Influence of Ouabain on Contractile Force, Resting Tension, Ca\(^{45}\) Entry and Tissue Ca Content in Rat Atria

By Gudrun Gersmeyer, M.D., and William C. Holland, M.D.

In 1958, Thomas and co-workers\(^1\) observed that ouabain caused an increased uptake of Ca\(^{45}\) in frog heart. Holland and Sekul\(^2\) later reported the same effect in rabbit atria and were able to establish a reasonably good correlation between Ca\(^{45}\) uptake and changes in resting tension induced by ouabain. Recently, Lillicmnn and Holland\(^3\) found a small but significant increment in an exchangeable Ca fraction in guinea pig atria during the positive inotropic effect of the drug.

It is known that the rat heart is very resistant to cardiac glycosides. However, Reiter in 1956,\(^4\) was able to show that if the concentrations of the glycoside were raised sufficiently both the positive inotropic effect and contracture (increase in resting tension) could be observed. In the light of these investigations we decided to study the effects of ouabain on Ca\(^{45}\) uptake and tissue Ca content in rat atria.

**Methods**

Measurements were made on spontaneously beating rat atria ranging in weight from 28 to 117 mg. The preparations equilibrated for at least 60 minutes in a modified Tyrode's solution of the following composition: NaCl 133 mm; KCl 5.4 mm; CaCl\(_2\) 2.2 mm; NaHCO\(_3\) 24 mm; succrose 12.5 mm; dextrose 10.9 mm; NaH\(_2\)PO\(_4\) 0.11 mm. The solutions were gassed with 95% O\(_2\)-5% CO\(_2\) at 30°C. In experiments in which Ca\(^{45}\) uptake was determined, atria with 1 g weight attached were suspended in 200 ml of modified Tyrode's solution. Not more than four atria were placed in each bath. Following a 60-minute equilibration period the atria were transferred to a second bath containing the same volume of modified Tyrode's solution labeled with Ca\(^{45}\) with and without varying concentrations of ouabain. After a one-hour incubation period they were transferred to a third beaker containing inactive solution of the same composition for 35 minutes. Tissue Ca was determined on a separate group of atria in order to avoid atmospheric contamination with isotope. The time schedule in the experiments in which tissue Ca was estimated was identical to that of Ca\(^{45}\) uptake studies with the exception that non-radioactive medium was used. The remaining procedures for determining the Ca\(^{45}\) uptake and tissue Ca content are exactly those described by Lillicmnn and Holland in an earlier series of studies.\(^3\) Ca\(^{45}\) uptake is expressed as cpm/kg wet tissue following a 15-minute washout period in non-radioactive Tyrode's solution adjusted to 100,000 cpm/ml of medium. Tissue Ca content is expressed in mEq/kg wet tissue corrected for extracellular space. For these calculations we have taken the inulin space measurements of Barclay, Hamley and Houghton.\(^5\) The value employed was 23 ml/100 g tissue. We have also determined the inulin space in rat atria by the method of Ross and Mokotoff\(^6\) and obtained a value of the same order of magnitude. Ouabain in concentrations used had no significant effect on this measurement. Meehanograms were obtained using a Grass strain gauge and Sanborn recorder. Atria were fixed to a glass rod and attached to the arm of the strain gauge and 1 g tension was applied. In this investigation 240 atria were used. Ca\(^{45}\) was obtained from the Oak Ridge National Laboratories as the chloride.

**Results**

In table 1 we have summarized our data on the effects of varying concentrations of ouabain on Ca\(^{45}\) uptake (column 3) and tissue Ca content (column 5). From these two values it was possible to calculate the specific activity of the tissue as cpm/Eq at the end of one hour's incubation (column 7). From the knowledge of the specific activity of the medium we then estimated the per cent of total tissue Ca that had exchanged with that in medium at the end of this interval (column 8). After one hour incubation, tissue Ca was
Effects of Varying Concentrations of Ouabain on Ca45 Uptake and Tissue Ca Content

<table>
<thead>
<tr>
<th>Ouabain M</th>
<th>Effect</th>
<th>cpm/kg × 10^6</th>
<th>P</th>
<th>Ca mEq/kg × 10^6</th>
<th>P</th>
<th>% exchange</th>
</tr>
</thead>
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<td>...</td>
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<td>...</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>1 × 10^-7</td>
<td>...</td>
<td>1.71 ± 0.09</td>
<td>...</td>
<td>11.61 ± 0.38</td>
<td>...</td>
<td>1.47 ± 7</td>
</tr>
<tr>
<td>6 × 10^-8</td>
<td>...</td>
<td>1.50 ± 0.09</td>
<td>&lt;0.01</td>
<td>11.88 ± 0.50</td>
<td>&lt;0.05</td>
<td>1.50 ± 6</td>
</tr>
<tr>
<td>1 × 10^-5</td>
<td>positive inotropic</td>
<td>2.33 ± 0.25</td>
<td>&lt;0.001</td>
<td>8.37 ± 0.45</td>
<td>&lt;0.001</td>
<td>2.8 ± 12</td>
</tr>
<tr>
<td>3 × 10^-5</td>
<td>positive inotropic</td>
<td>3.13 ± 0.16</td>
<td>&lt;0.001</td>
<td>12.59 ± 1.22</td>
<td>&gt;0.05</td>
<td>3.6 ± 12</td>
</tr>
<tr>
<td>6 × 10^-5</td>
<td>contractures</td>
<td>4.72 ± 0.48</td>
<td>&lt;0.001</td>
<td>13.14 ± 0.97</td>
<td>&gt;0.05</td>
<td>3.6 ± 17</td>
</tr>
</tbody>
</table>

Numbers in brackets: number of atria. Specific activity of medium: 20.8 × 10^6 cpm/mEq. Duration: one hour. Standard errors of specific activities are not presented since tissue counts and Ca content were determined on separate groups of atria.

Estimated to be 11.61 ± 0.38 mEq/kg wet tissue corrected for extracellular space. The percent of total tissue Ca that had exchanged with the Ca of the medium in this time interval was calculated to be 7%. Ouabain in concentrations of 10^-7 M and 6 × 10^-8 M, which had no effect on mechanical activity, caused a slight drop in Ca45 uptake and no significant effect on tissue Ca or on the percent Ca that exchanged during one hour. Ouabain at a concentration of 10^-5 M, which produced only a positive inotropic effect with no change in resting tension, caused a significant increase (36% to 42%) in Ca45 uptake and a drop in tissue Ca content. Similar changes in Ca45 uptake, but not tissue Ca, were noted with 3 × 10^-6 M ouabain. With this concentration both a positive inotropic effect and a slight increase (8% to 10%) in resting tension were observed. With doses (6 × 10^-5 M and 3 × 10^-5 M) producing severe contractures (30% to 50% increase in resting tension), a sharp rise in Ca45 uptake occurred (87% to 170% increase). There appeared to be a progressive rise in tissue Ca content to levels above the controls. However, these changes were not significant by statistical analysis.

In the present study, we noted no significant change in wet weights of atria during the positive inotropic effect or contracture. Furthermore, the concentrations of ouabain causing a positive inotropic effect had no effect on the spontaneous rate of beat. With the higher doses producing severe contracture, arrhythmias were noted.

Discussion

In an earlier study, Lemann and Holland observed a small but significant increase in the rate of Ca45 uptake in guinea pig atria during the positive inotropic effect of ouabain. With the onset of contracture, there was a sharp increase in Ca45 uptake and a small but significant rise in tissue Ca content. From the data it was estimated that approximately 18% of tissue Ca had exchanged with that in the medium at the end of one hour incubation in control atria under the conditions employed (see Methods). Values of 30% and 40% were obtained during the positive inotropic effect and contracture, respectively. Guinea pig atria are relatively sensitive to cardiac glycosides and can be grouped in the same class with dog, cat and rabbit. With these species, the positive inotropic effect is seen with doses of ouabain ranging from 10^-7 to 5 × 10^-4 M.

The rat, on the other hand, is considerably more resistant to the glycosides. However, we...
have observed, as did Reiter,\textsuperscript{4} that in concentrations ranging between $10^{-8}$ and $3 \times 10^{-9}$ an positive inotropic effect was noted. Thus, rat atria appear to be 100- to 200-fold more resistant to this agent than guinea pig atria. The difference in the LD$_{50}$ of ouabain for the two species is of the same order of magnitude.\textsuperscript{7}

As was the case with guinea pig,\textsuperscript{3} a small increase of Ca\textsuperscript{45} uptake was noted in rat atria during the positive inotropic effect of ouabain. In addition, we noted a drop in tissue Ca of the order of 20%. With contracture, uptake rates rose sharply and tissue Ca increased slightly, but the latter was not a significant change. At the end of the one-hour incubation period approximately 7% of tissue Ca had exchanged in control atria, and 13% and 17% during the positive inotropic effect and contracture, respectively.

The drop in tissue Ca content during the positive inotropic effect requires comment. A similar effect was noted by Klaus, Kuschinsky and Lüllmann.\textsuperscript{8} A smaller decrease was reported by Lüllmann and Holland\textsuperscript{3} in guinea pig atria. On comparing the data of Klaus et al. with those reported here, it can be seen that the Ca concentration in the tissue of both preparations is 2- to 3-fold greater than that in the medium. The lower gradient reported by Lüllmann and Holland might explain the smaller change in tissue Ca noted by these authors.

The drop in tissue Ca content could be interpreted as the binding of an extremely labile fraction of tissue Ca. Niedergerke\textsuperscript{9} proposed that the fraction of tissue Ca involved in the normal contractile response of the frog's heart was a rapidly exchanging one and probably located at or near the cell surface. Shanes\textsuperscript{10} theorized that this component was a part of the sarcoplasmic reticulum. It is possible that ouabain is affecting Ca exchange in these cellular fractions. However, no specific information is available on this point at the present time.

There has been considerable speculation on mechanisms responsible for species variation in sensitivity to cardiac glycosides. The only information we have on this problem is the observation that tissue Ca content of rat atria is almost double that of guinea pig atria, and that the exchange rate of Ca in rat atria is one-third that in guinea pig atria.

**Summary**

As was the case with guinea pig atria, ouabain induced a small but significant increment in Ca\textsuperscript{45} uptake and a drop of tissue Ca content in rat atria during the positive inotropic effect. In ouabain contractures, the uptake rate increased 87% to 190% and there was little increase in tissue Ca. Concentrations of the glycosides required to produce a positive inotropic effect and contracture in rat atria were 100- to 200-fold greater than those required in guinea pig atria.

**Reference**

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Content in Rat Atria

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