Binding of the drug to both preparations reached an equilibrium in 10 minutes. The table gives such an equilibrium value and demonstrates that the cell membrane exhibits very poor capacity for binding of ouabain and the capacity in the membrane is approximately one-tenth that found for the SR.

From the comparison of the results on heart muscle between Tables 1 and 2, it would be interesting to note that binding of ouabain to SR fr. (Table 1) was almost of same value regardless of whether ouabain was given to muscle extracellularly or intracellularly. On the basis of the relationship, it could be said that quantity of ouabain in cell membrane of the Langendorff heart must be very small at the positive inotropic stage.

How the present results should be integrated for understanding the mechanism in the positive inotropic effect of ouabain is at present not conclusive, but the following could be pointed out: 1) Bound ouabain in cell membrane cannot be excluded, since the drug is considered to exist there already at the stage with the positive inotropic effect alone. 2) SR-bound ouabain which reaches through cell membrane is also interesting, since not only the quantity is high to show an intense affinity, but also the SR is an important store-site of Ca that is considered to control the contractile machinery of muscle cell. The second view agrees well with the previous work (7) on ouabain-microinjection that, even in skeletal muscle in which ouabain does not permeate through cell membrane and not give a positive inotropic effect, the drug applied intracellularly can potentiate contractile responses.

REFERENCES


EFFECT OF COLD STRESS ON ACETYLCHOLINE CONTENT OF RAT MYOCARDIUM AND ITS MODIFICATION BY ATROPINE

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The present communication deals with the effect of cold stress on the acetylcholine content of rat myocardium and its modification by atropine.

Twenty-eight albino rats of both sexes, weighing between 90 and 150 g, were divided into 4 groups. Animals in the first group served as control. The rats of the second
group were exposed to cold stress by the method of Raab et al. (1). They were put in ice cold water (3°C±0.5°C) and allowed to swim thrice during 24 hours for 3 minutes each time. The third group received 1 mg/kg subcutaneously of atropine twice daily for three days. In the fourth group cold stress was given to rats pretreated with atropine on the third day as described in group second. On completion of the above procedures, the animals were sacrificed by stunning and the hearts were removed, cleaned and utilized for estimation of acetylcholine content by the method of Anand et al. (2). The extraction of acetylcholine was carried out in 5 ml of cserinized Ringer Solution at pH 4 at a temperature of 90–100°C. The assay was done biologically on the frog's rectus abdominis muscle preparation.

### Table 1. Acetylcholine content of rat's myocardium (μg/gm of fresh tissue).

<table>
<thead>
<tr>
<th>Group</th>
<th>Procedure</th>
<th>No. of rats</th>
<th>Mean</th>
<th>± S.E.</th>
<th>P* Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Control</td>
<td>8</td>
<td>3.01</td>
<td>0.09</td>
<td>—</td>
</tr>
<tr>
<td>II</td>
<td>Cold stress</td>
<td>8</td>
<td>4.54</td>
<td>0.09</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>III</td>
<td>Atropine</td>
<td>6</td>
<td>0.14</td>
<td>0.05</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>IV</td>
<td>Atropine + Cold stress</td>
<td>6</td>
<td>2.70</td>
<td>0.27</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

* Mean value of group II and III was compared with group I and that of group IV was compared with group II.

The results, as summarized in Table 1, indicate that cold stress produces a significant increase in the acetylcholine content of rat myocardium while atropine causes significant reduction in the acetylcholine content. The former results are in agreement with the findings of other workers in rats (3, 4) and in rabbits (5). A reduction in the brain acetylcholine level, caused by atropine, has been attributed to its ability to induce increased release and/or output of brain acetylcholine (6–8). It is, therefore, suggested that in the present series of experiments, a similar mechanism may be involved in causing reduction of acetylcholine content of the heart by atropine. Further, the present study reveals that atropine prevents the rise of acetylcholine in animals, exposed to cold stress.

### REFERENCES