Ionized Calcium and the Heart

Elucidation of in Vivo Concentration-Response Relationships in the Open-Chest Dog

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SUMMARY Pharmacological relationships between ionized calcium and cardiac electric and mechanical parameters were explored in the open-chest dog. A technique that allows the determination of reliable, repeatable concentration-response relationships in vivo was developed. This method consists of alternating infusions of sodium citrate and calcium gluconate and direct measurement of serum ionized calcium by an ion-specific electrode. With this technique, four consecutive curves for ionized calcium vs. the first derivative of the left ventricular pressure (dP/dt) were essentially superimposable within the limits of 2–10 mg/100 ml. Relationships between ionized calcium and the electrocardiograph (ECG) interval measured from the end of the S wave to the peak of the T wave (SaTc) proved to be superior to other ECG measurements as a correlate of ionized calcium. Ionized calcium correlated better than total calcium with physiological function in several situations. Post-surgical ionized calcium levels (4.92 ± 0.10 mg/100 ml) were consistently higher than pre-anesthetic values (4.57 ± 0.07 mg/100 ml, P < 0.025), whereas total calcium measurements were not significantly different (X = 5.3 ± 0.10 mEq/liter, X = 5.1 ± 0.10 mEq/liter, P < 0.1). This difference in ionized calcium was shown to be able to account for significant alterations in dP/dt, suggesting that fluctuations in ionized calcium may be involved in the regulation of the contractile state of the heart.

VARIATIONS in the extracellular concentration of ionized calcium (Ca\(^{2+}\)) have important effects on the electrical and mechanical function of the intact mammalian heart. In man, hypocalcemia associated with rapid transfusion of citrated blood has been implicated as a cause of decreased contractility, cardiovascular collapse, and cardiac arrest. Hypercalcemia has been associated with an increase in contractility in animals and conduction disturbances, ventricular irritability, and cardiac arrest have been described in man.

Recent studies have shown that the fraction of myocardial calcium that is correlated with contractility is readily exchangeable and in rapid equilibrium with plasma Ca\(^{2+}\). Since it has been suggested that alterations in cellular calcium levels are a common mediator of a variety of pharmacological influences on the contractile state of the heart, it is possible that minor as well as major changes in the extracellular Ca\(^{2+}\) can have important effects on cardiac function.

Because of the difficulty of directly measuring Ca\(^{2+}\), there have been no previous quantitative in vivo studies of Ca\(^{2+}\)-myocardial concentration-response relationships. The "safe" range of Ca\(^{2+}\) is unknown, as is the range over which significant inotropic effects occur.

Recent technological advances have made it possible to measure Ca\(^{2+}\) precisely and reliably. This study uses direct measurements of Ca\(^{2+}\) by an ion-specific electrode to determine quantitative relationships in vivo between Ca\(^{2+}\) and myocardial response throughout a wide range of serum Ca\(^{2+}\).

Methods

Twenty-two mongrel dogs weighing 14–30 kg were sedated with morphine sulfate (2 mg/kg) intramuscularly, then anesthetized with intravenous chloralose and urethane at doses of 85 mg/kg and 625 mg/kg, respectively. Ventilation was provided by a Harvard pump delivering room air through auffed endotracheal tube. Arterial blood gases were monitored frequently with a Corning model 165 gas analyzer and pH was maintained between 7.35 and 7.45, Po\(_2\) between 80 and 110 and PcO\(_2\) between 35 and 45 mmHg. Body temperature was maintained at 37–38° with a thermal blanket.

Infusion catheters were placed in the external jugular and femoral veins. Aortic pressure was monitored by means of a PE 240 polyethylene catheter inserted through the right carotid artery into the central aorta and connected to a Statham P23Db pressure transducer. This catheter was also used for serial sampling of arterial blood.

The chest was entered through a midline sternotomy and the heart was suspended in a pericardial cradle. A Micron MP10 implantable pressure transducer was placed in the left ventricle through a stab wound in the apical dimple. In some of the dogs (see below) an adjustable screw clamp was positioned around the descending aorta distal to the origin of the left subclavian artery through a small incision in the left chest wall so that aortic resistance could be varied. Pacing wires were sutured to the epicardium of the left ventricle near the apex and connected to a Grass model S4 stimulator. Ventricular rather than atrial
pacing was necessary because of atrioventricular (AV) conduction delays at high Ca\textsuperscript{2+} levels.

The zero for pressure measurements was considered to be the midchest level. All recordings were made with a Honeywell model 1508 Visicorder oscillograph. The mean aortic pressure was derived electronically and the first derivative of the left ventricular pressure (dP/dt) was obtained by an electronic differentiator circuit with a linear amplitude response to more than 500 Hz. This circuit was also equipped with an adjustable automatic cutoff so that dP/dt could be obtained at a predetermined point on the left ventricular (LV) pressure rise prior to the opening of the aortic valve, e.g., dP/dt 40 or dP/dt 65. Lead II of an external electrocardiogram was also recorded.

Hemodynamic variables and electrical intervals were measured at a paper speed of 100 or 200 mm/sec. Peak dP/dt values were discarded when the aortic and ventricular pressure curves were not superimposable and when maximum dP/dt did not occur during isovolumetric contraction. In paced preparations, the heart was driven at 10-20 beats/min above the spontaneous rate.

Calcium gluconate and sodium citrate were administered via the jugular and femoral veins, respectively, with a Harvard infusion pump. Concentration-response curves to Ca\textsuperscript{2+} were performed as follows: After instrumentation, hemodynamic parameters were measured every 5-10 minutes until three successive measurements showed that the preparation was stable. Reduction in the Ca\textsuperscript{2+} level was accomplished by the infusion of a 15% solution of sodium citrate that had been titrated to a pH of 7.4 with 1 N HCl and passed through a Millipore filter. The rate of administration was 5-10 mg/kg per min. The end point of the citrate infusion was evidence of impending circulatory collapse, defined as a decrease in arterial pressure >20 mmHg or the development of mechanical alternans. The infusion of citrate was then stopped, a 30-second period was allowed for equilibration, and the first point on the concentration-response curve was obtained by first withdrawing 5-6 ml of arterial blood from the aortic catheter and then rapidly making recordings. If the mean arterial pressure had not reached a value of 10 mmHg below control level, it was temporarily brought to that level by means of the aortic screw clamp. After the initial recording, an infusion of 10% calcium gluconate was begun at 2-4 mg/kg per min. Arterial samples and recordings were taken every 1-2 minutes until two successive recordings yielded similar values of dP/dt. At this point, the infusion rate was doubled. The maximum response (100%) was considered to be the point at which increasing the infusion rate produced no further increase in dP/dt or when the S-T segment began to lengthen in the hypercalcemia range (see below). This usually occurred after 2-5 minutes of an infusion of 40 mg/kg per min. Blood that was withdrawn was replaced with an equal volume of 0.45 % NaCl containing 5 mEq/KCl/liter.

After reaching the maximum point on the concentration-response curve, the calcium infusion was abruptly stopped and the citrate infusion was reinstalled. The total time to perform curves in this manner varied between 30 and 45 minutes, with a mean of 38 minutes/curve. The maximum number of curves performed for any one preparation was four and the maximum number of blood samples per dog was 40. Hematocrits did not significantly differ from the beginning of the experiment (X = 41 ± 2) to the end (X = 42 ± 3). Left atrial pressure was monitored in the first five animals studied and did not significantly change throughout the experiment.

When multiple ionized calcium levels were determined prior to initiation of a citrate or calcium infusion, the following designations were used: preanesthesia, blood drawn prior to administration of general anesthesia; postanesthesia, drawn approximately 10 minutes after administration of anesthesia and just prior to sternotomy; control, drawn just prior to beginning citrate or calcium infusion. Ionized calcium was measured in sera with the AMT electron system, model auto I (Applied Medical Technology). Briefly, this is an automated system with temperature and pH control. It makes use of serum standards and two solid state dip calcium electrodes used simultaneously. This system has been shown to be accurate and reliable for use with serum. In the range of Ca\textsuperscript{2+} measured in this study, the standard deviation of this method for the same sample is less than 1.2%.

Electrical intervals were measured as follows: QTc, interval in seconds measured from the beginning of the Q wave of the electrocardiogram to the end of the T wave and divided by the square root of the R-R interval (\sqrt{R-R}); QTc\textsubscript{V}, directly measured as the interval between the beginning of the Q wave and the upstroke of the T wave divided by \sqrt{R-R}; S\textsubscript{Tc} (or S-T segment) either directly measured as the interval between the end of the S wave and the apex of the T wave divided by \sqrt{R-R}; S\textsubscript{Tc} (or S-T segment) either directly measured as the interval between the end of the S wave and the upstroke of the T wave divided by \sqrt{R-R} or obtained through the formula S\textsubscript{Tc} = (Q\textsubscript{Tc} - ORS)/\sqrt{R-R}. All measurements were determined from the mean of at least three complexes recorded at 100 mm/sec; all contractility measurements were also the mean of at least three successive complexes.

Concentration-response data were organized as follows: Following a plot of the non-normalized data (e.g., peak dP/dt or dP/dt 65 vs. log Ca\textsuperscript{2+}; S\textsubscript{Tc} vs. log Ca\textsuperscript{2+}), percent change from 0-100% was determined for each concentration-related variable. For mechanical parameters, e.g., peak dP/dt or dP/dt 65, the minimum or zero value was considered to be the lowest value reached throughout the course of the experiment. The 100% or “maximum” response was taken as the point in the hypercalcemic range at which dP/dt did not increase despite a subsequent increase in Ca\textsuperscript{2+}, provided that left ventricular pressure recordings were acceptable. For electrical measurements reflecting the plateau phase of the action potential, the minimum value was the longest rate-corrected value and the maximum was the shortest obtained at any time on any curve during the experiment. This method was necessary because of the tendency of all the electrical parameters to progressively shorten and then lengthen with increasing Ca\textsuperscript{2+}; the maximum shortening on any one curve could therefore be missed by successive sampling.

Ionized magnesium (Mg\textsuperscript{2+}) was approximated from the formula Mg\textsuperscript{2+} = (0.6 Mg) (Ca\textsuperscript{2+})/Ca, where Mg = total magnesium expressed as mEq/liter, Ca\textsuperscript{2+} = ionized cal-

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cium expressed as mg/100 ml, and Ca = total calcium expressed as mEq/liter. This formula is valid only when the complexing agent responsible for changes in the total calcium and Ca²⁺ is citrate and is based on the observation that the dissociation constant (K) for calcium citrate and magnesium citrate is the same, 10⁻¹².¹⁶ Total calcium and magnesium were measured by atomic absorption by Bio-Science Laboratories, Van Nuys, California, who also performed analyses for Na⁺, K⁺, and glucose.

Statistical analyses employed included the following: the correlation coefficient of the linear regression line as defined as:

\[
 r = \frac{\Sigma xy - (\Sigma x)(\Sigma y)/n}{\sqrt{[(\Sigma x)^2 - (\Sigma x)^2/n]([\Sigma y]^2 - (\Sigma y)^2/n)]}
\]

where x = the raw, "non-normalized" values of dP/dt, S₄Tₑ, QTₑ, S₆Tₑ, or QₓTₑ and y = log ionized calcium; unpaired Student's t-test and paired t-test.¹⁷

Results

DEFINITION OF THE Ca²⁺ CONCENTRATION-RESPONSE CURVE

To establish feasibility of the methods and stability of the preparation, five paced dogs were studied with blood samples and recordings taken during the calcium infusion. The only electrical interval representative of the plateau phase of the action potential that could be measured reliably in paced dogs was the S₄Tₑ.

Figure 1 presents data from two individual experiments and shows four successive concentration-response curves expressing the increase in peak dP/dt with infusion of calcium gluconate. Figure 2 gives mean curves for dP/dt and S₄Tₑ vs. Ca²⁺ in five consecutive preparations treated in this manner. The concentration-response curves for increase in peak dP/dt are remarkably similar with an EC₅₀ (Ca²⁺ level at 50% of maximum response) variation of less than 0.4 mg/100 ml. The mean S₄Tₑ curves are also nearly superimposable, with a range of EC₅₀ of 3.75-4.50 mg/100 ml. Both variables are linearly related to log Ca²⁺ from 2 to 7.50 mg/100 ml.

Correlation coefficients (r) of the linear regression line were determined for log Ca²⁺ vs. non-normalized peak dP/dt and S₄Tₑ values in the five paced dogs. These results are given in Table 1. Peak dP/dt and S₄Tₑ show a significant correlation with log Ca²⁺ in the range of 1-6 mg/100 ml.
Between 6 and 10 mg/100 ml the correlation of Ca^2+ and dP/dt is nonlinear because of "plateauing" of the concentration-response curves. At Ca^2+ concentrations greater than 10 mg/100 ml dP/dt tended to decrease with increasing Ca^2+, and the r value changes sign to negative.

Serum Na and K, total Ca, and total Mg were measured at the beginning of each concentration-response curve (EC0), near the EC50, and at maximum (EC100). These results appear in Table 2. Na rises slightly throughout the course of the four concentration-response curves. At the EC50, Na+ is 155 on curve 1 and 160 on curve 4, with a calculated Mg^2+ gradually falls from a baseline value of 0.55 mg/100 ml for the EC50 of curve 1 to 0.26 mg/100 ml for curve 4.

### Table 1 Correlation Coefficient (r) of the Linear Regression Line of Log Ca^{2+} vs. dP/dt and S_{Tc} in Five Paced Dogs with Four Concentration-Response Curves per Dog

<table>
<thead>
<tr>
<th>Ca^{2+} (mg/100 ml)</th>
<th>dP/dt</th>
<th>S_{Tc}</th>
</tr>
</thead>
<tbody>
<tr>
<td>r</td>
<td>n</td>
<td>P</td>
</tr>
<tr>
<td>1-3</td>
<td>+0.353</td>
<td>30</td>
</tr>
<tr>
<td>3-6</td>
<td>+0.328</td>
<td>59</td>
</tr>
<tr>
<td>6-10</td>
<td>+0.224</td>
<td>43</td>
</tr>
<tr>
<td>10-30</td>
<td>-0.477</td>
<td>25</td>
</tr>
</tbody>
</table>

### Table 2 Serum Electrolytes and Glucose at the Beginning (EC0), Midpoint (EC50), and End (EC100) of Four Consecutive Ca^{2+} Concentration-Response Curves in Five Dogs

<table>
<thead>
<tr>
<th>Curve</th>
<th>Na^+ (mEq/liter)</th>
<th>K^+ (mEq/liter)</th>
<th>Glucose (mg/100 ml)</th>
<th>Total Ca (mEq/liter)</th>
<th>Ca^2+ (mg/100 ml)</th>
<th>Total Mg (mEq/liter)</th>
<th>Mg^{2+} (mg/100 ml)</th>
<th>Total Ca/ Ca^{2+}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>148</td>
<td>3.6</td>
<td>164 ± 21</td>
<td>5.3</td>
<td>4.80</td>
<td>1.6</td>
<td>0.87</td>
<td>1.1</td>
</tr>
<tr>
<td>Curve 1</td>
<td>EC0</td>
<td>158</td>
<td>3.7</td>
<td>170 ± 23</td>
<td>4.9</td>
<td>1.95</td>
<td>1.4</td>
<td>0.33</td>
</tr>
<tr>
<td></td>
<td>EC50</td>
<td>155</td>
<td>3.7</td>
<td>166 ± 23</td>
<td>8.3</td>
<td>5.37</td>
<td>1.4</td>
<td>0.55</td>
</tr>
<tr>
<td></td>
<td>EC100</td>
<td>149</td>
<td>3.5</td>
<td>167 ± 20</td>
<td>14.0</td>
<td>9.93</td>
<td>1.4</td>
<td>0.59</td>
</tr>
<tr>
<td>Curve 2</td>
<td>EC0</td>
<td>161</td>
<td>3.4</td>
<td>155 ± 18</td>
<td>7.8</td>
<td>2.38</td>
<td>1.4</td>
<td>0.25</td>
</tr>
<tr>
<td></td>
<td>EC50</td>
<td>157</td>
<td>3.4</td>
<td>144 ± 12</td>
<td>10.2</td>
<td>5.78</td>
<td>1.3</td>
<td>0.46</td>
</tr>
<tr>
<td></td>
<td>EC100</td>
<td>152</td>
<td>3.4</td>
<td>150 ± 17</td>
<td>15.7</td>
<td>10.31</td>
<td>1.3</td>
<td>0.57</td>
</tr>
<tr>
<td>Curve 3</td>
<td>EC0</td>
<td>164</td>
<td>3.6</td>
<td>159 ± 15</td>
<td>9.0</td>
<td>2.17</td>
<td>1.3</td>
<td>0.18</td>
</tr>
<tr>
<td></td>
<td>EC50</td>
<td>159</td>
<td>3.6</td>
<td>152 ± 12</td>
<td>11.8</td>
<td>5.21</td>
<td>1.2</td>
<td>0.33</td>
</tr>
<tr>
<td></td>
<td>EC100</td>
<td>155</td>
<td>3.4</td>
<td>156 ± 14</td>
<td>19.4</td>
<td>10.01</td>
<td>1.4</td>
<td>0.38</td>
</tr>
<tr>
<td>Curve 4</td>
<td>EC0</td>
<td>167</td>
<td>3.5</td>
<td>157 ± 16</td>
<td>10.9</td>
<td>2.06</td>
<td>1.3</td>
<td>0.14</td>
</tr>
<tr>
<td></td>
<td>EC50</td>
<td>160</td>
<td>3.6</td>
<td>155 ± 20</td>
<td>14.8</td>
<td>5.30</td>
<td>1.2</td>
<td>0.26</td>
</tr>
<tr>
<td></td>
<td>EC100</td>
<td>158</td>
<td>3.7</td>
<td>162 ± 22</td>
<td>26</td>
<td>11.36</td>
<td>1.2</td>
<td>0.34</td>
</tr>
</tbody>
</table>
IN VIVO PHARMACOLOGY OF Ca\textsuperscript{2+}/Bristow et al.

Table 4

<table>
<thead>
<tr>
<th>Ca\textsuperscript{2+} (mg/100 ml)</th>
<th>dP/dt</th>
<th>O\textsubscript{T} \textsuperscript{0}</th>
<th>QT</th>
<th>S\textsubscript{T} \textsuperscript{0}</th>
<th>S\textsubscript{aT}</th>
<th>S\textsubscript{aT}\textsuperscript{c}</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-3</td>
<td>+ 0.27</td>
<td>35 &gt; 0.05</td>
<td>-0.356 55 &lt; 0.01</td>
<td>-0.366 29 &lt; 0.05</td>
<td>-0.283 35 &gt; 0.05</td>
<td>-0.482 34 &lt; 0.01</td>
</tr>
<tr>
<td>3-6</td>
<td>+ 0.603 80 &lt; 0.001</td>
<td>-0.123 75 &gt; 0.05</td>
<td>-0.437 75 &lt; 0.001</td>
<td>-0.137 75 &gt; 0.05</td>
<td>-0.448 79 &lt; 0.001</td>
<td></td>
</tr>
<tr>
<td>6-10</td>
<td>+ 0.37 55 &lt; 0.01</td>
<td>-0.175 54 &gt; 0.05</td>
<td>+0.005 51 &gt; 0.05</td>
<td>-0.226 54 &gt; 0.05</td>
<td>-0.257 58 &lt; 0.05</td>
<td></td>
</tr>
<tr>
<td>10-30</td>
<td>+ 0.178 21 &gt; 0.05</td>
<td>+0.368 54 &lt; 0.01</td>
<td>+0.305 26 &gt; 0.05</td>
<td>+0.215 35 &gt; 0.05</td>
<td>+0.655 35 &lt; 0.001</td>
<td></td>
</tr>
</tbody>
</table>

The heart was not paced but rate did not change significantly during the infusions. The \( r \) values for peak dP/dt and \( S_{aT} \) vs. log Ca\textsuperscript{2+} and total calcium are given in Table 5. Note that Ca\textsuperscript{2+} correlates positively with peak dP/dt and negatively with the \( S_{aT} \) in the expected manner. The total calcium, on the other hand, correlates in the reverse direction for each of these variables.

**EFFECT OF PRIOR EXPOSURE TO CITRATE ON THE Ca\textsuperscript{2+} CONCENTRATION-RESPONSE CURVE**

Since it was noted that citrate infusions lead to small changes in the serum Na\textsuperscript{+} and Mg\textsuperscript{2+} concentrations, we studied five dogs in which Ca\textsuperscript{2+} concentration-response curves were performed without prior exposure to citrate and then repeated during and following citrate infusion. The sequence of infusions and measurements in these experiments, therefore, was: calcium gluconate, sodium citrate, and then calcium gluconate again. Results of these experiments are given in Figure 4.

For peak dP/dt (Fig. 4A), the calcium concentration-response relationships, determined before citrate was in-

**Figure 3** Data for a dog in which a simultaneous infusion of calcium gluconate (4.4-8.6 mg/kg per min) and sodium citrate (9.4-19.8 mg/kg per min) was adjusted to produce a reduction in ionized calcium but an increase in total calcium (A). Effect of these alterations on peak dP/dt (B) and \( S_{aT} \) (C).
Table 5  Correlation Coefficient (r) of Linear Regression Line of Log Ca$^{2+}$ or Total Calcium vs. dP/dt and $S_Tc$, in a Dog Receiving a Simultaneous Citrate and CalciumInfusion

<table>
<thead>
<tr>
<th></th>
<th>$dP/dt$</th>
<th></th>
<th>$S_Tc$</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r</td>
<td>n</td>
<td>P</td>
<td>r</td>
</tr>
<tr>
<td>Ca$^{2+}$</td>
<td>+0.610</td>
<td>9</td>
<td>&lt;0.025</td>
<td>-0.700</td>
</tr>
<tr>
<td>Total Ca$^{2+}$</td>
<td>-0.590</td>
<td>9</td>
<td>&lt;0.025</td>
<td>+0.750</td>
</tr>
</tbody>
</table>

fused, are significantly to the right of the curve performed following citrate administration, whereas in Figure 1 all curves are superimposable. (EC$_{50}$ is used instead of EC$_{90}$ because the baseline Ca$^{2+}$ value was greater than the EC$_{50}$ in some experiments.) The EC$_{60}$ of these two curves vary by 1.55 mg/100 ml with a $P$ value of <0.025 by paired $t$ analysis. In contrast, the $S_Tc$ concentration-response curves (Fig. 4B) are not significantly different ($P$ > 0.05), as the curves are nearly superimposable.

Figure 4A shows that the concentration-response curve of Ca$^{2+}$-peak dP/dt, determined during the transition from hypercalcemia to hypocalcemia as citrate is infused, is slightly to the right of the subsequent curve (from hypo- to hypercalcemia) determined during calcium infusion, but the difference at the EC$_{50}$ is not significant ($P$ < 0.2) by paired $t$ analysis. In contrast, the Ca$^{2+}$-$S_Tc$ curve performed during citrate infusion (Fig. 5B) is slightly to the left, but again with no significant difference in the EC$_{50}$ ($P$ < 0.5).

**TOTAL CALCIUM AND Ca$^{2+}$ CONCENTRATION-RESPONSE RELATIONSHIPS IN THE ABSENCE OF CITRATE**

Table 6 gives the $r$, $n$, and $P$ values for Ca$^{2+}$ and total calcium, as well as the total calcium-Ca$^{2+}$ ratios for the first curves shown in Figure 4, when calcium gluconate was infused in the absence of prior citrate administration. The total-Ca$^{2+}$ ratios increase from baseline to EC$_{75}$ to EC$_{100}$; analysis of the increase from baseline to the EC$_{100}$ reveals a significant difference ($P$ < 0.01) by paired $t$ analysis. Also, ionized but not total calcium is correlated significantly with the non-normalized dP/dt values.

**PREANESTHESIA, POSTANESTHESIA, POSTSURGICAL AND POSTCONTROL Ca$^{2+}$ VALUES**

Table 7 gives the total and Ca$^{2+}$ values from blood drawn prior to chloralose-urethane anesthesia (but after morphine), just after anesthesia and carotid artery-femoral vein cannulation but prior to open-chest surgery, after surgery, and at the end of the 30- to 45-minute control period. The Ca$^{2+}$ values prior to anesthesia are significantly less than those obtained after anesthesia or surgery. Although preanesthesia values were obtained from venous blood and samples that follow from arterial blood, a comparison of eight consecutive postsurgical venous and arterial samples revealed no significant difference (venous $X$ = 4.88 ± 0.10 mg/100 ml; arterial $X$ = 4.82 ± 0.15 mg/100 ml). There was a great deal of variation in the Ca$^{2+}$
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Table 6 Linear Regression Data (r) for Log Ca²⁺ (mg/100 ml) and Log Total Calcium (mEq/liter), vs. dP/dt in Six Dogs That Received an Infusion of Calcium Gluconate without Prior Exposure to Citrate; Ca Total/Ca²⁺ Ratios at Baseline and EC₁₀₀ in These Same Curves

<table>
<thead>
<tr>
<th></th>
<th>r</th>
<th>n</th>
<th>P</th>
<th>Baseline</th>
<th>EC₁₀₀</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ca²⁺</td>
<td>0.356</td>
<td>42</td>
<td>&lt; 0.05</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Ca total</td>
<td>0.288</td>
<td>42</td>
<td>&gt; 0.05</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Ca total/Ca²⁺ ± SEM</td>
<td>–</td>
<td>–</td>
<td>&lt; 0.01</td>
<td>1.09 ± 0.05</td>
<td>1.30 ± 0.08</td>
</tr>
</tbody>
</table>

values drawn at the end of the control period, with some dogs showing a significant decrease from postsurgical values and others continuing to increase. For example, in Figure 1B, X₁ is the Ca²⁺ and peak dP/dt just after surgery and instrumentation was completed, and X₂ gives the concentration-response relationships 45 minutes later. The difference in the two values for Ca²⁺ is 0.40 mg/100 ml and for dP/dt 2,000 mm Hg/sec. A line drawn between these points is nearly parallel to the family of curves that follows. This suggests that the difference in Ca²⁺ and dP/dt during the control period are causally related. This experiment and the plot of the mean curves in Figure 2 show that the mean preanesthetic—postsurgical difference in Ca²⁺ (0.35 mg/100 ml) could account for a significant change in contractility, a dP/dt difference of approximately 600–800 mm Hg/sec in Figure 1B.

In contrast to Ca²⁺, the postsurgical total calcium value is less than the value obtained prior to anesthesia (5.0 ± 0.1, 5.3 ± 0.1, respectively) but the P value is not significant (<0.01).

Ca²⁺-peak dP/dt relationships produced by disappearance of citrate vs. infusion of calcium gluconate

If only Ca²⁺ is physiologically active, then concentration-response relationships generated by calcium infusions should be the same as those produced by effectively increasing the Ca²⁺ by allowing citrate to be eliminated from plasma. Figure 5 shows results of an experiment in which calcium gluconate was infused initially and the Ca²⁺ then lowered by a citrate infusion. At this point the dog was allowed to recover spontaneously from an infusion of citrate that was begun immediately after the control period. Citrate is immediately effective in reversing the effects of Ca²⁺ on the heart, in contrast to allowing Ca²⁺ to be reduced by redistribution and elimination, which takes several minutes. Note that citrate will paradoxically produce a shortening of the S₉Tₐ, if this electrical parameter is on the part of its curve where it is positively correlated with Ca²⁺.

Kinetics of citrate removal

Figure 6 is a plot of log Ca²⁺ vs. time for two dogs allowed to recover spontaneously from an infusion of citrate that was begun immediately after the control period and with the total and Ca²⁺ in the normal range. The curve of the recovery of Ca²⁺, reflecting disappearance of citrate, follows two-compartment kinetics—an initial fast distribution phase with a t₁ of 8.76 ± 0.53 minutes and an elimination phase with a t₁ of 76.50 ± 5.3 minutes.

Efficacy of citrate in reversing hypercalcemic effects on the heart

In five dogs a 2-ml “bolus” of citrate was given at the peak of the calcium infusion curve and a recording taken 30 seconds later. The calculated Ca²⁺-S₉Tₐ relationships from one such experiment are shown in Figure 7. Citrate is immediately effective in reversing the effects of Ca²⁺ on the heart, in contrast to allowing Ca²⁺ to be reduced by redistribution and elimination, which takes several minutes. Note that citrate will paradoxically produce a shortening of the S₉T, if this electrical parameter is on the part of its curve where it is positively correlated with Ca²⁺.

Discussion

In vivo data reflecting dose-response relationships to calcium have been previously reported for animals. Surawicz reduced plasma Ca²⁺ in open-chest dogs with EDTA and then produced hypercalcemia by calcium chloride infusions. He had no way of measuring Ca²⁺ directly but the qualitative changes noted in the study were similar to our results. Seifen et al. also reported electrical and mechanical effects of alterations in calcium with EDTA and calcium infusions. These investigators measured total calcium by EDTA titration, but since the method measures total and not Ca²⁺, the concentration-response data are not valid.

Alterations in Ca²⁺ occur in many clinical situations, including hypoparathyroidism, neonatal hypocalcemia, renal failure, rapid transfusion with citrated blood, malabsorption, hyperparathyroidism and neoplastic disease. Quantitative effects on contractility have not been de-

Table 7 Preanesthesia, Postanesthesia, Pre- and Postsurgery, and Final Control Sera

<table>
<thead>
<tr>
<th></th>
<th>Preanesthesia, venous (n = 17)</th>
<th>Postanesthesia, arterial (n = 6)</th>
<th>Presurgical, arterial (n = 17)</th>
<th>Postsurgical venous (n = 17)</th>
<th>Control, arterial (n = 17)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ca²⁺ (mg/100 ml)</td>
<td>4.57 ± 0.07</td>
<td>4.93 ± 0.15</td>
<td>4.92 ± 0.10</td>
<td>4.89 ± 0.12</td>
<td>4.93 ± 0.12</td>
</tr>
<tr>
<td>Total Ca (mEq/liter)</td>
<td>5.47 ± 0.16</td>
<td>~</td>
<td>5.19 ± 0.12</td>
<td>~</td>
<td>~</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± SEM.
spontaneous increase in ionized calcium following citrate infusion in two individual preparations: •—•, dog in which ionized calcium was reduced from a baseline level of 5.49 to 2.35 mg/100 ml; O—O, dog in which ionized calcium was reduced from 4.82 (XJ to 1.65 mg/100 ml.

This study establishes that log serum Ca²⁺ and the rate of cardiac muscle tension development (dP/dt) are related linearly from 2 to 7.5 mg/100 ml, a range that encompasses most values of Ca²⁺ encountered clinically. Rapid fluctuations in Ca²⁺ were well tolerated in our preparations, with most dogs tolerating a reduction in Ca²⁺ to a level of 2 mg/100 ml before a significant reduction in mean arterial pressure occurred, and all dogs tolerating reduction to 2.5 mg/100 ml. The EC₅₀ of the mean Ca²⁺-dP/dt concentration-response curves in paced preparations ranged from 4.60 to 4.95; this coincides with the range of baseline Ca²⁺ prior to anesthesia and surgery (4.61–4.95). This observation suggests that homeostatic mechanisms operate to keep the Ca²⁺ at a level of maximum safety where the potential for modification of the contractile response by increasing and decreasing Ca²⁺ is equal in both directions.

The quantitative effects of Ca²⁺ on electrical parameters have not been described and no effects on hemodynamic parameters have been reported.

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The relationship between serum Ca²⁺ and dP/dt is such that concentration-response curves are repeatable and superimposable over four full curves determined over a 2.5-hour period. The actual effect on dP/dt that is measured during a calcium infusion is no doubt the product both of increases in ionized calcium and catecholamine release by calcium. This is shown by the effect of the β-blocking agent practolol on the ionized calcium-dP/dt relationship.

The quantitative effects of Ca²⁺ on electrical measurements that reflect the plateau phase of the action potential are also precise and repeatable. The electrical interval S₆T₆ was the easiest to measure and correlated best with Ca²⁺ in all ranges that are likely to be encountered clinically (Ca²⁺ of 1–3, 3–6, 6–10 mg/100 ml). This is consistent with Surawicz's observation that most of the shortening in the ECG intervals that occurs as calcium is increased is between the S wave and the apex of the T wave. The nature of the superior correlation of the S₆T₆ is either that it can be measured more precisely or that it is positioned on the ECG in such a way to reflect more of the plateau phase of the action potential, or both. We believe that S₆T₆ should be given consideration as a replacement for the Q₆T₆ as the preferred ECG correlate of plasma Ca²⁺.

Sudden death in hypercalcemia, presumably from ventricular fibrillation, was not seen in our studies within clinically encountered ranges of Ca²⁺; no dog died of ventricular fibrillation at a Ca²⁺ of less than 10 mg/100 ml. Repeat citrate and calcium infusion were well tolerated by the dogs in this study. Two electrolyte abnormalities appeared throughout the course of the experiment, an increase in serum sodium and a reduction in Mg²⁺. The slight increase in serum sodium was not accompanied by metabolic alkalosis and such small increases (increase of 5 mEq/liter from the EC₅₀ of curve 1 to curve 4) in serum sodium would not be expected to account for a noticeable effect on the contractile state. The 2-fold reduction in Mg²⁺ from the EC₅₀ of curve 1 to curve 4 would theoretically be enough to account for a small alteration in Ca²⁺-dP/dt relationships, and it is perhaps surprising that the
concentration-response curves do not progressively shift to the left as Mg²⁺ is gradually reduced. However, the effect of alterations in Mg²⁺ levels on calcium concentration-response curves in preparations in vitro apparently is somewhat species-specific with not all species showing an effect. An even more likely explanation for the lack of effect of gradually decreasing Mg²⁺ is that any effect of the small decrease that occurs is obscured by desensitization of the preparation with time, possibly related to decreasing levels of circulating catecholamines.

Slightly different dP/dt-Ca²⁺ relationships were obtained depending on the manner in which the curve was determined. Ca²⁺-dP/dt relationships derived after citrate administration were left-shifted relative to relationships obtained prior to citrate exposure. Possible explanations for this include differences in adrenergic state, differences in electrolytes (slight increase in Na⁺, decrease in ionized magnesium after citrate infusion), or an effect of citrate itself. Support for a reduction of ionized magnesium as the mediator of this effect is given in a companion publication.

Although it is difficult to extrapolate from animal studies, the ease and speed with which one is able to alter Ca²⁺ with sodium citrate and the resultant control that one has over Ca²⁺ concentration-response relationships suggest that citrate infusions monitored by frequent Ca²⁺ measurements would be an ideal acute treatment in hypercalcemia, for which there is a paucity of safe measures that can quickly lower plasma Ca²⁺. If the infusion were continued for several hours, the large sodium load in Na₃ citrate would probably cause metabolic alkalosis (not seen in this study) and would have to be dealt with. Magnesium losses would have to be replaced as Mg citrate is excreted.

This study provides in vivo support for the McLean-Hastings hypothesis that only Ca²⁺ is physiologically active in tissue. Although both total calcium and Ca²⁺ correlate with dP/dt and the S₄Tₙ, as calcium is infused, during simultaneous citrate and calcium infusion total calcium correlates paradoxically in the reverse direction with dP/dt and S₄Tₙ, while Ca²⁺ correlates in the expected manner. The finding of a consistently elevated Ca²⁺ following anesthesia or surgery to an extent that would account for a significant change in dP/dt suggests a previously unsuspected method of control of the myocardial contractile state—a homeostatic mechanism by which Ca²⁺ can be rapidly modulated. Although diffusible and nondiffusible compounds that can bind Ca²⁺ were not specifically measured in this study, it seems likely that an alteration of a substance in the diffusible (nonprotein) fraction would be the most likely mediator of this mechanism. This substance would need to be metabolized or excreted rapidly to account for the acute effect on Ca²⁺; fluctuations in plasma citrate levels could possibly account for such a mechanism.

A consistent increase in the total calcium-Ca²⁺ ratio as calcium is infused suggests that an endogenous complexing agent is being produced in response to either an increase in Ca²⁺ or an increase in contractile force, a situation that would have biological advantage. Again, a fluctuation in serum citrate levels through alterations in hepatic production and metabolism offers an attractive theoretical homeostatic mechanism to account for this finding. Specific data in support of this notion are lacking, however.

Throughout all aspects of our study, Ca²⁺ proved to be better correlated with myocardial physiological relationships than total calcium. There are many clinical situations in which Ca²⁺ and total calcium yield markedly different values, such as after massive transfusion of citrated blood. The better correlation of Ca²⁺ in situations where Ca²⁺ and total calcium should be changing proportionately, such as during calcium infusion, may be a function of a more precise measurement of Ca²⁺ or better physiological correlation, or both. The superiority of Ca²⁺ over total calcium measurements has been noted by others, including Gordon and Vaughan. We agree with these authors when they state that “serum ionized calcium determinations should eventually replace the conventionally measured serum total calcium concentration, since the two yield disparate values in many important clinical situations.” Since reliable measurements of ionized calcium are available, we believe that ionized calcium should replace total calcium as a routine clinical test.

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Effect of D600, Practolol, and Alterations in Magnesium on Ionized Calcium Concentration-Response Relationships in the Intact Dog Heart

MICHAEL R. BRISTOW, JOHN R. DANIELS, ROBERT S. KERNOFF AND DONALD C. HARRISON

SUMMARY The effect of practolol, D600, and alterations in ionized magnesium concentration on the cardiovascular pharmacology of calcium was examined in vivo in the open-chest dog. In the presence of practolol the ionized calcium-dP/dt (first derivative of left ventricular pressure) concentration-response curve was shifted to the right, with a decreased slope and maximum. Practolol also shifted ionized calcium-SaTc (S wave to peak of T wave on ECG) relationships to the right but in a parallel fashion. Compound D600 produced a parallel shift to the right of ionized calcium-dP/dt relationships but only at maximal doses (0.8 mg/kg). In the presence of practolol, 2.5 mg/kg, D600 shifted ionized calcium-dP/dt relationships to the right at doses (0.8 and 0.25 mg/kg) that had no effect in the absence of practolol. Ionized calcium-dP/dt relationships were the same before and after chelation with EGTA, in contrast to results of the same experiment performed with sodium citrate as the chelating agent. In the intact canine heart, practolol appears to act as a noncompetitive antagonist, D600 as a competitive antagonist, and ionized magnesium as an antagonist of the effects of ionized calcium on dP/dt. D600 in doses of 0.25 mg/kg and 0.80 mg/kg produced hyperglycemia, and this effect was prevented by prior administration of practolol.

IN A PREVIOUS study we described a method for determining precise in vivo concentration-response relationships between ionized calcium (Ca²⁺) and the mechanical and electrical activity of the heart. With this method Ca²⁺ raised or lowered by infusions of calcium gluconate or sodium citrate and then directly measured by an ionic-specific electrode. With this technique Ca²⁺-dP/dt (first derivative of left ventricular pressure rise) relationships are precise and reliable between 2 and 10 mg/100 ml, and four curves from measurements repeated over a 2½-hour interval are superimposable.

In our present study we have used this method to define pharmacological relationships between Ca²⁺ and members of three classes of agents that are thought to alter the effects of Ca²⁺ on the heart: alterations in the level of ionized magnesium (Mg²⁺), β-adrenergic blockade with practolol, and "calcium antagonism" with compound D600.

Methods

Thirty mongrel dogs weighing between 14 and 25 kg were used in this study. Materials and methods were identical to those previously described. Briefly, concentration-response curves between Ca²⁺ and dP/dt or SaTc (ECG measurement from S wave to peak of T wave = √R−T interval) were performed by infusing calcium gluconate after first producing hypocalcemia to the point of circulatory depression with an infusion of sodium citrate. Twenty-seven dogs were paced with an electrode sutured to the left ventricular epicardium near the apex, and three were studied at a spontaneous rate. Practolol was prepared before each experiment by dissolving it in 5.0 ml of 0.1 N HCl and then adding 5.0 ml of 0.1 N NaOH to give 10 ml of a solution of 10 mg/ml. Compound D600 (methoxyverapamil) was prepared by dissolving it in 0.9% NaCl to give a final concentration of 2 mg/ml. Ethyleneglycol-bis(β-aminoethyl ether)-N,N′-tetraacetic acid (EGTA) was prepared as a 10% solution in water and passed through a 0.45-μm Millipore filter. Practolol was administered iv over 5-10 minutes; the infusion of practolol was completed 15 minutes prior to the determination of a Ca²⁺ concentration-response curve. If repeat Ca²⁺ curves were determined in the presence of practolol, the drug was administered at a rate of 0.5 mg/kg per hour following the initial dose. D600 was given slowly iv over 5-30 minutes at a rate not exceeding 0.1 mg/kg per min. EGTA was infused with a Harvard pump at a rate of 4.0-8.0 mg/kg per min. Sodium citrate as a 15% solution was prepared before each experiment by dissolving it in 5.0 ml of 0.1 N HCl and then adding 5.0 ml of 0.1 N NaOH to give 10 ml of a solution of 10 mg/ml. Compound D600 (methoxyverapamil) was prepared by dissolving it in 0.9% NaCl to give a final concentration of 2 mg/ml. Ethyleneglycol-bis(β-aminoethyl ether)-N,N′-tetraacetic acid (EGTA) was prepared as a 10% solution in water and passed through a 0.45-μm Millipore filter. Practolol was administered iv over 5-10 minutes; the infusion of practolol was completed 15 minutes prior to the determination of a Ca²⁺ concentration-response curve. If repeat Ca²⁺ curves were determined in the presence of practolol, the drug was administered at a rate of 0.5 mg/kg per hour following the initial dose. D600 was given slowly iv over 5-30 minutes at a rate not exceeding 0.1 mg/kg per min. EGTA was infused with a Harvard pump at a rate of 4.0-8.0 mg/kg per min. Sodium citrate as a 15% solution was prepared and administered as previously described. In A PREVIOUS study we described a method for determining precise in vivo concentration-response relationships between ionized calcium (Ca²⁺) and the mechanical and electrical activity of the heart. With this method Ca²⁺ raised or lowered by infusions of calcium gluconate or sodium citrate and then directly measured by an ionic-specific electrode. With this technique Ca²⁺-dP/dt (first derivative of left ventricular pressure rise) relationships are precise and reliable between 2 and 10 mg/100 ml, and four curves from measurements repeated over a 2½-hour interval are superimposable.

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