The Effect of Complete Ischemia on the Intracellular Electrical Activity of the Whole Mammalian Heart

By Milton Karleskind, M.D., Charles E. Hogue, M.D., and Richard J. Bing, M.D.

Spontaneous ventricular electrical potentials of whole mammalian hearts subjected to complete ischemia were investigated by means of the intracellular microelectrode. Spontaneous membrane action potentials persist for 20 min. following the onset of anoxia. Early, the duration of the action potential shortens, followed by a progressive fall in the amplitude of the spike. The resting potential shows a diminution to 65 per cent of the control value. The relationship of these effects to change in membrane ionic transports and metabolic processes are discussed.

Survival of any organ is a graded process, depending on the function and composition of the structures of which it is composed. In the case of the heart, a differentiation can be made between functions depending on the survival of the muscle proteins, the oxidative enzymes and the cell membrane. Recent studies from this laboratory have shown that the contractile proteins prepared from human hearts retain their contractility as late as 12 hours after the death of the patient. Ulrici, Lentini, and Summers have demonstrated that trabecular muscle strips, obtained from hearts after death, retain their excitability and contractility for 6 hours. In 1903, Kuliabko, using a Langendorff preparation, reported the revival of human hearts 20 to 30 hours post mortem. Taussig and Meserne, using strips of human ventricular muscle prepared in oxygenated Ringer's solution for 4 hours after death, described maintenance of the contractility of these strips for 3 hours. Upon perfusion of the coronary arteries of human hearts obtained 3 hours after death, Wearn was able to produce regular spontaneous atrial and ventricular contractions. Recently Lawrie has shown that the onset of rigor mortis in hearts obtained from horses does not appear until 50 minutes after death.

Work from this laboratory has also demonstrated that the oxidative enzymes in heart muscle remain fully active for several hours after the interruption of coronary circulation. Bernheim and Bernheim have found that anoxic rat heart tissue slices were able to oxidize pyruvate and lactate as readily as normal heart tissue for as long as 4 hours. These findings were confirmed by the studies of Webb, Saunders, and Thienes who used similar tissue preparations. Govier has shown that in dog hearts, the coenzyme I content decreased only 25.9 per cent and the coenzyme II only 13.7 per cent during 1 hour of complete anoxia. Wachstein and Miesel, using human hearts obtained at post mortem, reported a satisfactory staining for succinic dehydrogenase for as long as 3 hours after death. Yokoyama, Jennings, Clabough, and Wartman allowed dog hearts to undergo autolysis in situ. Using tissue stains, they were able to show the presence of glycogen for at least 5 hours after cessation of coronary circulation. After the incubation of dog hearts at 37 C. in closed containers, Kent reported no histologic changes in the staining characteristics for desoxynucleic acid as late as 6 hours after death.

The influence of anoxia upon the cell membrane electrical properties, as studied with...

the intracellular microelectrode, has previously been described on stimulated mammalian cardiac tissue. Webb and Hollander have shown that upon stimulation, anoxic rat atria elicit action potentials for 17 min.14 Trautwein and Dudel, using isolated cat papillary muscles, have reported survival of the action potentials for approximately 32 min.15 The present study is concerned with the effects of complete ischemia on the spontaneous ventricular action and resting potentials as recorded from ventricular cells of the isolated whole dog's and rabbit's heart.

**METHOD**

Seventeen rabbits and 4 small dogs, weighing between 400 and 1,000 Gm., were anesthetized by the intravenous injection of sodium pentobarbital (35 mg./Kg.). Whole hearts, weighing 3 to 4 Gm., were quickly removed from the animals and stripped of their pericardium. The time interval between the interruption of the circulation and the onset of perfusion was kept at less than 90 seconds. The hearts were perfused by means of a modified Langendorff preparation (fig. 1). This resulted in immediate forceful muscular contractions. Perfusions were maintained at a constant pressure, using oxygenated blood diluted with an equal amount of Tyrode's solution and maintained at a temperature of 36 to 38° C. The hearts were bathed in normal Tyrode's solution at a constant temperature of 38° C.

Spontaneous left ventricular membrane action and resting potentials were obtained by means of glass capillary microelectrodes, as described by Ling and Gerard.16 The microelectrodes were pulled from soft glass capillary tubing to a diameter of less than 1 μ and filled with 3 M potassium chloride by boiling, followed by cooling in a vacuum chamber. Only microelectrodes with a resistance of 10 to 100 megohms were used. Due to the forceful activity of the contracting heart muscle, the microelectrodes were connected to the cathode tube follower by means of a short 1-mil (0.001 in.) tungsten wire, according to the method of Woodbury and Budy.17 This flexible mounting permitted the electrode to move easily with the contracting tissue, without causing tissue damage or becoming dislodged. The membrane potentials were recorded on the screen of a DuMont Dual-Beam Cathode Ray Oscillograph (type 322-A) and photographed with a DuMont Oscilloscope Record (type 297) polaroid camera. To minimize the injurious effects of prolonged maintenance of the microelectrode within a single cell, several different ventricular sites were selected for penetration during each experiment.

Following a period of stabilization of the preparation, the perfusion was suddenly stopped and the bathing solution replaced with Tyrode's solution rendered oxygen free by prior saturation.
INTRACELLULAR ELECTRICAL ACTIVITY OF ISCHEMIC HEART

TABLE 1.—Effects of Anoxia on Ventricular Cell Membrane Action and Resting Potential

<table>
<thead>
<tr>
<th></th>
<th>Control Value</th>
<th>6 min.* of anoxia</th>
<th>12 min.* of anoxia</th>
<th>18 min.* of anoxia</th>
<th>At disappearance of action potentials</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rest. potential (mv.)</td>
<td>85</td>
<td>70</td>
<td>-18</td>
<td>66</td>
<td>-22</td>
</tr>
<tr>
<td>Act. potential (mv.)</td>
<td>97</td>
<td>78</td>
<td>-19</td>
<td>74</td>
<td>-24</td>
</tr>
<tr>
<td>Overshoot (mv.)</td>
<td>12</td>
<td>9</td>
<td>-25</td>
<td>8</td>
<td>-33</td>
</tr>
<tr>
<td>Act. potential duration (msec.)</td>
<td>255</td>
<td>213</td>
<td>-16</td>
<td>195</td>
<td>-24</td>
</tr>
<tr>
<td>No. of hearts</td>
<td>17</td>
<td>13</td>
<td></td>
<td>13</td>
<td></td>
</tr>
</tbody>
</table>

*Mean time following cessation of perfusion.

with nitrogen. For the remainder of the experiment, nitrogen was continuously bubbled through the bathing solution.

RESULTS

Figure 2.1 illustrates a normal spontaneous ventricular action potential. The average transmembrane resting potential of ventricular cells was found to be 85 mv. Upon arrival of the activation process, the cells depolarized quickly, resulting in a very rapid disappearance and subsequent reversal of this potential. This phase represents an average change in potential of 97 mv. with an overshoot of 12 mv. during depolarization. Subsequent repolarization slowly restored the potential across the cell membrane to its resting value. The average duration of spontaneous ventricular action potentials was found to be 255 msec. These values are similar to those reported by other observers using different species.14, 15 The effects of interruption of coronary circulation are illustrated in figures 2B and 3A. The amplitude of the action potentials gradually diminished after the introduction of nitrogen and the cessation of coronary perfusion. The progressive changes in the characteristics of the action potentials are shown in table 1. Within an average time of 13 min. there was a loss of overshoot. Following that period, the spike of the action potential never reached the isoelectric line (fig. 3A and table 1). Complete failure to record action potentials from 21 hearts, occurred between 15 to 26 min. (average 20 min.) after the onset of complete ischemia of the heart. The resting potential fell to an average of 65 per cent of the control value (85 mv.) at the time of complete cessation of electrical activity (table 1, fig. 3B). The duration of the action potentials decreased progressively during the first 5 min. of ischemia (a fall of 16 per cent from the control); after that period, the action potential shortened more rapidly (36 per cent of the control value, see table 1). Troutwein and Dudel,15 using cat papillary muscles, and Webb and Hollander,14 using rat atrial, obtained similar results.

The amplitude of the action potentials declined as the rate and strength of muscular contraction diminished. At no time did cessation of perfusion induce any irregularities in rhythm. Following complete arrest of muscle activity for 10 min., reperfusion with oxygenated blood resulted in rapid recovery of all elements of the action potential.

DISCUSSION

The results described in this report deal with the survival time of action potential, the changes in amplitude and duration in action potential, and the magnitude of the resting potential induced by complete ischemia of the whole heart.

Twenty minutes following the cessation of coronary perfusion, no further spontaneous action potentials occurred. This survival time is in line with the observations made by others who used a similar technique.14, 15, 18 The diminishing amplitude of the action potential, as shown in figure 3A and table 1, can be related to the effects of a lowered resting potential. Voltage clamp studies by Weidman using mammalian Purkinje fibers have shown that the capacity for an increase in sodium ion permeability is related to the
level of the membrane potential prior to stimulation.19

Sudden exposure of the whole heart to anoxia, as reported in this series of experiments, markedly shortens the duration of the action potential 4 min. after interruption of the coronary circulation. This is due to a shortening of the repolarization phase (fig. 2B) and suggests a more rapid passive transport of K+ ions outwardly across its concentration gradient. Weidman speculates that this shortening of repolarization during anoxia is due to the decreasing K+ ionic inflow which is in contrast to the usual increased K+ influx occurring during the metabolically active phase.20

The amplitude of the resting potential exhibits relatively less severe changes throughout the period of observation (fig. 3B). It is likely that the duration of ischemia is too brief (approximately 20 min.) to result in a marked derangement of the sodium-potassium pumps. Changes in resting potential during anoxia may be the result of an increased uptake of sodium and loss of potassium by the cell. This has been demonstrated in the rat diaphragm, rat ventricular muscle strips, and the dog heart.21-23 Stimulation of anoxic ventricular muscle strips enhances this sodium influx. Exposure of these anoxic muscle strips to a medium of low sodium content for 1 hour does not lower the intracellular sodium below that of the external medium. In contrast, well oxygenated muscle strips suspended in a low sodium environment demonstrate an effective mechanism for extrusion of sodium ions.22 These findings suggest that it is the active sodium pump mechanism that is injured by anoxia. The membrane resting potential of mammalian cardiac fibers is eventually lowered considerably by anoxia and by metabolic inhibitors.20-24 Injury to the sodium pump results in a smaller number of sodium ions extruded from the fiber per unit of time, which produces a partial lowering of the resting potential. According to Weidman, this renders the heart incapable of responding to stimuli.25

It is possible that the changes observed in the membrane resting potential are due to the inhibition of oxidative phosphorylation produced by anoxia. Thus, a decline in the
forces of contraction of anoxic cat papillary muscles has been reported to accompany the fall in concentration of adenosine triphosphate.\textsuperscript{24} In addition, L\textsuperscript{ing} and Gerard,\textsuperscript{25} using anoxic single frog sartorius fibers, have shown a parallel fall in creatine phosphate and membrane potential during a 3 hour period of observation.

\textbf{SUMMARY}

The effects of complete ischemia on the spontaneous ventricular membrane action and resting potentials of the whole mammalian heart were investigated by means of the intracellular microelectrode. Rabbit and dog hearts were perfused with a modified Langendorff preparation. A completely ischemic state was produced by cessation of coronary circulation and the continuous bubbling of nitrogen through the bathing solution.

Spontaneous membrane action potentials persisted for 20 min following the cessation of coronary perfusion. The earliest change in the configuration of the action potential was a shortening of its duration. This was followed by a progressive fall in the amplitude of the spike. The membrane resting potential showed a diminution to 65 per cent of the control value.

The relationship of these findings to the changes in the membrane ionic transport and permeability is discussed in the light of current concepts. The effects of anoxia and metabolic inhibitors on electrical potentials are so similar as to suggest a derangement in oxidative phosphorylation.

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