediately to muscle baths containing Krebs-Henseleit solution with 100 mg/100 ml of dextrose bubbled with 95% O₂/5% CO₂. The muscle baths were kept at a temperature of 32°C. After removal of the papillary muscle, the free right ventricular wall and the left ventricle with the septum were blotted and weighed. The isotonic lever system and isometric force transducer used in this laboratory have already been described in detail (Shimosato et al., 1973). Platinum field electrodes were placed parallel to the long axis of each muscle. Stimulation (Grass model S8C isolation unit) was introduced at a frequency of 0.2 Hz with a square wave of 5 msec duration at a voltage 15-20% above threshold. Each muscle was lightly preloaded (<0.4 g/mm²) and allowed to contract isotonically for 2 hours before the studies were performed. The contractile performance had reached a stable level by this time and continued for 5-6 hours. All experiments were performed during this stable period. At the end of each experiment, the muscle between the spring clips was weighed and the cross-sectional area was calculated by dividing the muscle mass by its specific gravity (1.05) and length, assuming a cylindrical model. Measurements of force of contraction and changes in muscle length, along with their derivatives obtained by R-C differentiating circuits and stimulation artefact, were recorded on a multi-channel Sanborn direct writing recorder (model 7700 series) at a high paper speed (100 mm/sec). A series of isotonic contractions was produced by increasing the load (afterload) in a stepwise manner until the muscle was unable to shorten and maximal isometric force (Fm) was reached. Force-velocity curves were obtained by plotting peak velocity of shortening against force. The velocity of shortening at 0.4 g/mm² (Vmax) was used as an approximate value of the maximal velocity of shortening to avoid the errors inherent in extrapolation to zero load. Time to maximal isometric force (TTFm) was measured from the stimulus artefact to maximal force. Velocity of shortening was expressed in units of muscle length per second (ML/sec), while force was expressed in grams per unit of cross-sectional area.

After control measurements, 10% or 20% C₃H₄ (v/v) in 95% O₂/5% CO₂ were administered from a Quantiflex rigid frame anaesthesia machine. The carbon dioxide was added in order to maintain a normal Pco₂ value. Both concentrations of C₃H₄ were administered for 30 min and this period was considered to be sufficient to attain a new steady state. Before each measurement, C₃H₄ concentrations (mg/100 ml) in the muscle bath were determined by gas chromatography. After obtaining further control measurements, the muscle was exposed to 10 or 20% N₂ plus 5% CO₂ in oxygen in a similar manner. Gas tensions and pH of the bathing solution were determined throughout the study with Radiometer O₂, CO₂ and pH electrodes.

All results have been expressed as mean values with the standard error of the mean unless stated otherwise. Student's t-test for paired and unpaired data was used for statistical analysis of the data. The levels of significance used were P<0.05 to P<0.02, probably significant; P<0.01, significant; P<0.001, highly significant.

RESULTS

Only papillary muscles of similar cross-sectional area were compared in order to eliminate changes in mechanics due to changes in muscle thickness. Thus, 10 papillary muscles excised from 9 animals with CHF were investigated and compared with 10 muscles excised from 8 normal cats. Table I summarizes the haemodynamic data under pentobarbitone anaesthesia (30 mg/kg body weight) and the physical characteristics of the hearts in both groups of animals. The average cardiac index in the CHF group was about 45% less than that in the control group (P<0.001). The mean right ventricular pressure, right ventricular end-diastolic pressure and central venous pressure were significantly elevated in the CHF animals (P<0.001, P<0.001, P<0.01), while heart rate and mean aortic pressure did not differ significantly. In the CHF group, right ventricular weight and the right ventricular weight/kg body weight ratio were increased to approximately twice the control group values (P<0.001), whereas left ventricular weight was not changed. These findings are similar to those reported by Gold and co-workers (1970).

The mean values for contractile performance in
both groups of papillary muscles before administration of $C_2H_4$ and $N_2$ are shown in Table II. $V_{o4}$, Fm and maximal first time derivative of isometric force (maximal dF/dt) were significantly lower in the muscles of cats with CHF ($P<0.001$, $P<0.01$, $P<0.001$). While TTFm appeared slightly longer in the CHF group, the difference was not statistically significant. Administration of either $C_2H_4$ or $N_2$ caused a dose-dependent depression in the contractile performance of both groups. There were no significant differences in the changes between groups except $V_{o4}$ and maximal dF/dt with 10% $C_2H_4$ (Table III). Although per cent changes in $V_{o4}$, Fm and maximal dF/dt from control were small (3.0–5.8%) during administration of 10% $N_2$, paired analysis revealed statistically significant differences. TTFm was decreased but the difference was not significant. Figure 1 illustrates force-velocity relations in both groups of papillary muscles before and after administration of $C_2H_4$ and $N_2$. The curves were shifted to the left and downwards with increasing concentration. There were definite correlations between the per cent changes in $V_{o4}$ and Fm and concentrations of either $C_2H_4$ or $N_2$ (fig. 2). These are valid only within the anaesthetic range studied. $N_2$ depressed myocardial performance directly in the range of concentrations studied. Therefore, the changes observed in myocardial performance produced by $C_2H_4$ were partly the result of a decrease in $P_{o4}$.

Averaged $P_{o4}$ values of the bathing solution were $578 \pm 3.5$, $516 \pm 2.9$ and $457 \pm 2.5$ mm Hg for control, 10% and 20% $C_2H_4$, and $583 \pm 3.3$, $521 \pm 4.4$ and $454 \pm 3.5$ mm Hg for control, 10% and 20% $N_2$. Averaged $P_{co2}$ and pH values were $39 \pm 0.2$ mm Hg and 7.395 $\pm$ 0.002 respectively. The mean $C_2H_4$ concentrations were $3.2 \pm 0.1$ and 6.3 $\pm$ 0.1 mg/100 ml for 10% and 20% $C_2H_4$.

### Table I. Haemodynamic data and physical characteristics (mean±SE).

<table>
<thead>
<tr>
<th></th>
<th>Normal</th>
<th>CHF</th>
<th>Significant difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (kg)</td>
<td>2.7±0.3</td>
<td>2.0±0.2</td>
<td>n.s.</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>209±11</td>
<td>205±6</td>
<td>n.s.</td>
</tr>
<tr>
<td>Mean aortic pressure (mm Hg)</td>
<td>148±11</td>
<td>149±6</td>
<td>n.s.</td>
</tr>
<tr>
<td>Mean right ventricular pressure (mm Hg)</td>
<td>12.1±0.6</td>
<td>26.7±2.1</td>
<td>P&lt;0.001</td>
</tr>
<tr>
<td>Right ventricular end-diastolic pressure (mm Hg)</td>
<td>0.1±0.6</td>
<td>6.0±0.6</td>
<td>P&lt;0.001</td>
</tr>
<tr>
<td>Central venous pressure (mm Hg)</td>
<td>0.1±0.3</td>
<td>3.6±1.0</td>
<td>P&lt;0.01</td>
</tr>
<tr>
<td>Cardiac index (ml/min/kg)</td>
<td>233±10</td>
<td>130±5</td>
<td>P&lt;0.001</td>
</tr>
<tr>
<td>Right ventricular weight (g)</td>
<td>1.68±0.18</td>
<td>2.95±0.24</td>
<td>P&lt;0.001</td>
</tr>
<tr>
<td>Left ventricular weight (g)</td>
<td>6.64±0.61</td>
<td>6.12±0.42</td>
<td>n.s.</td>
</tr>
<tr>
<td>Right ventricular weight/kg body weight</td>
<td>0.63±0.04</td>
<td>1.10±0.07</td>
<td>P&lt;0.001</td>
</tr>
<tr>
<td>Cross-sectional area of papillary muscle (mm²)</td>
<td>1.24±0.12</td>
<td>1.28±0.12</td>
<td>n.s.</td>
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</tbody>
</table>

CHF=congestive heart failure; n.s.=not significant.

### Table II. Control mean values (±SE) of contractile performance.

<table>
<thead>
<tr>
<th></th>
<th>Velocity of shortening at 0.4 g/mm² (ML/sec)</th>
<th>Maximal isometric force (g/mm²)</th>
<th>Maximal dF/dt (g/mm²/sec)</th>
<th>Time to maximal isometric force (msec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal (n=10)</td>
<td>1.11±0.12</td>
<td>3.9±0.4</td>
<td>20.3±3.3</td>
<td>276±8</td>
</tr>
<tr>
<td>CHF (n=10)</td>
<td>0.65±0.06</td>
<td>2.6±0.3</td>
<td>11.0±1.4</td>
<td>306±18</td>
</tr>
</tbody>
</table>

Significant difference P<0.001, P<0.01, P<0.001, P<0.01, P<0.001, n.s.

CHF=congestive heart failure; n=number of papillary muscles; n.s.=not significant.

### Table III. Effects of cyclopropane and $N_2$ on contractile performance of papillary muscles (values are mean per cent depressions from control±SE).

<table>
<thead>
<tr>
<th></th>
<th>$C_2H_4$</th>
<th>$N_2$</th>
<th>$C_2H_4$</th>
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<th>$C_2H_4$</th>
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<th>$C_2H_4$</th>
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<tr>
<td>Normal (n=10)</td>
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<tr>
<td>10%</td>
<td>15.9±0.9</td>
<td>3.3±0.7</td>
<td>21.4±1.8</td>
<td>3.6±0.6</td>
<td>20.7±1.8</td>
<td>3.0±0.7</td>
<td>2.5±0.8</td>
<td>0.4±0.8</td>
</tr>
<tr>
<td>20%</td>
<td>28.4±1.3</td>
<td>7.5±1.1</td>
<td>34.4±2.8</td>
<td>9.5±1.4</td>
<td>35.5±2.5</td>
<td>9.2±1.7</td>
<td>5.5±1.4</td>
<td>1.5±1.0</td>
</tr>
<tr>
<td>CHF (n=10)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>10%</td>
<td>19.8±1.5</td>
<td>3.8±0.9</td>
<td>22.0±2.4</td>
<td>3.2±0.8</td>
<td>28.6±2.8</td>
<td>5.8±1.5</td>
<td>1.1±0.7</td>
<td>2.1±1.0</td>
</tr>
<tr>
<td>20%</td>
<td>33.5±2.4</td>
<td>8.9±1.4</td>
<td>34.7±3.4</td>
<td>9.5±2.2</td>
<td>42.9±3.1</td>
<td>9.8±1.7</td>
<td>4.0±1.0</td>
<td>2.7±1.5</td>
</tr>
</tbody>
</table>

CHF=congestive heart failure; n=number of papillary muscles; * significant difference (P<0.05) from the corresponding value for normal group.
CONTRACTILE PERFORMANCE OF CARDIAC MUSCLE

Fig. 1. Representative force-velocity relations in isolated papillary muscles from normal cats and those with CHF before and after administration of 10 and 20% \( \text{C}_3\text{H}_6 \) and \( \text{N}_2 \). Note the parallel shift in the force-velocity curves.

**CYCLOPROPANE and \( \text{N}_2 \) CONCENTRATION (%)**

Fig. 2. Effect of \( \text{C}_3\text{H}_6 \) and \( \text{N}_2 \) on velocity of shortening at 0.4 g/mm\(^2\) (\( V_{0.4} \)) and maximal isometric force (\( F_m \)). The regression lines compare per cent depressions in \( V_{0.4} \) (left panel) and \( F_m \) (right panel) versus different concentrations of \( \text{C}_3\text{H}_6 \) and \( \text{N}_2 \). All regression lines showed good correlation (\( r \) values were greater than 0.743, \( P<0.001 \)). Heavy lines represent the actual effect of \( \text{C}_3\text{H}_6 \) (measured values of \( \text{C}_3\text{H}_6 \), minus values of \( \text{N}_2 \)). Regression equation of \( V_{0.4} \) normal: \( y=-1.1x-0.6(r=-0.957, P<0.001) \); CHF: \( y=-1.2x-0.9(r=-0.940, P<0.001) \). Regression equation of \( F_m \) normal: \( y=-1.3x-2.0(r=-0.906, P<0.001) \); CHF: \( y=-1.2x-1.5(r=-0.847, P<0.001) \). Comparison of corresponding lines showed no significant difference.
DISCUSSION

In the present study, a significant depression of contractile performance in the papillary muscles of cats with CHF was observed together with an elevated right ventricular pressure and a reduction in cardiac index. In some recent studies on intact hearts with CHF, it was found that myocardial contractility, as reflected by the force-velocity curve and the length-active tension curve, were clearly depressed below normal. However, cardiac output and stroke volume, which represent the pumping action of the whole heart, were maintained at close to normal levels (Hugenholtz et al., 1970; Spann et al., 1972). The performance of the pump is maintained by compensatory mechanisms such as an increase in muscle mass (Meerson, 1965), the Frank-Starling mechanism (Spann et al., 1972) and augmented sympathetic stimulation (Chidsey, Braunwald and Morrow, 1965). On the other hand, the severity of depression in the contractile state appears to be related to the severity of the stress (Spann et al., 1967). Spann and co-workers (1972) suggested that at some point in a reduced contractile state, perhaps after a decrease in catecholamine stores in the cardiac muscle, circulatory compensation can no longer be maintained and cardiac output falls with the clinical manifestation of overt congestive failure.

Prasad and Callaghan (1969) estimated that cat papillary muscle with a diameter of less than 0.93 mm can be adequately supplied with O\(_2\), by simple diffusion, in an atmosphere of 95% O\(_2\) at 37°C and at high stimulation frequencies of 1 Hz. The cross-sectional area of the muscles used in the present experiments varied from 0.56 to 1.66 mm\(^2\) (diameter 0.84 to 1.45 mm). Because our muscle was relatively thick, we used a bath temperature of 32°C in order to improve the oxygenation of the central core of the muscle. When changes in mechanical performance in the CHF group exposed to N\(_2\) were compared with those of the normal group, no difference between the groups was observed (fig. 2). Similar findings have been reported in the rat heart muscle (Bing et al., 1971b). Since it has been reported in vitro and in vivo that an unusual alteration of mechanical properties of heart muscle was observed during recovery from acute hypoxia (Bing et al., 1971a; Tyberg, Parmley and Sonnenblick, 1969), muscles were not exposed to any higher concentrations of the C\(_3\)H\(_8\) and N\(_2\) in this study.

The present study showed that C\(_3\)H\(_8\) induced a direct negative inotropic effect on isolated papillary muscles of normal cats and those with CHF. These changes were dose-related and reversible within the anaesthetic range studied. Although TTFm did not show any apparent change, the mechanical behaviour of papillary muscle under C\(_3\)H\(_8\) anaesthesia could be considered qualitatively similar to other anaesthetics (Sugai, Shimosato and Etsten, 1968; Shimosato, Sugai and Etsten, 1969; Shimosato et al., 1969; Iwatsuki and Shimosato, 1971). These findings were in general agreement with those of Brown and Crout (1971) in comparative studies of anaesthetics on cat heart muscle. In addition, Brown and Crout (1971) suggested that the relative potency of anaesthetics in the mammalian heart may be similar from species to species. When 9.2% C\(_3\)H\(_8\) (minimum alveolar concentration (MAC) in man (Saidman et al., 1967)) was used as a point on our linear regression lines the decreases in V\(_{0.4}\) and Fm were 10.8% and 14.0% in the normal group, 12.3% and 12.5% in the CHF group, respectively (fig. 3). When changes in V\(_{0.4}\) and Fm in muscles with CHF were compared with the control values of normal muscles at MAC, depressions were 48.9% and 43.7% respectively. These effects of C\(_3\)H\(_8\) and the pathological state of muscle in combination were less than those of isoflurane (Forane) (Kemmotsu, Hashimoto and Shimosato, 1973) and halothane (Shimosato et al., 1973) (fig. 4).

It should be pointed out that the frequency of contraction (0.2 Hz), the bath temperature of 32°C, and the provision of substrate and oxygen by diffusion are among the factors which limit the application of these data to the intact ventricle. However, Spann and co-workers (1972) recently compared the contractile state of myocardium, both in the intact ventricles and the isolated papillary muscles.
removed from these ventricles, in cats with CHF and normal heart muscle. They reported that the marked depressions of the contractile state in the intact heart and the isolated muscle were qualitatively similar. Therefore, the results of this study and our previous experiment (Shimosato, Shanks and Etsten, 1970) suggest that C₃H₆ produces less influence on the contractile performance than that of isoflurane or halothane in either normal hearts or those with chronic congestive failure.

ACKNOWLEDGEMENTS

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REFERENCES


**EFFETS EXERCES PAR LE CYCLOPROPANE SUR LE POUVOIR DE CONTRACTION DU MYOCARDE EN PRESENCE D'UNE INSUFFISANCE CARDIAQUE CHRONIQUE EXPERIMENTALE**

**SOMMAIRE**

Les effets exercés par le cyclopropane sur le pouvoir contractile des muscles papillaires obtenus à partir de chats atteints d'insuffisance cardiaque congestive engendrée par une ligature de l'artère pulmonaire, ont été étudiés et comparés aux données enregistrées chez des animaux normaux non opérés. Une dépression significative du pouvoir contractile des muscles papillaires provenant de chats atteints d'insuffisance cardiaque congestive, a été observée parallèlement à une augmentation de la pression interventriculaire droite et une réduction de l'indice cardiaque. Le cyclopropane a engendré au niveau des deux groupes de muscles, un déplacement de la courbe force/vitesse vers la gauche et en bas. Pour une concentration anesthésique équiptopente (MAC) de cyclopropane, la réduction du pouvoir contractile s'est avérée être similaire au niveau des deux types de muscles. Lorsque l'on a procédé à la comparaison des changements intervenus quant à la vitesse de la force de contraction et de la force isométrique maximale propre aux muscles, en présence d'une insuffisance cardiaque congestive, avec les données de contrôle obtenues à partir de muscles normaux et pour des concentrations anesthésiques équipotentes (MAC), la dépression enregistrée a été respectivement de 49 et 44%. Néanmoins, les effets combinés du cyclopropane et de l'altération musculaire pathologique ont été significativement moindres avec l'isoflurane et l'halothane.

**ZUSAMMENFASSUNG**


**RESUMEN**

En gatos con descompensación cardíaca congestiva, inducida por constricción de la arteria pulmonar, se estudió el efecto del ciclopropano sobre la función contráctil de los músculos papilares, comparando los resultados con los que se aprecian en animales normales no operados. Se observó una depresión importante de la función contráctil en los músculos papilares de gatos con descompensación cardíaca congestiva, juntamente con una elevación de la presión ventricular derecha y reducción de índice cardíaco. En ambos grupos de músculos, el ciclopropano producía una desviación de la curva potencia-velocidad hacia la izquierda y abajo. Para una concentración equipotente de ciclopropano (MAC), la reducción de la capacidad contráctil era similar en ambos músculos. Cuando se comparaban los cambios de la velocidad de acortamiento y fuerza máxima isométrica en los músculos con descompensación cardíaca congestiva, con los valores de control en los músculos normales para MAC, la depresión fue 49 y 44% respectivamente. Sin embargo, el efecto combinado del ciclopropano y el estado patológico del músculo era significativamente menor que el del isoflurano y halotano.