Acetylcholine and Electrolyte Metabolism in the Various Chambers of the Frog and Turtle Heart

By Paul Mazel, M.S., and William C. Holland, M.D.

Evidence has accumulated suggesting acetylcholine may be involved in initiation of the heart beat. Acetylcholine and electrolyte metabolism in the various chambers of the heart have been studied. The area of highest intrinsic rhythm (sinus) contained greater amounts of acetylcholine equivalents, true cholinesterase, choline acetylase and sodium; all of these decreasing in amount in areas of lower intrinsic rhythm. It is concluded that intrinsic rhythm may be directly correlated with overall acetylcholine metabolism and sodium content and inversely related to overall energy metabolism and potassium concentration.

Over the past two decades evidence has accumulated which suggests that acetylcholine plays a role in the initiation of the heart beat. As early as 1937, Sachs found that small doses of acetylcholine exerted a stimulant effect on rabbit atria and Welsh in 1938 reported that small amounts of acetylcholine increased the rate of the crab heart. Later Abdon and Hammarskjold reported on an acetylcholine precursor which yielded free acetylcholine upon heating or acidification. The mechanical activity of the heart appeared to vary directly with the amount of precursor present. The findings have been extended and confirmed by Burn, Spadolini and coworkers. It has been suggested by the latter group of investigators that acetylcholine induces the automatic rhythm and regulates the rate and force of beat.

Recently, it has been postulated by Holland that acetylcholine might act by virtue of its ability to increase membrane permeability to sodium and potassium ions. Thus the intrinsic metabolism of acetylcholine in pacemaker tissue may be of such a nature that periodic changes in permeability occur which give rise to impulses at regular intervals. If the above hypothesis be true then one would expect that the region of the heart with the higher intrinsic rhythm would exhibit a greater overall metabolism of acetylcholine.

There have been scattered observations on acetylcholine metabolism in the various regions of the heart. In general, these studies were incomplete in that most of them have been confined to atria and ventricles. In order to pursue the matter further we have made a study of the acetylcholine and electrolyte metabolism in the several chambers of the frog and turtle heart.

METHODS

The turtle (Pseudemys scripta) and the bull frog (Rana catesbiana) were used in all experiments. In these studies pooled sinuses, atria and ventricles were used. Therefore, in a number of cases, only mean values are presented and no statistical analysis of the data was undertaken. The acetylcholine content of the various chambers was determined by quickly excising the tissue, weighing and homogenizing in ice cold 10 per cent trichloroacetic acid (TCA) 3 to 4 ml./Gm. tissue. After centrifugation, TCA was removed by extraction with ether and the solution then freed of ether by aeration. Aliquots of the extract were neutralized with a few drops of saturated sodium bicarbonate solution just prior to assay on the eserinized (1.0 X 10⁻⁵) frog rectus abdominis muscle. The acetylcholine equivalent was determined using known amounts of acetylcholine bromide as standard. Precautions of Feldberg and Hebb were observed and the extract again tested after alkalization with 1.0 N NaOH and heating.

Choline acetylase activity was determined by a modification of the method described by Hebb and Waites. Acetone dried powders were pre-
Table 1.—*Acetylcholine Metabolism of the Sinus, Atria and Ventricle of the Turtle Heart.
Average of 5 Observations

<table>
<thead>
<tr>
<th>Turtle tissue</th>
<th>ACh equiv. (µg./Gm. (Wet weight))</th>
<th>Cholinesterase activity µg. Ch. equiv./Gm./hr.</th>
<th>Choline acetylase µg. ACh equiv. formed/Gm. powder/hr.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sinus venosus</td>
<td>5.0 (3.15 - 8.30)</td>
<td>480</td>
<td>280 (263 - 291)</td>
</tr>
<tr>
<td>Atria</td>
<td>2.0 (1.53 - 3.0)</td>
<td>154</td>
<td>188 (126 - 316)</td>
</tr>
<tr>
<td>Ventricle</td>
<td>0.99 (.51 - 2.29)</td>
<td>81</td>
<td>104 (54.0 - 160.0)</td>
</tr>
</tbody>
</table>

Results

The inherent rhythmicity of various heart areas has been studied for many years. It is now generally accepted that the rate of intrinsic beat of the sinus is greater than that of the atria while the latter is, in turn, above that of the ventricle.

The quantity of acetylcholine (ACh), cholinesterase and choline acetylase activities in the various chambers of the frog and turtle heart are summarized in tables 1 and 2. It should be noted that the ACh equivalent in the sinus is more than twice that of the atria and the atria, in turn, greater than the ventricle. The cholinesterase activities show a similar distribution pattern as did choline acetylase activity.

The results on the frog shown in table 2 presented a similar pattern. Acetylcholine equivalent in the sinus was greater than that of the atrium and the atrium above that of the ventricle. The frog ventricle displayed a proportionately greater ACh equivalent than did the turtle ventricle.

Table 3 summarizes the studies on cation distribution in the turtle heart. It should be noted that the sodium content of the sinus is much greater than the atria and the atria.
TABLE 3.—Cation Distribution in the Various Chambers of the Turtle Heart, Determinations Made on Trichloroacetic Acid Extract of 6 Pooled Sinuses, Atria and Ventricles

<table>
<thead>
<tr>
<th>Turtle tissue</th>
<th>Na⁺ (mM/Kg., tissue)</th>
<th>K⁺ (mM/Kg.)</th>
<th>Na⁺/K⁺</th>
<th>Ca²⁺ (mM/Kg.)</th>
<th>Mg²⁺ (mM/Kg.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sinus venosus</td>
<td>129.5</td>
<td>56.8</td>
<td>2.29</td>
<td>3.5</td>
<td>2.3</td>
</tr>
<tr>
<td>Atria</td>
<td>64.00</td>
<td>57.8</td>
<td>1.11</td>
<td>3.5</td>
<td>3.2</td>
</tr>
<tr>
<td>Ventricle</td>
<td>49.8</td>
<td>66.0</td>
<td>0.75</td>
<td>1.4</td>
<td>5.0</td>
</tr>
</tbody>
</table>

in turn, greater than the ventricles. The reverse is true with potassium; potassium being greatest in the ventricle but approximately the same in the atria and sinus. The sodium-potassium ratio shows a progressive decline from sinus to atria to ventricle. Similar sodium and potassium distributions were observed in the frog heart (table 4).

The high concentration of Na in the sinus could be the result of a large extracellular space in this tissue. This seems unlikely in view of the data presented in table 5. In these experiments, sinuses and atria were incubated for one hour in an Na deficient medium (28 mMolar). Isotonicity was maintained by the addition of sucrose. Even though incubating the tissues in a low Na medium reduces the Na content in both chambers, the ratio of Na concentration in the sinus to that in the atria remains essentially the same. (Compare data in table 5 with those in table 3.)

Calcium was found to be identical in amount in the sinus and atria with far less in the ventricle. With respect to magnesium, the content was similar in the sinus and atria but greater in the ventricle.

Figure 1 is a summary of some of the data. Here acetylcholine equivalents, cholinesterase and choline acetylase activities and Na/K ratios are plotted against intrinsic rhythm of the various chambers. It should be noted that the tissue with a higher inherent rhythm (sinus) contains a greater amount of ACh equivalents, cholinesterase and choline acetylase activity and higher sodium-potassium ratio; all of these factors decreasing in amount in those areas of lowered inherent rhythm (atria and ventricle).

**DISCUSSION**

On more than one occasion, attempts have been made to correlate intrinsic rhythm of the various chambers of the heart with metabolic patterns in these separate regions. For example, glycogen is said to be highest in pacemaker tissue. On the other hand, an inverse correlation has been found for succinic dehydrogenase, oxygen consumption, and content of adenosine and its derivatives.

**TABLE 4.—Cation Distribution in the Various Chambers of the Frog Heart, Determinations Made on Trichloroacetic Acid Extract of 6 Pooled Sinuses, Atria and Ventricles**

<table>
<thead>
<tr>
<th>Frog tissue</th>
<th>Na⁺ (mM/Kg.)</th>
<th>K⁺ (mM/Kg.)</th>
<th>Na⁺/K⁺</th>
<th>Ca²⁺ (mM/Kg.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sinus venosus</td>
<td>124</td>
<td>44.5</td>
<td>2.79</td>
<td>1.8</td>
</tr>
<tr>
<td>Atria</td>
<td>87</td>
<td>42.2</td>
<td>1.97</td>
<td>1.8</td>
</tr>
<tr>
<td>Ventricle</td>
<td>49.7</td>
<td>65.2</td>
<td>0.78</td>
<td>2.0</td>
</tr>
</tbody>
</table>

**TABLE 5.—The Effect of Na Deficient Medium on the Na and K Content of Sinuses and Atria of the Turtle Heart. Temperature 27 C. Na Concentration in Medium: 28m Molar. Duration of Incubation 1 Hour. Medium Changed Every 10 Minutes**

<table>
<thead>
<tr>
<th>Tissue</th>
<th>No. of experiments</th>
<th>Cations (Mm/Kg., wet tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Na</td>
</tr>
<tr>
<td>Sinus</td>
<td>8</td>
<td>68.6 ± 12</td>
</tr>
<tr>
<td>Atria</td>
<td>8</td>
<td>36.4 ± 8</td>
</tr>
</tbody>
</table>
In fact, the work load of the several chambers bears a direct correlation with overall energy metabolism. In the present study evidence is presented which shows a direct relation between the rate of intrinsic rhythm and acetylcholine metabolism. On the other hand, these investigations may well represent a comparison of the distribution of nervous elements in these regions of the heart. This is reasonable in light of the known role of acetylcholine in nervous activity. However, it has been shown histochemically and enzymatically that pacemaker and Purkinje tissue contain large amounts of acetylcholine. In addition, acetylcholine is not always associated with nervous tissue as evident by the large amounts found in the placenta and spleen. Burn has recently reported the presence of the acetylcholine-cholinesterase system in the gill plates of the mussel Mytilus edulis, a nerveless structure. Girvin and Stevenson have described a choline acetylating system in Lactobacillus plantarum.

The presence of large amounts of sodium in pacemaker tissue is very interesting. Similar findings have been described by Davies et al. for the ox heart. This could be the result of the high intrinsic metabolism of acetylcholine in the sinus venosus as it is known that acetylcholine will release potassium from and cause an uptake of sodium by myocardial tissue. However, the full significance of these findings requires further study.

Although the evidence at present is circumstantial in nature, it is quite possible that synthesis and destruction of acetylcholine with its known effects on cell permeability may play a fundamental role in the genesis of the heart beat.

**SUMMARY**

Acetylcholine metabolism, including cholinesterase and choline acetylase, in the various chambers of the frog and turtle heart have been studied. Cation distribution (Na⁺, K⁺, Ca²⁺, Mg²⁺) have been included. In both species the ACh equivalents (Gm/wet wt.) were as follows: turtle, sinus 5.0, atria 2.0, ventricle 0.99; frog, 5.0, 3.1, 2.9. True cholinesterase values (µl/CO₂/Gm./hr.) were: turtle, 480, 154, 81; frog, 794, 280, 132, respectively. Choline acetylase activities (µg/ACh formed/Gm. powder/hr.) in the turtle were: 280, 188, and 31.8. Sodium content for both species was highest in the sinus, less in the atria and least in the ventricle. The reverse was true for potassium. Calcium and magnesium contents of both sinus and atria were approximately the same. It is concluded that intrinsic rhythmicity of the various chambers may be directly correlated with over-all ACh metabolism and sodium content, and inversely related to over-all energy metabolism and potassium concentration.
REFERENCES
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