Effects of Calcium and Sodium on Cardiac Contractility and Heat Production in Rabbit Papillary Muscle

By J. B. Chapman, C. L. Gibbs, and W. R. Gibson

ABSTRACT

The effects of altering the concentrations of sodium (Na+) and calcium (Ca2+) ions on the isometric energy output of rabbit papillary muscle at room temperature were examined by a myothermic technique. The well-known inotropic changes resulting from these alterations were not correlated with parallel changes in the magnitude of the tension-independent heat. Instead, the magnitude of the tension-independent heat was directly correlated with both increasing Na+ concentration and with increasing Ca2+ concentration, and these effects were additive. The energetics of ions pumping in cardiac muscle are discussed quantitatively and it is suggested that the combined enthalpy consumption of both the Na+ and the Ca2+ pumps constitutes the major determinant of cardiac tension-independent heat production.

ADDITIONAL KEY WORDS

sodium-calcium antagonism
active transport energetics
myothermic technique peak tension
tension-independent heat

The myothermic technique has yielded much information about the energetics of cardiac muscle beating normally and under the influence of inotropic agents. The initial study of cardiac energetics by Gibbs, et al. (1) has recently been extended by Gibbs and Gibson (unpublished observations) while the effects of calcium, temperature, epinephrine, ouabain, and heart rate on cardiac heat production have all been established (2-5). In this paper these basic investigations are completed by studying the effects of variations of the extracellular [Ca2+]/[Na+]2 on cardiac heat production and their relation to the well-known effects on myocardial contractility (6-8).

Methods

The mechanical and myothermic methods and the experimental procedures for obtaining isometric heat-tension curves have been described in the previous paper (5). The rabbit papillary muscles used had a mean weight of 5.7 mg and a mean length of 7.7 mm under a resting load of 1.0 g. Their mean cross-sectional area was .73 mm² and ranged from .35 to 1.17 mm². The experiments were conducted at temperatures ranging from 20.2 to 22.0°C. The stimulus rate was ¾ seconds in all experiments. The average heat loss was 16.5% sec⁻¹.

Five solutions that differed in their content of sodium and calcium ions (see Table 1) were used. After dissection, the preparations were allowed to contract isotonically in normal Tyrode's solution until contractility stabilized. This usually took 1 to 2 hours. Each of the 10 muscles was equilibrated for 30 minutes in any given solution before heat and mechanical measurements were attempted. Note that solutions A, B, D, E constitute a factorial arrangement of treatments (chap. 11, ref. 9); i.e., there are two levels of calcium concentration (factor 1) associated with each of two levels of sodium concentration (factor 2). Also note that the [Ca2+]/[Na+]2 for both treatments A and D is 1, and for both treatments B and C, is 4.

The muscles were placed in the different solutions in a planned order, so that there would be no confounding of the effects of the solutions with effects due to temporal changes in the properties of the muscle preparations, and so that the likelihood of carry-over effects of the solutions affecting estimates of treatment effects would be minimized. The experimental material was arranged in two 5 × 5 latin squares, each having five columns (i.e., muscle preparations) and five
TABLE 1

Composition of Solutions

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Solution A</th>
<th>Solution B</th>
<th>Solution C</th>
<th>Solution D</th>
<th>Solution E</th>
</tr>
</thead>
<tbody>
<tr>
<td>[Ca^{2+}]/[Na^{+}]²</td>
<td>Normal Tyrode</td>
<td>1</td>
<td>4</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>NaCl</td>
<td>154.00</td>
<td>71.00</td>
<td>154.00</td>
<td>71.00</td>
<td>154.00</td>
</tr>
<tr>
<td>KCl</td>
<td>2.68</td>
<td>2.68</td>
<td>2.68</td>
<td>2.68</td>
<td>2.68</td>
</tr>
<tr>
<td>CaCl₂</td>
<td>1.80</td>
<td>1.80</td>
<td>7.20</td>
<td>0.45</td>
<td>0.45</td>
</tr>
<tr>
<td>MgCl₂</td>
<td>1.05</td>
<td>1.05</td>
<td>1.05</td>
<td>1.05</td>
<td>1.05</td>
</tr>
<tr>
<td>NaHCO₃</td>
<td>11.90</td>
<td>11.90</td>
<td>11.90</td>
<td>11.90</td>
<td>11.90</td>
</tr>
<tr>
<td>NaH₂PO₄</td>
<td>0.40</td>
<td>0.40</td>
<td>0.40</td>
<td>0.40</td>
<td>0.40</td>
</tr>
<tr>
<td>Glucose</td>
<td>5.56</td>
<td>5.56</td>
<td>5.56</td>
<td>5.56</td>
<td>5.56</td>
</tr>
<tr>
<td>Sucrose</td>
<td>0</td>
<td>154.00</td>
<td>0</td>
<td>154.00</td>
<td>0</td>
</tr>
</tbody>
</table>

All values are expressed in millimoles per liter; all solutions were aerated with 95% O₂:5% CO₂.

Results

INOTROPIC EFFECTS

Table 2 shows the means and analysis of variance for the effects of the various treatments on the mean peak tension generated in a block of 16 contractions at l₀, the resting length under a load of 1 g. Reduction of the Na⁺ concentration from normal to ½ normal levels increased the mean peak tension by an average of 29% (P<0.001), whereas reduction of the Ca²⁺ concentration from normal to ½ normal levels reduced the tension by 39% (P<0.001). These effects of different [Ca²⁺] and [Na⁺] levels were additive (i.e., [Ca²⁺] × [Na⁺] interaction was not significant).

Solutions B and C, which both had [Ca²⁺]/[Na⁺]² of 4 × normal, both produced similar increases in peak tension. The apparent tendency for solution C to produce higher tensions was not significant (0.1 > P > 0.05). However, muscles developed 22% less tension in solution D than in solution A (P < 0.01), although both solutions had the same [Ca²⁺]/[Na⁺]².

EFFECTS ON THE HEAT-TENSION RELATIONSHIP

Table 3 shows the means and analysis of variance for the effects of the various treatments on the intercept (tension-independent heat), slope and curvature of the heat-tension relationship for the 10 muscles.

Tension-Independent Heat.—Tension-independent heat production showed significant differences between preparations (P<0.001) and within preparations during the course of an experiment (P<0.05). Reduction of the Na⁺ concentration from normal to ½ normal levels decreased the tension-independent heat by an average of 9% (P<0.05), whereas reduction of the Ca²⁺ concentration from normal to ½ normal levels reduced the tension-independent heat by 22% (P<0.001). These effects of different Ca²⁺ and Na⁺ levels were additive (i.e., [Ca²⁺] × [Na⁺] interaction was not significant).
TABLE 2

Peak Tension (g · cm⁻²) Produced during Isometric Contractions in Solutions Containing Different Concentrations of Calcium and Sodium Ions

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Solution</th>
<th>Mean peak tension at 1 sec for 10 muscles (g · cm⁻²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>[Ca²⁺]/[Na⁺]² (× normal)</td>
<td>A Normal</td>
<td>400</td>
</tr>
<tr>
<td></td>
<td>B ½ Na⁺</td>
<td>508</td>
</tr>
<tr>
<td></td>
<td>C 4 Ca²⁺</td>
<td>553</td>
</tr>
<tr>
<td></td>
<td>D ½ Na⁺ · ¼ Ca²⁺</td>
<td>313</td>
</tr>
<tr>
<td></td>
<td>E ½ Ca²⁺</td>
<td>238</td>
</tr>
</tbody>
</table>

Analysis of Variance

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>DF</th>
<th>MS (172,062)</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Muscle preparation</td>
<td>9</td>
<td>89,752</td>
<td>35.85*</td>
</tr>
<tr>
<td>Period of day (rows)</td>
<td>4</td>
<td>4,017</td>
<td>1.60</td>
</tr>
<tr>
<td>Solutions</td>
<td>4</td>
<td>(172,062)</td>
<td></td>
</tr>
<tr>
<td>A, B vs. D, E (normal Ca²⁺ vs. ½ Ca²⁺)</td>
<td>1</td>
<td>318,286</td>
<td>127.11*</td>
</tr>
<tr>
<td>A, E vs. B, D (normal Na⁺ vs. ½ Na⁺)</td>
<td>1</td>
<td>83,723</td>
<td>33.44†</td>
</tr>
<tr>
<td>Ca²⁺ × Na⁺ interaction</td>
<td>1</td>
<td>2,756</td>
<td>1.10</td>
</tr>
<tr>
<td>B vs. C</td>
<td>1</td>
<td>10,125</td>
<td>4.04</td>
</tr>
<tr>
<td>D vs. A</td>
<td>1</td>
<td>37,758</td>
<td>15.08‡</td>
</tr>
<tr>
<td>Residual (error)</td>
<td>32</td>
<td>2,504</td>
<td></td>
</tr>
</tbody>
</table>

DF = degrees of freedom; MS = mean squares.

*P < 0.001; †P < 0.01.

Solution C gave values of tension-independent heat 24% higher than did solution B (P < 0.001), each solution having a [Ca²⁺]/[Na⁺]² of 4 × normal, and solution A gave values 42% higher than did solution D (P < 0.001), each of these latter solutions having the normal [Ca²⁺]/[Na⁺]². Sample records of tension-independent heat production obtained from one of the muscles in the five solutions are shown in Figure 1.

Effects on Slope and Curvature of Polynomials.—Muscle preparations differed in the slope (P < 0.05) and curvature (P < 0.01) of the heat-tension relationship, but there were no temporal changes in these parameters. Changes in both the Ca²⁺ and Na⁺ concentrations had no detectable effect on curvature, but lowering the Ca²⁺ concentration from normal to ½ normal levels increased the slope by 40% (P < 0.01). The five "averaged polynomials" are plotted over a low range of tensions in Figure 2.

Discussion

INOTROPIC EFFECTS

The present results show the expected effects of sodium and calcium ions on myocardial contractility and they are in broad agreement with previous studies for the amphibian heart (6, 7) and those for the mammalian heart (8). The preparations in the present work were relatively more sensitive to changes in Ca²⁺ concentration than to changes in Na⁺ concentration, so that the proportionality between contractile force and external [Ca²⁺]/[Na⁺]² described by Lüttgau and Niedergerke (7) did not hold exactly. However, the work of Teiger and Farah (8) showed that the approximation to this proportionality for rabbit heart muscle depended on the stimulus rate, the closest approximation being attained at low rates of 0.1 to 0.2/sec at 37.5°C (see Table 3, ref. 8). Thus the absence of an exact proportionality between the contractile force and external [Ca²⁺]/[Na⁺]² in the present work may be related to the stimulus frequency (0.25/sec) and to the temperature (20 to 22°C) used.

EFFECTS ON THE HEAT-TENSION RELATIONSHIP

Effects on Slope and Curvature of Polynomials.—In two previous studies we have...
### TABLE 3

Averaged Polynomials Describing the Heat-Tension Relationship during Isometric Contractions of Muscles Bathed in Solutions Containing Different Concentrations of Calcium and Sodium Ions

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Normal</th>
<th>h Na⁺</th>
<th>h Ca²⁺</th>
<th>%Na⁺; %Ca²⁺</th>
<th>%Ca²⁺</th>
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<tbody>
<tr>
<td>[Ca²⁺/Na⁺]²</td>
<td>1</td>
<td>4</td>
<td>4</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Averaged polynomial*</td>
<td>h = 0.6715</td>
<td>h = 0.6271</td>
<td>h = 0.7762</td>
<td>h = 0.4722</td>
<td>h = 0.5348</td>
</tr>
<tr>
<td></td>
<td>+0.002878T</td>
<td>+ 0.00347T</td>
<td>+0.003241T</td>
<td>+0.004018T</td>
<td>+0.004865T</td>
</tr>
<tr>
<td></td>
<td>+0.0001254T²</td>
<td>+0.0000667T²</td>
<td>+0.0000621T²</td>
<td>+0.00000786T²</td>
<td>+0.00001026T²</td>
</tr>
</tbody>
</table>

**Sources of variation**

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>DF</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
</tr>
</thead>
<tbody>
<tr>
<td>Muscle preparation (columns)</td>
<td>9</td>
<td>46,809</td>
<td>7.06†</td>
<td>4,257</td>
<td>2.68‡</td>
<td>24,486</td>
</tr>
<tr>
<td>Period of day (rows)</td>
<td>4</td>
<td>21,837</td>
<td>3.29†</td>
<td>2,235</td>
<td>1.41‡</td>
<td>12,503</td>
</tr>
<tr>
<td>Solutions</td>
<td>4</td>
<td>(140,340)</td>
<td>21.15†</td>
<td>(5,995)</td>
<td>3.77</td>
<td>(7,051)</td>
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<tr>
<td>A,B vs. D,E (normal Ca²⁺ vs. %Ca²⁺)</td>
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<td>212,504</td>
<td>32.03‡</td>
<td>16,067</td>
<td>10.12§</td>
<td></td>
</tr>
<tr>
<td>A,E vs. B,D (normal Na⁺ vs. %Na⁺)</td>
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<td>28,649</td>
<td>4.32</td>
<td>162</td>
<td>&lt; 1</td>
<td></td>
</tr>
<tr>
<td>Ca²⁺ × Na⁺ interaction</td>
<td>1</td>
<td>838</td>
<td>&lt; 1</td>
<td>5,181</td>
<td>3.26</td>
<td></td>
</tr>
<tr>
<td>B vs. C</td>
<td>1</td>
<td>111,154</td>
<td>16.75‡</td>
<td>262</td>
<td>&lt; 1</td>
<td></td>
</tr>
<tr>
<td>D vs. A</td>
<td>1</td>
<td>196,502</td>
<td>29.94</td>
<td>6,498</td>
<td>4.09</td>
<td></td>
</tr>
<tr>
<td>Residual (error)</td>
<td>32</td>
<td>6,634</td>
<td>1,588</td>
<td>5,500</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Analysis of Variance**

<table>
<thead>
<tr>
<th>Coefficient</th>
<th>Intercept</th>
<th>Slope</th>
<th>Curvature</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MS × 10⁶</td>
<td>F</td>
<td>MS × 10⁹</td>
</tr>
<tr>
<td>Muscle preparation (columns)</td>
<td>46,809</td>
<td>7.06†</td>
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</tr>
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</tr>
<tr>
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<td>196,502</td>
<td>29.94</td>
<td>6,498</td>
</tr>
<tr>
<td>Residual (error)</td>
<td>6,634</td>
<td>1,588</td>
<td>5,500</td>
</tr>
</tbody>
</table>

*Each coefficient is the mean of values obtained for the 10 muscles; †P < 0.001; ‡P < 0.05; §P < 0.01.

Abbreviations as in Table 2.
CALCIUM, SODIUM AND CARDIAC HEAT PRODUCTION

FIGURE 1
Tension-independent heat production in: (a) normal; (b) % Ca\(^{2+}\): % Na\(^{+}\); (c) % Ca\(^{2+}\); (d) 4 Ca\(^{2+}\); and (e) % Na\(^{+}\), Tyrode's solution. The muscle weighed 7.2 mg. Time calibration (horizontal bar) = 10 seconds; Heat calibration (vertical bar) = 5.60 mcal/g muscle. The broken lines in the bottom half indicate the tension-independent heat produced in normal Tyrode's solution (i.e., same level as reached in a).

been unable to detect any statistically significant slope changes in the heat versus tension relationship when papillary muscles had their contractility altered by changes in heart rate (5) or by the action of ouabain (4). In the present study only the low Ca\(^{2+}\) solution, E, had any significant effect on slope. Low Ca\(^{2+}\) appears to have made the muscles "less efficient" in that it is energetically more costly to generate a given level of tension. At present we have no explanation for this effect but heat versus tension-integral plots, which were not made in this series of experiments, may help to elucidate the phenomenon.

Tension-Independent Heat.—The present work contains the first demonstration of a definite lack of correlation between inotropic changes and changes in the tension-independent heat production of cardiac muscle, thereby strengthening an assumption that has existed since the first study by Gibbs et al. (1), namely that the tension-independent heat is associated with processes underlying the activation mechanism and is largely uncontaminated by activity of the myofilaments (12). We are not denying, however, that some of the tension-independent heat may be due to the degraded internal work done by the contractile proteins against the z lines or against the encapsulation of the muscle epicardium. Our point is that such an effect must be small, probably less than 0.1 mcal/g muscle, as in muscles being shortened down to the same length to eliminate external tension development, heat production is not correlated with the intrinsic cardiac contractility.

It has been shown elsewhere (13) that the activation heat of skeletal muscle as defined by Hill (14) can probably be identified almost entirely with the enthalpy of hydrolysis of adenosine triphosphate (ATP) by the sarcocytotic calcium pump. However, the present results indicate that this cannot be the sole identity of the cardiac tension-independent heat. According to current theories of excitation-contraction coupling in cardiac muscle (15, 16), the inotropic changes reported in this paper would have been associated with changes in the amount of Ca\(^{2+}\) reaching and

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being transported from the myofilaments. That these changes were not correlated in all cases with parallel changes in the tension-independent heat suggests either that the transport of Ca\(^{2+}\) in cardiac muscle is not an appreciable determinant of tension-independent heat at all, or that some other process is also an important determinant, and under the conditions peculiar to the present experiments, it was altered in such a way as to mask the changes in the enthalpy expended by the Ca\(^{2+}\) pump.

Because of the long duration of the cardiac action potential and the small surface-to-volume ratio of cardiac muscle fibers by comparison with skeletal muscle fibers, it is necessary to consider the energy cost of electrical excitation as a significant determinant of cardiac tension-independent heat production. Langer (17) has estimated the Na\(^{+}\) flux associated with each stimulus to be 6.6 \times 10^{-8} \text{ mole per gram of muscle for dog ventricle at 23 to 24°C}. This estimate is based on tracer flux experiments and is close to the value computed by Noble (18) for Purkinje fibers. Only about one-sixth of this flux is necessary to discharge the membrane capacitance during the action potential and so the remainder of the Na\(^{+}\) flux is electrically balanced by K\(^{+}\) or Cl\(^{-}\) movements. Langer and Brady (19) detected no change in K\(^{+}\) flux above or below the resting level that could be associated with excitation. Other workers have produced widely different estimates of the K\(^{+}\) efflux associated with excitation (see review by Langer [16]) but the general pattern of their results suggests that whatever net K\(^{+}\) efflux occurs above the resting level as a result of excitation, it is considerably smaller than the K\(^{+}\) flux necessary to repolarize the membrane at the end of the action potential. This can only mean that the K\(^{+}\) efflux falls below resting levels during the plateau of the action potential, a result which agrees with electrophysiological conductance measurements demonstrating a fall in membrane potassium conductance (g_k) during the plateau (18, 20, 21). Thus the principal ionic movement that electrically neutralizes the excess Na\(^{+}\) influx must be inward movement of chloride.

Now the free energy changes associated with ion movements during the action potential (\(\sum \Delta G\), total change in free energy due to movements of all ion species) are balanced thermodynamically by changes in entropy (\(\sum \Delta S\)) such that the total enthalpy change (\(\sum \Delta H\)) of excitation is zero in the first approximation. The electrical work dissipated by local circuit currents \(W = \sum j_i^2 R \cdot dt\) is balanced by an absorption of heat (Q) such that \(\Delta H = Q + W = 0\), i.e., the excitatory event is thermally neutral insofar as ion movements are concerned. (See also Hodgkin [22].)

However, to conserve a steady state across the cell membranes, electrochemical work must be done to reverse the flow of ions that occurs during the action potential. Almost all of this work is expended on Na\(^{+}\) pumping because the small amount of K\(^{+}\) per excitation that might have to be pumped back into the cell will be pumped against a resting electrochemical gradient of approximately zero compared with the gradient for Na\(^{+}\) pumping; also the chloride ions will be distributed passively.

The electrochemical work per mole of Na\(^{+}\) extruded is given by:

\[
W = RT \cdot \ln \left( \frac{[Na^+]_0}{[Na^+]_e} \right) + zF \cdot E_r,
\]

where
\[
R = \text{gas constant} = 8.31 \text{ J/mole/°K},
\]
\[
T = \text{absolute temperature} = 296°K,
\]
\[
[Na^+]_0/[Na^+]_e = 5 (16),
\]
\[
z = \text{valence} = 1 \text{ g equiv/mole},
\]
\[
F = \text{faraday} = 9.65 \times 10^4 \text{ coul/g equiv},
\]
\[
E_r = \text{resting membrane potential} = 90 \text{ mV}.
\]

Thus\( W = 3.02 \times 10^3 \text{ cal/mole}.\) If the Na\(^{+}\) flux is \(6.6 \times 10^{-8} \text{ mole per gram of muscle per beat}\) (17), then the amount of electrochemical work performed by the Na\(^{+}\) pump per beat in maintaining a steady state is approximately 0.2 mcal/g.

This figure can possibly be regarded as an upper limit, as the process of exchange diffusion may contribute to some extent to the total efflux value for Na\(^{+}\) as estimated from tracer studies. Exchange diffusion is an energy-independent process and if it constituted a significant portion of the total flux,
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then the calculated energy requirement would have to be reduced accordingly.

Langer (16) has discussed the energy requirement for calcium pumping in heart muscle using the following information: The work of Weber et al. (23, 24) on both skeletal and cardiac muscle indicates that about $10^{-8}$ mole of Ca$^{2+}$ must be removed from 1 g Ca$^{2+}$-saturated myofibrillar protein to achieve relaxation; cardiac muscle contains about 0.05 g of actomyosin/g of muscle tissue (25, 26); about 5 kcal of energy is required to transport 1 mole of Ca$^{2+}$ to the sarcoplasmic reticulum, assuming the efficiency of the calcium pump to be 40 to 50% (23, 27). This means that for every maximal contraction $5 \times 10^{-8}$ mole of Ca$^{2+}$ per gram of muscle would have to be removed from the Ca$^{2+}$-saturated contractile protein by the sarcoreticular Ca$^{2+}$ pump which would therefore consume energy at the rate of 0.25 mcal per gram per beat at a pumping efficiency of 40 to 50%. The word “efficiency” in this context is not a true thermodynamic efficiency because the true extent of the electrochemical work of Ca$^{2+}$ pumping and the correct value of the free energy of ATP hydrolysis in vivo are not known. However, on the basis of a Ca$^{2+}$/ATP of two for the Ca$^{2+}$ pump (28) and a value of 11 kcal/mole for the enthalpy of hydrolysis of ATP in vivo (29), one can deduce that the ΔH of ATP hydrolysis due to the Ca$^{2+}$ pump will be $11 \times 10^8$ cal/mole $\times 0.5 \times 5 \times 10^{-8}$ mole/g, i.e., about 0.25 mcal/g, and all this enthalpy will appear as part of the tension-independent heat.

The remainder of the tension-independent heat will consist mainly of the ΔH of ATP hydrolysis due to activity of the Na$^+$ pump in maintaining a steady state against the increased Na$^{2+}$ influx associated with excitation. The electrochemical work involved per beat, calculated above to be about 0.2 mcal/g, represents the minimum amount of free energy of ATP hydrolysis necessary to maintain this steady state. As the free energy per mole of ATP hydrolyzed in vivo is uncertain, one can only say that the corresponding enthalpy change associated with performing this amount of work will be of the same order of magnitude, say 0.1 to 0.3 mcal/g.

Thus the major ion transport mechanisms, Na$^+$ pumping and Ca$^{2+}$ pumping, will hydrolyze ATP with an enthalpy change of 0.35 to 0.55 mcal per gram per beat and all of this will appear as tension-independent heat. This value applies to a maximally activated cardiac contraction in a muscle that is maintaining an ionic steady state. However, three important considerations could drastically reduce the tension-independent heat production: (1) If the activation is not maximal as a result of lesser amounts of Ca$^{2+}$ reaching the myofibrils, then less Ca$^{2+}$ pumping will have to be performed. It takes some 50 μmoles of Ca$^{2+}$/kg of muscle to saturate the actomyosin of cardiac muscle but even with a high external calcium concentration (5mM), the sarcotubules of dog papillary muscle have only about 25 μmoles of Ca$^{2+}$/kg bound to them. Thus if only this amount was released to the myofilament upon excitation, the energy required for calcium transport would be only half the value calculated previously, i.e., half of 0.25 mcal/g; (2) If the isolated muscle is not maintaining a steady state but is gradually losing K$^+$ and gaining Na$^+$ then, although the electrophysiological and mechanical consequences of this will be slight and will develop slowly over a period of hours, the energy requirement for Na$^+$ pumping could conceivably become only a fraction of that necessary to maintain a steady state; (3) Recent work by Baker et al. (30) has shown that there is a component of sodium efflux which depends on the presence of external calcium. This calcium-dependent efflux is small under resting conditions but is increased by raising internal sodium or external calcium, or by lowering external sodium. These authors also showed that the calcium influx behaved in a parallel fashion to the sodium efflux and following a suggestion made by Reuter and Seitz (31), they proposed that calcium may be pumped out of cells by a mechanism whereby external sodium is exchanged for internal calcium. Their work suggests that some of the free energy released by Na$^+$ moving down its
electrochemical gradient could be used for calcium pumping in squid axon. If a similar process occurs in cardiac muscle, it would decrease the tension-independent energy utilization (in the form of ATP hydrolysis), and because such a process would be thermally neutral, there would be a decrease in the tension-independent heat production.

In myothermic experiments at 23°C, the tension-independent heat production of a cardiac contraction ranges between 0.4 to 1.0 mcal/g. Thus, considering that part of this is probably recovery heat (1), there is good agreement between the orders of magnitude for the experimentally measured tension-independent heat and that predicted from thermodynamic considerations. In practice, this heat component will depend on the thermodynamic efficiency of the Na⁺ pump, the degree of activation, and how effectively the muscle is maintaining an ionic steady state.

The changes in myocardial contractility induced by changes in the external [Ca²⁺]/[Na⁺]² have been suggested to occur by Na⁺ and Ca²⁺ competing for a membrane carrier system (7, 32-34). There is good correlation in terms of calcium exchange kinetics between the "superficial" membrane site of Niedergerke (32), the "intermediary locus" of Winegrad and Shanes (35) and the "phase 2" fraction of Langer and Brady (36) and Shelburne et al. (37). Now if there is antagonism between Ca²⁺ and Na⁺ movements during excitation, this would be expected to result in a reciprocal relation between the quantities of Na⁺ and Ca²⁺ to be transported during recovery and hence there would be a reciprocal relation between the amount of work to be performed by each of the Na⁺ and Ca²⁺ pumps. Therefore, the lack of correlation between the tension-independent heat component of cardiac muscle and changes in the external [Ca²⁺]/[Na⁺]² reported in this work could be explained by supposing that the tension-independent heat is the sum of the enthalpy requirements for both Na⁺ and Ca²⁺ pumping. For example, any extra energy required for increased Ca²⁺ transport that occurs in % Na⁺ solution will be balanced by a decreased energy requirement for Na⁺ transport, because the Na⁺ influx associated with excitation will be reduced in % Na⁺ solution.

This argument is supported by the strong direct correlation between Na⁺ and Ca²⁺ concentration and the tension-independent heat shown in the present work. The interpretation can also explain the consistent trend for diminished tension-independent heat production in % Na⁺ : % Ca²⁺ solution. Under these conditions the slightly diminished mechanical output indicates a slightly diminished Ca²⁺ turnover. But the reduced Na⁺ concentration would be expected in this case to result in a net reduction of the total electrochemical work of Na⁺ and Ca²⁺ pumping and this would be reflected in a diminished tension-independent heat production.

The argument used above may be extended to explain the relatively small effects of cardiac glycosides or increased heart rate on tension-independent heat production compared with the large effects of epinephrine (3-5).

According to a recent theory of cardiac Na⁺, K⁺ and Ca²⁺ exchanges and their relationships to myocardial contractility (16), it is supposed that any sodium pump lag (which will presumably occur on increasing frequency or administering cardiac glycosides) will result in a net shift of Na⁺ to the more intracellular side, or "inside" (16) of membranes (sarcosomal or sarcotubular) to which Ca²⁺ and Na⁺ are bound competitively. This results in further Ca²⁺ binding to the sites from which Na⁺ is displaced and so causes an increased mechanical output following subsequent depolarization. It is easy to visualize that under such conditions of sodium pump lag, there will be a reduced transmembrane Na⁺ gradient and so the Na⁺ influx per excitation will be diminished. The consequent reduction of energy expended by the Na⁺ pump will thus tend to counterbalance the increase in tension-independent energy utilization associated with increased Ca²⁺ turnover under the influence of cardiac glycosides or increased beat rate.

One outstanding problem relates to the widely different shape changes produced in...
the cardiac twitch myogram by various inotropic agents. For example, the twitch potentiated by epinephrine has a much shorter contraction time than a twitch of similar amplitude potentiated by raised Ca^{2+} concentration, or lowered Na^{+} concentration. The rate at which the contractile event occurs in cardiac muscle may possibly have important effects on the thermodynamic efficiency of both the ion pumps and the contractile mechanism. Steps towards resolving these difficulties would be to investigate any possible interference by epinephrine with either the Ca^{2+}/ATP of the cardiac calcium pump or with the actomyosin ATPase activity. A direct quantitative study of the relative increments in Ca^{2+} turnover produced by the various inotropic agents would also be of interest.

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