Studies of Cat Heart Muscle during Recovery after Prolonged Hypothermia

HYPERPOLARIZATION OF CELL MEMBRANES AND ITS DEPENDENCE ON THE SODIUM PUMP WITH ELECTROGENIC CHARACTERISTICS

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ABSTRACT

Cat heart muscles preserved in normal Tyrode’s solution at 4°C for prolonged periods were investigated by recording the transmembrane potentials after rewarming to 37°C. The cell membranes were gradually hyperpolarized during rewarming, the maximum hyperpolarization being reached within a few hours. The amplitude of resting potentials obtained at a maximally hyperpolarized stage was largest for ventricular muscle that had been preserved for 20 hours at 4°C and for atrial muscle that had been preserved for about 50 hours at 4°C. The maximum potentials averaged 267.7 mv for the ventricles and 184.4 mv for the atria. KCl at 10× normal and epinephrine and norepinephrine in final concentrations of 2.5×10⁻⁷ g/ml brought about a marked hyperpolarization of the cell membrane when the membrane potential of the muscles was declining. The cells of muscles loaded with Li had a greater than normal membrane potential for an hour during gradual equilibration over a range of 4 to 22°C before rewarming. When muscles were rewarmed to 37°C in Li-Tyrode’s solution the membrane potential gradually decreased to 5 to 23 mv for the right atrium and 13 to 34 mv for the right ventricle. Ouabain 10⁻⁵ and 10⁻⁶ M abolished the hyperpolarization and subsequently depolarized the cell membrane. DNP (0.2 and 0.6 mM) and sodium azide (6 mM) also had an inhibiting effect on the hyperpolarization. The observations are consistent with a hypothesis of an electrogenic sodium pump, which produces hyperpolarization whose magnitude depends largely on the previous length of time the tissue was preserved in the hypothermic state.

ADDITIONAL KEY WORDS

recovery course during rewarming maximum hyperpolarization nonsteady state preservation period

Preservation of the heart by prolonged cooling is a problem to cardiac surgeons interested in cardiac transplantation. Because of this, we undertook an in vitro study of the heart during recovery after prolonged preservation at 4°C. The first step was an investigation of the hyperpolarization of the cell membrane that occurred during rewarming.

Recently it has been emphasized that the hyperpolarization of muscle and nerve cells enriched with Na⁺ during recovery is presumably due to an “electrogenic” sodium pump which increases the negative transmembrane potential by moving net electrical charges out of the cell. Experiments supporting such a hypothesis have been performed, especially in frog sartorius muscle, during rewarming in recovery fluids containing a high concentration of K⁺ (10 mM). Under these conditions the membrane potential is greater than the potassium equilibrium potential, a marked extrusion of sodium takes...
place, and the difference between these potentials is reduced or abolished by treatments which interfere, directly or indirectly, with the sodium pump (1-8). Experiments on post-tetanic hyperpolarization in nonmyelinated nerves (9-11) and with the injection of Na ions into nerve cells of snails (12) have also given evidence for an electrogenic sodium pump. Page and co-workers (13, 14) have presented evidence for a sodium pump in the papillary muscle of cat heart loaded with Na⁺ by preincubation at 2 to 3°C; they followed the membrane potential after abrupt rewarming to 27 to 28°C and suggested that rewarming may activate a ouabain-sensitive electrogenic mechanism, most probably that concerned with the net active transport of Na⁺ out of the cell, and that net K⁺ uptake follows passively.

In the present experiments, unlike those of the other authors, cat's heart muscle was incubated in normal Tyrode's solution at 2 to 5°C for several hours to 10 days and then rewarmed in the same solution without increasing the [K⁺]. The original purpose of the experiments was to learn how long the muscle could be preserved in the hypothermic state with subsequent survival and what events would happen during recovery. This paper describes a marked hyperpolarization of the cell membranes during rewarming and its dependence on the electrogenic sodium pump.

Methods

Whole hearts from either kittens or adult cats anesthetized with pentobarbital were excised and transferred for cooling into a beaker of oxygenated normal Tyrode's solution. The beaker containing the heart was covered with a sheet of vinyl before transferring it into a refrigerator at 4°C. Throughout the preservation period, the temperature of the solution was maintained between 2 and 5°C. After a desired period, either the right atrium or a strip of the free wall of the right ventricle was dissected. Usually these tissues were placed in cold normal Tyrode's solution, which was continuously oxygenated and then gradually warmed to room temperature over a period of 30 minutes or more. Then the preparation was fixed in the chamber, with its endocardial surface upward; it was warmed to 37°C ± 0.5 and kept at that temperature. The solution was aerated with 97% O₂-3% CO₂. The preparation was illuminated from below to permit microscopic observation. After warming to 37°C and occasionally after warming to room temperature, microelectrode measurements were made.

Floating microelectrodes filled with 3 x 10⁻⁴ M KCl and with a resistance of about 30 megohms were used to measure membrane potentials. The tip potential of the electrodes was not always checked because of the evidence that the potential usually was changed by only 2 mv or less by fiber impalement (15, 16). The membrane potentials were displayed on a dual beam oscilloscope (Nihonkoden VC-6) and photographed on 35-mm film; they were also recorded on an inkwriting recorder.

The normal Tyrode's solution contained, in mM, 136.8 NaCl, 2.68 KCl, 1.8 CaCl₂, 0.49 MgCl₂, 11.9 NaHCO₃, 3.3 NaH₂PO₄, and 5.6 glucose. Various solutions affecting the sodium pump or metabolism of the cells were made up as follows. High K⁺-Tyrode's solution was made by adding KCl and decreasing the NaCl concentration by the same amount. For Li⁺-Tyrode's solution LiCl completely replaced NaCl. Epinephrine and norepinephrine were injected into the chamber after stopping the flow to produce a final concentration of either 10⁻⁶ or 10⁻⁷ g/ml (corresponding to approximately 6 x 10⁻⁴ and 6 x 10⁻⁷ M). Ouabain (G-strophanthin) was also injected in the same way, except that the final concentration was either 10⁻⁵ or 10⁻⁶ M. DNP (2-4 dinitrophenol) and sodium azide were dissolved in Tyrode's solution to produce final concentrations in the reservoir of 0.2 or 0.6 mM for the former and of 6 µM for the latter.

Results

I. HYPERPOLARIZATION DURING REWARMING

To follow the recovery of the right atrial and ventricular muscles in normal Tyrode's solution during rewarming to 37°C after different periods of preservation at 4°C, the resting potentials were measured. Examples for muscles stored at 4°C are shown in Figure 1. In this figure, as well as in all the following figures, mean and maximum values of resting potential obtained at each recovery stage are shown by solid and broken lines, respectively (for the dispersion of the values, see Discussion). Zero time on the graphs shows the time when the preparation was taken out of the refrigerator. Usually the amplitude of the resting potentials was smaller before, and for a period after, rewarming than in control muscles (Fig. 1, A and C), though in some
preparations the resting potentials were larger, even at a lower temperature than the controls (Fig. 1, B and D). After this initial period, the cell membranes gradually hyperpolarized with time, the maximum hyperpolarization being reached within a few hours after the muscle was rewarmed. Following this increase, the resting potentials tended to decrease and continued to do so for several hours; a typical recovery course is shown in Figure 1, A. The time required to attain the maximum hyperpolarization was usually less than 3 hours, though the time varied with the period at 4°C or according to the preparation. After longer periods at 4°C, more time was required within a limit, in both atrial and ventricular muscles, before the maximum resting potentials were obtained. The maximum hyperpolarization obtained during recovery from cooling was much greater than that reported by any other authors.

The mean resting potential value at the maximally hyperpolarizing stage during rewarming varied from 67.4 ± 1.1 to 267.7 ± 16.4 mv in both atrial and ventricular muscles (Table 1). Such a marked hyperpolarization led us to consider an electrogenic sodium pump as its basis, since the membrane potential is too high to be due to the potassium equilibrium potential alone. Table 1 shows that the maximum hyperpolarization was greatest at about 20 hours for the ventricular muscle and at about 50 hours for the atrial (the data of Figure 1, C are excluded from Table 1), while at the shorter periods in the cold it was at the level of approximately

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normal resting potential for both preparations. In hyperpolarized cells that were spontaneously active, action potentials with or without an overshoot were observed. There were more spontaneously active cells among those having resting potentials close to normal values than among either the hyperpolarized or depolarized cells; resting potentials obtained from spontaneously active cells are shown by the closed circles in all the figures. An exceptional case in which almost all the hyperpolarized cells were spontaneously active is given in Figure 1, C.

II. HYPERPOLARIZING EFFECTS ON THE CELL MEMBRANES

Excess external K+ stimulates the sodium pump (17, 18), and epinephrine and norepinephrine increase cellular metabolism (19). To examine the effect of excess K+ on the resting potential, we used right atria stored for 175 hours at 4°C in which the muscle cells were only slightly hyperpolarized during rewarming. When the cells were in the declining phase of resting potentials during the rewarming period, the perfusing fluid was switched to the solution with excess K+ (26.8 mm). The cell membranes were depolarized from 67.0 to 56.1 mv resting potential immediately after changing the solution and then again hyperpolarized by 40 mv (Table 2 and Figure 2). This shows that the marked hyperpolarization is not due to the passive distribution of potassium. After the maximum

TABLE 1
Membrane Potentials (mv) of Ventricular and Atrial Muscles Obtained at a Maximally Hyperpolarizing Stage during Recovery at 37°C

<table>
<thead>
<tr>
<th>Hours preserved at 4°C</th>
<th>R. ventricle (11 cats)</th>
<th>R. atrial appendage (10 cats)</th>
<th>S-A and A-V nodes (9 cats)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>87.3 ± 0.9 (16)</td>
<td>83.7 ± 1.2 (34)</td>
<td>67.4 ± 1.1 (19)</td>
</tr>
<tr>
<td>4.5</td>
<td>71.1 ± 1.7 (19)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12.5</td>
<td>106.5 ± 2.2 (17)</td>
<td>80.5 ± 1.7 (15)</td>
<td>73.5 ± 2.2 (9)</td>
</tr>
<tr>
<td>19</td>
<td>267.7 ± 16.4 (10)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>107.3 ± 7.5 (10)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>20.3</td>
<td>229.9 ± 13.7 (17)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>23.4</td>
<td>145.6 ± 4.5 (21)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>23.7</td>
<td>207.3 ± 5.2 (23)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>26</td>
<td>212.7 ± 19.4 (10)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>43</td>
<td>103.7 ± 3.2 (8)</td>
<td>112.5 ± 2.8 (6)</td>
<td></td>
</tr>
<tr>
<td>44.3</td>
<td>148.3 ± 6.6 (15)</td>
<td>103.9 ± 15.5 (4)</td>
<td></td>
</tr>
<tr>
<td>46</td>
<td>157.1 ± 5.2 (9)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>50</td>
<td>179.2 ± 2.7 (14)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>51</td>
<td>184.4 ± 3.2 (18)</td>
<td>151.4 ± 5.4 (13)</td>
<td></td>
</tr>
<tr>
<td>74</td>
<td>168.6 ± 1.5 (10)</td>
<td>105.3 ± 4.8 (6)</td>
<td></td>
</tr>
<tr>
<td>144</td>
<td>156.8 ± 4.5 (11)</td>
<td>114.7 ± 3.9 (8)</td>
<td></td>
</tr>
<tr>
<td>169</td>
<td>105.1 ± 3.4 (11)</td>
<td>76.1 ± 7.5 (14)</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ±SE. Numbers in parentheses are number of measurements. Each line represents measurements made on a different cat except for 0 and 15 hours, when 2 cats were studied (1 for measurements on the ventricle and 1 on the atrium and the nodal tissues).

TABLE 2
Hyperpolarizing Effect of Excess KCl (28.8 mm) on Atrial Muscle Cells Preserved for 175 Hours at 4°C

<table>
<thead>
<tr>
<th>Membrane potentials (mv)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before adding KCl</td>
</tr>
<tr>
<td>67.0 ± 4.4 (15)</td>
</tr>
<tr>
<td>After adding KCl</td>
</tr>
<tr>
<td>0-10 min</td>
</tr>
<tr>
<td>58.1 ± 6.1 (9)</td>
</tr>
<tr>
<td>10-40 min</td>
</tr>
<tr>
<td>85.0 ± 3.7 (29)</td>
</tr>
<tr>
<td>47-80 min</td>
</tr>
<tr>
<td>108.9 ± 2.3 (29)</td>
</tr>
<tr>
<td>110-120 min</td>
</tr>
<tr>
<td>89.5 ± 3.8 (15)</td>
</tr>
</tbody>
</table>

Values are mean ±SEM. Numbers in parentheses are number of measurements.
potential was reached, it tended to decline gradually.

The effects of norepinephrine \((2.5 \times 10^{-7} \text{ g/ml})\) on atria that had been stored for 50 to 61 hours are shown in Figure 3, A and B, respectively. Because the measurement of resting potentials in these cases was started about 2 hours after the preparations were taken out of the refrigerator, the cells were initially in a hyperpolarized state. After adding norepinephrine in the phase of declining potentials, the resting potentials showed both rapid (Fig. 3, A) and delayed (B) increases, but in both cases they reached a maximum of approximately 235 mV. Occasionally, after norepinephrine was added, muscle activity was increased. During this time, it was possible to record regular and full spike activity in any area of the muscle. Similar results, which are not shown, were obtained with epinephrine.

These facts indicate that potassium and norepinephrine may act directly or indirectly on the sodium pump mechanism to make the pump more electrogenic.

III. DEPOLARIZING EFFECTS ON THE CELL MEMBRANES

Procedures that interfere with the sodium pump were tried on preparations that showed pronounced maximum hyperpolarization during rewarming. Li ions easily permeate the cell membrane of cardiac muscles (20) and of a variety of other tissues (18), but they are not pumped out of the cell rapidly (21).

In Figure 4, the atrial and ventricular muscles had been stored at \(4^\circ\text{C}\) for 44 and 22 hours, respectively, in a Li\(^+\)-Tyrode's solution, and 90\% of the Na\(^+\) had been replaced with Li\(^+\). The resting potentials were fol-
Resting potential of right atrial (A) and ventricular (B) muscles loaded with lithium before and during rewarming in Li+ Tyrode's solution and after return to normal solution. The atria were preserved at 4°C in Li+ Tyrode's solution and the ventricles for 20 hours. Mean and SEM are given in Table 3.

allowed during the gradual change of the temperature from 4 to 22°C and then to 37°C. In both cases, when the cells were inactive immediately after being rewarmed, the resting potentials which initially were 144.1 and 103.8 mv tended to decline and eventually reached 13.8 and 46.3 mv, respectively, after about 1 hour (Table 3). Return to normal Tyrode's solution from Li-Tyrode's solution brought about either immediate (Fig. 4, B) or delayed recovery (Fig. 4, A) of the resting potentials, depending on the time of the return; the cell membranes were again hyperpolarized. For most cells in the right ventricle, however, the resting potentials returned to a normal level (89.5 ± 4.8 mv), and the cells were spontaneously active, as indicated by the closed circles in B of Figure 4. Further, it should be noticed that the cell membranes in Li+ Tyrode's solution were depolarized at 37°C, but hyperpolarized at 4°C when the sodium pump is considered not to be actively working.

Ouabain, a cardiac glycoside that has been shown to be a specific inhibitor for the sodium pump (12, 22-25), was added to the chamber about 1 hour after warming final concentrations of 10^-5 and 10^-4 M. Examples of its effect was given in Figure 5. With both concentrations of ouabain, the hyperpolarization was immediately depressed and the cell membranes were depolarized. The lowered resting potentials did not recover even after the preparation was returned to normal Tyrode's solution. They declined in a stepwise fashion until they reached approximately 10 mv.

Finally, the effects of uncoupling agents, dinitrophenol and sodium azide, were studied (Table 4). These metabolic inhibitors were

<table>
<thead>
<tr>
<th>Table 3</th>
<th>Depolarizing Effect of Lithium Ions on Membrane Potentials of Ventricular and Atrial Muscle Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Preparation</strong></td>
<td><strong>In Li+ Tyrode's solution</strong></td>
</tr>
<tr>
<td></td>
<td>Min after rewarming to 37°C</td>
</tr>
<tr>
<td>R. ventricle</td>
<td></td>
</tr>
<tr>
<td>(20 hr at 4°C)</td>
<td>0</td>
</tr>
<tr>
<td>0-30</td>
<td>73.7 ± 5.5 (23)</td>
</tr>
<tr>
<td>30-60</td>
<td>48.3 ± 4.1 (48)</td>
</tr>
<tr>
<td>R. atrium</td>
<td></td>
</tr>
<tr>
<td>(44 hr at 4°C)</td>
<td>0</td>
</tr>
<tr>
<td>0-30</td>
<td>148.2 ± 6.7 (17)</td>
</tr>
<tr>
<td>30-60</td>
<td>32.2 ± 6.9 (17)</td>
</tr>
</tbody>
</table>

Values are mean ±SEM. Numbers in parentheses refer to number of measurements.
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### Table 4

<table>
<thead>
<tr>
<th>Drug added</th>
<th>Min from drug</th>
<th>Membrane potentials at 37°C</th>
<th>Hour</th>
<th>Membrane potentials at 37°C</th>
<th>Hour</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaCl (0.2 mM)</td>
<td>155</td>
<td>235 ± 2.3</td>
<td>5-30</td>
<td>183 ± 0.9</td>
<td>0.30</td>
</tr>
<tr>
<td>DNP (0.6 mM)</td>
<td>110</td>
<td>66 ± 3.1</td>
<td>30-60</td>
<td>101.0 ± 4.8</td>
<td>0.30</td>
</tr>
<tr>
<td>NaN₃ (6 mM)</td>
<td>110</td>
<td>25.9 ± 2.9</td>
<td>50-120</td>
<td>101.0 ± 4.8</td>
<td>0.30</td>
</tr>
<tr>
<td>6 mM NaN₃</td>
<td>10</td>
<td>42.4 ± 4.0</td>
<td>30-60</td>
<td>39.8 ± 4.7</td>
<td>40-100</td>
</tr>
</tbody>
</table>

*After rewarming, just before addition of drug*.

### Figure 5

**Effect of 10⁻⁵ and 10⁻⁶ m ouabain on the resting potential of rewarmed right ventricles.**

Applied to the muscle preparations immediately after rewarming in 0.2 mM DNP and also during the course of recovery in 0.6 mM DNP and 6 mM NaN₃. In both cases, the agents either considerably depressed the hyperpolarization or depolarized the cell membranes. The effects of these agents were not as intense as those produced by Li or ouabain. DNP changed the recovery course of the resting potentials during rewarming by depressing the hyperpolarization. The muscle, which had been held for a relatively short period in the cold, developed a tendency to recover when the agents were applied in low concentrations; then, when switched to the normal Tyrode's solution, the cell membrane again hyperpolarized or recovered. Approximately the same result was obtained with the application of sodium azide.
Discussion

It is known that muscle cells in vitro at a low temperature gain Na\(^+\) and lose K\(^+\). On the other hand, there is evidence for many cells, including cardiac muscle, that the active transport of Na\(^+\) is stimulated by increasing Na\(^+\) concentration within the cell and is controlled by the intracellular concentration (26-29). The muscle cells of the heart in this experiment, therefore, are considered to have gained Na\(^+\) and to have lost K\(^+\) in the cold (even though the muscle previously was not soaked in Na\(^+\)-rich and K\(^+\)-free solution) and to have had their Na pumps activated during rewarming.

HYPERPOLARIZATION AND RECOVERY COURSE DURINGREWARMING

Both the grade of hyperpolarization and the recovery course were considerably different from the results given by other authors. Page and Storm (14), using cat papillary muscle, Adrian and Slayman (1), using frog muscle, and Kerkut and Thomas (12), using snail nerve cells, showed a hyperpolarization of 25.7, 14 to 34 and 31 mv respectively (calculated from the data in these papers) that could be attributed to the sodium pump and maximum resting potentials attaining about 100 mv.

The discrepancy between our results and those of Page and Storm obtained with the same species requires explanation. First, the discrepancy in hyperpolarization may be due largely to the difference between the periods at the low temperature and at warm temperature. Page and Storm kept the tissues in the cold for only 1 or 2 hours; we preserved them for several hours to 10 days. Table 1 shows that the hyperpolarization was maximum at about 20 hours for ventricular preparations and at about 50 hours for atrial ones, while at the shorter periods the resting potential was approximately normal for both preparations, as Page and Storm observed. Further, the muscle preserved for such long periods might be in a nonsteady state during rewarming to a greater extent than it would be after a very short period in the cold. It has been suggested that the cells enriched in Na\(^+\) by any means would be in a nonsteady state and the electrogenic sodium pump would be more active than in a steady state (12, 14). The resting potentials obtained during rewarming were far greater in amplitude than normal (Fig. 1 and Table 1). Therefore, this would indicate that the hyperpolarization must be mainly due to the electrogenic sodium pump acting during extremely nonsteady states, and the discrepancy may be due to the difference of the state of the cells.

The second reason for the discrepancy in the degree of hyperpolarization could come from both the difference in the size of the preparations and the region in the heart. Because the amplitude of resting potentials varied in cells from different areas of the muscle, this could also be one of the reasons for dispersion of resting potentials. Other causes for the dispersion may be the length of the preservation period, i.e., depth at which the cells were located and the degree of injury to the cells.

Third, problems related to the origin of the hyperpolarization itself, and discussed by the other authors, may account for the discrepancy. Before discussing these problems, the recovery course of resting potentials as shown in Figure 1, will be discussed briefly here.

Page and Storm (14) observed that in papillary muscle incubated at 2 to 3°C for 1 to 2 hours, the recovery of membrane potential was complete within 10 minutes, although the intracellular K\(^+\) concentration did not show parallel recovery. Frumento (3) obtained similar results in frog skeletal muscle kept overnight in the cold. On the other hand, the figures given by Cross, Keynes and Rybova (2) and by Adrian and Slayman (1) for frog skeletal muscle soaked for 25 hours at approximately 0°C and for 1 or 2 days at 0 to 2°C, respectively, apparently show that the maximum hyperpolarization was reached within an hour after rewarming. In the present report on cat heart muscle, the time required to attain the maximum hyperpolarization after rewarming to 37°C was usually within 3 hours. It was longer than the time reported.
by the authors mentioned above, although the temperature of our rewarmed solution was higher than that of their recovery solution used for cat papillary (14) and frog skeletal (1, 3) muscles. Moreover, the time to maximum resting potential varied with changes of either the soaking period in the cold or the preparation. Since the longer the soaking period, the longer was the time required up to a limit, it might be that this limiting time would be needed to drive the electrogenic sodium pump maximally at a given temperature.

DEPENDENCE OF THE HYPERPOLARIZATION ON ELECTROGENIC SODIUM PUMP

Although the cellular ion contents were not measured, it seems certain that the maximum hyperpolarization (more than 200 mv) cannot possibly be interpreted by the potassium equilibrium potential given by $E_k = 61.4 \log \left[\frac{[K]}{[K]_o}\right]$, at $37°C$. If the potential were governed only by the passive distribution of potassium, the ratio of $[K]_o$ to $[K]$ for the resting potential amplitude of 300 mv would be in the order of $10^{-4}$ at which $[K]_o$ must be nearly zero at the cell surface even in the normal Tyrode’s solution. Further, this conclusion is supported by the enhancing action of the high KCl solution on the resting potential, which had been approximately at the normal level (Fig. 2). Kerkut and Thomas (12) observed an inhibiting action of reducing external potassium concentration on the hyperpolarization in nerve cells enriched in Na$^+$. On the other hand, many authors have observed that during recovery the values of $[K]^+_i$ and $[Na]^+_i$ were not restored to physiological levels long after the membrane potential had been returned to normal and the internal potential had become more negative than the estimated $E_k$. It becomes evident, therefore, that the hyperpolarization, presumably the large part of it, could not be due to a chemically coupled or neutral sodium pump. However, the potassium ions would at least partly contribute to the hyperpolarization. The strongly activated Na$^+$ extrusion, whether K$^+$ uptake is passively or actively coupled to it, would make K$^+$ concentrations immediately inside and outside of the membrane considerably different from the concentrations in the bulk of the cytoplasmic and external solutions. Therefore, the actual K$^+$ gradient across the membrane will be much greater than the apparent gradient. This condition could be brought about as a result of a diffusion barrier in the extracellular space, as suggested by Page (30), who partitioned the space into two compartments, i.e., a diffusion channel and an intermediate extracellular component. The latter, which may correspond to a special region whose volume was estimated as 1/500 to 1/200 of the single muscle fiber and in which K$^+$ ions can be retained for a short time in a fall of $[K]_o$ (31), would play an important role.

A correlation between the membrane potential and chloride was not observed, and this agrees with the suggestion by other authors (6, 12), who have found that chloride does not contribute much to the hyperpolarization but moves passively toward equilibrium like potassium at the end of the period of Na$^+$ extrusion. In addition, it should be important to make sure of an implication of other ions such as Ca$^{2+}$ and H$^+$ in the phenomenon.

Burnstock (32) postulated that epinephrine acts by stimulating a process involving the electrogenic extrusion of sodium, thus raising the resting potential. Epinephrine and norepinephrine both exert an indirect influence on the membrane potential by stimulating cell metabolism (19, 33). The effect of norepinephrine (Fig. 3, A) indicates that the hyperpolarization is strongly dependent on the electrogenic sodium pump because it is not conceivable that a passive K redistribution, corresponding to the potential change, could occur so promptly. In this case, the action of the agent on the membrane itself, especially the possibility of an increase in the membrane resistance should also be considered. This was not measured in the present experiment.

For Li-loaded heart muscle the amplitude of the resting potential was greater than nor-
mal immediately after the preparation was taken out of the refrigerator at 4°C and then during equilibration at 4 to 22°C (Fig. 4). Subsequently, when it was rewarmed to 37°C the resting potential gradually decreased, reaching 5 to 23 mv for the right atrium and 13 to 34 mv for the right ventricle, though all of the cells were inactive. Kerkut and Thomas (12) observed in snail nerve cells that a cell injected with LiCl was hyperpolarized by 20 mv in the first 10 minutes, but this then slowly declined. In the present experiments, the hyperpolarization in Li⁺-Tyrode's solution before rewarming was probably caused by a mechanism similar in nature to that suggested by them. The depolarization in rewarmed Li⁺-Tyrode's solution could be due to accumulation of Li ions and depletion of K ions in the cell interior (20). The difference of recovery after return to the normal Tyrode's solution (Fig. 4) may be due to a difference in the tissue and in the time it was preserved at 4°C, and the reappearance of hyperpolarization seems to imply that the cell membrane still could become electrogenic.

Although the effects of DNP and azide were not as intense as those of ouabain, the muscle recovered after its return to the normal solution (Table 4). These metabolic inhibitors have produced little or no change in the resting potentials of frog ventricle and of atria in a steady state (34). Therefore, it could be shown that the cell membrane came into a highly nonsteady state and that the sodium pump became electrogenic during rewarming.

It was concluded that after prolonged preservation in the hypothermic state, the heart muscle enters into a highly nonsteady state during rewarming, resulting in the hyperpolarization of the membranes due to the electrogenic sodium pump.

Acknowledgments

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HEART MUSCLE AFTER PROLONGED HYPOTHERMIA


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Studies of Cat Heart Muscle during Recovery after Prolonged Hypothermia: HYPERPOLARIZATION OF CELL MEMBRANES AND ITS DEPENDENCE ON THE SODIUM PUMP WITH ELECTROGENIC CHARACTERISTICS
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