Ventricular Fibrillation and Ion Transport


Ventricular fibrillation has been studied by driving the ventricles of the isolated rabbit heart electrically and observing whether fibrillation persisted after stimulation was stopped. The hearts were perfused by solutions of different ionic composition and the proportion of hearts in which persistent fibrillation was seen was determined for each solution. The proportion was controlled from 0 to 100 per cent according to the amount of K⁺, hearts fibrillating spontaneously in 0.25 N K⁺. A similar study was made by varying Ca⁺². Fibrillation was arrested by ATP and prolonged by dinitrophenol. Fibrillating hearts lost more K⁺ than when they were not fibrillating. Fibrillation appeared to depend on disturbances of the metabolic processes concerned with ion movements.

A METHOD has been described for producing atrial fibrillation in the heart-lung preparation of the dog in such a way that the fibrillation was started and stopped at will. Acetylcholine was infused at a constant rate into the blood going to the heart and electric stimuli were applied for 2 minutes to the right atrial appendix. The stimuli induced fibrillation and this was then maintained for as long as the acetylcholine was infused. In seeking to explain the action of the acetylcholine, attention was drawn to the change it had produced in the shape of the atrial action potential. The action potential consists of a sharp rise in which depolarization takes place and a much more gradual fall in which repolarization takes place. Acetylcholine was shown to accelerate this fall. In nerve the fall has been shown to be due to the passage of potassium ions out of the cell, and if this is also true for heart muscle, acetylcholine would appear to facilitate the passage of potassium ions out of the cell. If the maintenance of fibrillation by acetylcholine is related to this action, it should be possible to arrest the fibrillation by raising the concentration of potassium ions in the blood. This would make it more difficult for potassium ions to leave the cell. Burn, Gunning and Walker showed that fibrillation was arrested in this way and that when the potassium concentration was so raised a persisting fibrillation could no longer be induced. These observations suggested a close relation between fibrillation and the movement of ions across the cell membrane.

In order to study ventricular fibrillation, the heart-lung preparation cannot be used since the flow of blood through the coronary system stops when fibrillation occurs. We have therefore used the isolated rabbit heart perfused through the coronary vessels by Langendorff's method.

METHOD

The rabbit heart was perfused through a cannula tied in the aorta with a solution recently described by McEwen. It contained: NaCl, 7.7 Gm.; KCl, 0.42 Gm.; CaCl₂, 0.24 Gm.; NaH₂PO₄•2H₂O, 0.143 Gm.; NaHCO₃, 2.1 Gm.; dextrose, 2.0 Gm.; sucrose, 4.5 Gm.; and 1000 ml. distilled water, so that the Na⁺ concentration was 163 mM/L., the K⁺ concentration 5.6 mM/L. and the Ca⁺² concentration 2.2 mM/L.*

The solution was saturated with oxygen and 5 per cent CO₂ before being placed in an apparatus of conventional pattern. The solution was again aerated as it passed out of the Marriotte bottle. The solution entering the heart was maintained constant at 37 C. at all rates of coronary flow by using the device described by Saxby. A pair of platinum electrodes were inserted in the ventricles through which square wave pulses of 1 ma. strength and of 0.75 msec. duration could be applied at varying rates. A second pair of electrodes were inserted in the ventricles on the opposite side as leads to a Cossor model 1314 electrocardiograph. A mechanical record was also taken by a thread attached to the apex of the ventricle. When a heart was set up it was perfused with the solution for 30 min. to ensure a steady state. When desired, by turning a tap, a modification of the solution with a different ionic composition or with various substances added in known concentra-

* Dr. Baird Hastings has drawn our attention to the calcium concentration, which should be 1.1 mM/L. to be normal.
tion was perfused through the heart for 30 min. Stimulation was applied beginning at a rate of about 200/min. and the rate was increased until fibrillation was observed.

RESULTS

Variation in Potassium Concentration. The ventricles usually fibrillated when the stimulation rate was between 500 and 700/min. When fibrillation began, stimulation was continued for 5 min. at this rate. On cessation of stimulation one of two things happened. In the majority of hearts, fibrillation either reverted to normal rhythm within the next 5 min., or else it persisted until observation was discontinued at the end of 30 min. Only a few hearts reverted to normal rhythm in the period between 5 and 30 min. An arbitrary distinction was therefore made. Hearts which reverted to normal rhythm within 5 min. were said not to have fibrillated while those which fibrillated for a period between 5 and 30 min. were counted as hearts which had fibrillated.

When observations were made in this way on hearts perfused with the normal solution it was found that fibrillation occurred in 11 out of 28 hearts (39 per cent). When the potassium concentration was reduced as little as 25 per cent, fibrillation occurred to 20 out of 26 hearts (77 per cent). The results are given in table 1 and are expressed graphically in figure 1. They indicate that the proportion of hearts fibrillating could be raised to include all hearts, or lowered to include none, by varying the potassium concentration in the perfusing fluid. The two extremes of the curve were of special interest. When the potassium concentration was twice that of the normal solution, 3 of the 4 hearts tested were observed to fibrillate spontaneously without the application of any stimulation. The electrocardiogram of one of these hearts appears in figure 2.

Variation in Calcium Concentration. Having made observations on potassium, we next examined the effect of changes in calcium concentration. Grumbach, Howard and Merrill have observed that the rapid injection of a dose of 50 mg. calcium chloride into the fluid perfusing a heart caused it to fibrillate. We were therefore not surprised to find that when the calcium concentration was doubled, fibrillation occurred in every heart tested. The reduction of calcium below the normal proportion to 50 per cent and then to 25 per cent gradually increased the proportion of hearts fibrillating. This reached a peak when the calcium concentration was reduced to 12.5 per cent, that is, to 0.275 mM/L. The proportion of hearts fibrillating rose to 80 per cent at this point. Further reductions in calcium then

<table>
<thead>
<tr>
<th>K⁺ concentration mM/L</th>
<th>Proportion of hearts fibrillating</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>11.2</td>
<td>0 of 7</td>
<td>0</td>
</tr>
<tr>
<td>5.6</td>
<td>11 of 28</td>
<td>39</td>
</tr>
<tr>
<td>4.2</td>
<td>20 of 26</td>
<td>77</td>
</tr>
<tr>
<td>2.8</td>
<td>10 of 11</td>
<td>90</td>
</tr>
<tr>
<td>1.4</td>
<td>4 of 4</td>
<td>100</td>
</tr>
</tbody>
</table>

Fig. 1. Effect of K⁺ on fibrillation. Ordinate, percentage of hearts in which fibrillation persisted after electric stimulation. Abscissa, amount of K⁺ in the perfusion fluid expressed in terms of the normal amount which is 5.6 mM/L.
TABLE 2.—Effect of Changes in Calcium Concentration on Proportion of Hearts Fibrillating

<table>
<thead>
<tr>
<th>Ca concentration mM/L.</th>
<th>K 5.6 mM/L</th>
<th>K 4.2 mM/L</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Proportion fibrillating</td>
<td>Percentage</td>
</tr>
<tr>
<td>4.4</td>
<td>19 of 20</td>
<td>95</td>
</tr>
<tr>
<td>2.2</td>
<td>11 of 28</td>
<td>39</td>
</tr>
<tr>
<td>1.65</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>1.1</td>
<td>6 of 13</td>
<td>46</td>
</tr>
<tr>
<td>0.55</td>
<td>7 of 12</td>
<td>55</td>
</tr>
<tr>
<td>0.275</td>
<td>16 of 20</td>
<td>80</td>
</tr>
<tr>
<td>0.137</td>
<td>6 of 10</td>
<td>60</td>
</tr>
<tr>
<td>0.068</td>
<td>4 of 10</td>
<td>40</td>
</tr>
</tbody>
</table>

Fig. 2. Electrocardiogram of isolated rabbit heart, a, immediately after the perfusion fluid was changed from normal to one with 0.25 K+ (= 1.4 mM/L); b, 5 min. later, signs of irregularity; c, 20 min. later, spontaneous ventricular fibrillation.

Fig. 3. Electrocardiogram of isolated rabbit heart; a, fibrillation initiated by stimulation during perfusion solution containing 0.25 Ca++ (= 0.068 mM/L); b, normal rhythm when perfusion was continued with Ca++-free solution.

diminished the proportion of hearts fibrillating, until, in the absence of calcium, hearts did not fibrillate at all. Our observations on the effect of reducing calcium to zero were chiefly made by changing the perfusion solution passing through a fibrillating heart to a solution which was calcium-free. Fibrillation was then always arrested, as shown in figure 3. The effect of changes in the calcium concentration was observed not only in perfusing solutions containing the normal amount of potassium, but also in solutions containing 75 per cent of the normal potassium. These observations supported those made with normal potassium in showing a similar rise in the proportion of hearts fibrillating when the calcium was reduced to 0.275 mM/L. The results are given in table 2 and are expressed graphically in figure 4.

Results with Adenosine Triphosphate. In pursuance of the idea that fibrillation was related to the transport of ions across the cell membrane, particularly to the transport of potassium ions, it seemed that electric stimulation at a high rate might evoke fibrillation because the loss of potassium ions during contractions exceeded the replacement of potassium ions between contractions. We therefore considered that adenosine triphosphate (ATP), a substance capable of supplying energy, should be tested to see if it could arrest fibrillation.
We found that ATP was able to do this (fig. 5). In section a of the figure the normal electrocardiogram record is shown. Fibrillation was then produced by stimulation, and a record was taken 20 min. later (b). The solution perfusing the heart which contained normal concentrations of potassium and calcium was then changed to one containing ATP 40 /µg./ml. The fibrillation changed to a fast regular rhythm (c). On changing to a solution without ATP, the fibrillation returned (d). Perfusion with a higher concentration of ATP (100 /µg./ml.) then caused reversion to normal rhythm (e), and again when the ATP was omitted, fibrillation returned (f). Finally, perfusion with ATP in the higher concentration again caused reversion to normal rhythm (g).

Having observed in several experiments that ATP abolished fibrillation, we wanted to discover whether other related substances had a similar action. We tested adenosine monophosphate and inosine triphosphate. ATP is known to bind calcium ions; ethylenediamine tetra-acetate (Versene) a substance which chelates many metal ions, was therefore included in our observations. These substances were tested in solutions which contained both high calcium (2.2 to 4.4 mM/L) and low...
TABLE 3—Effects of ATP, AMP, ITP and Versene

<table>
<thead>
<tr>
<th>Molar concentration</th>
<th>Proportion of hearts in which fibrillation was arrested</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2.2-4.4 (mM Ca⁺ concentration)</td>
</tr>
<tr>
<td>ATP</td>
<td>1.6 X 10⁻⁴</td>
</tr>
<tr>
<td>AMP</td>
<td>2.9 X 10⁻⁴</td>
</tr>
<tr>
<td>ITP</td>
<td>5.8 X 10⁻⁴</td>
</tr>
<tr>
<td>Versene</td>
<td>8.7 X 10⁻⁴</td>
</tr>
<tr>
<td></td>
<td>1.6 X 10⁻⁴</td>
</tr>
<tr>
<td></td>
<td>3.2 X 10⁻⁴</td>
</tr>
</tbody>
</table>

Adenosine Triphosphate and Magnesium. Magnesium ions may potentiate the action of ATP either by inhibiting the enzyme which destroys ATP, or by taking part in the formation of the active complex. Experiments were carried out to see if the effect of ATP in abolishing fibrillation was potentiated by magnesium ions. The results are shown in table 4. A concentration of ATP was chosen which, by itself, did not arrest fibrillation, but higher concentrations of Versene and AMP were required to abolish fibrillation.

TABLE 4.—Effect of Magnesium in Potentiating ATP, Perfusion fluid 75 per cent K, 12.5 per cent Ca, the Same Hearts were Used in Both Series

<table>
<thead>
<tr>
<th>Molar concentration</th>
<th>Molar concentration Mg</th>
<th>Proportion of hearts in which fibrillation abolished</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6.4 X 10⁻⁴</td>
<td>—</td>
<td>0/7</td>
</tr>
<tr>
<td>6.4 X 10⁻⁵</td>
<td>1.6 X 10⁻⁴</td>
<td>4/7</td>
</tr>
</tbody>
</table>

calcium (0.275 to 0.55 mM/L). The results are summarized in table 3.

In high calcium concentrations fibrillation was abolished by ATP only, whereas in low calcium concentrations, ATP, AMP, ITP and Versene were effective. ATP and ITP were effective in 1.6 X 10⁻⁴ molar concentration, but higher concentrations of Versene and AMP were required to abolish fibrillation.

**Effect of Dinitrophenol.** The effect of dinitrophenol was observed in 5 hearts which continued in fibrillation for less than 5 min. after the stimulation was stopped (table 5). When DNP in a concentration of 3 X 10⁻⁶ M or 5 X 10⁻⁶ M was added to the perfusion fluid and the hearts were driven again to induce fibrillation, it continued for a much longer time.

On changing back to the original perfusion solution, hearts 2, 4 and 5 reverted to normal rhythm, although in one experiment the normal rhythm did not return for 69 min. Hearts 1 and 3 which reverted to normal rhythm in the presence of DNP were then driven again in the original perfusion solution without DNP and, as at the beginning of the experiment, reverted to normal rhythm within 5 min. after the stimulation was stopped.

**Loss of Potassium During Fibrillation.** A few preliminary experiments were made to see whether there was a greater loss of potassium from the heart during fibrillation. Samples of perfusate from normally beating hearts were collected, fibrillation was then induced and two more samples were taken; the first during the 5 min. period of fibrillation during stimulation and the second after stimulation was stopped. The fibrillation was finally abolished by injecting 10 mg. KCl into the cannula. Normal rhythm was usually well established after a further 15 min., and a final sample of perfusate

<table>
<thead>
<tr>
<th>Exp. no.</th>
<th>Before DNP (min.)</th>
<th>In presence of DNP (min.)</th>
<th>Molar concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>16</td>
<td>3 X 10⁻⁶</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>&gt;35</td>
<td>3 X 10⁻⁴</td>
</tr>
<tr>
<td>3</td>
<td>4</td>
<td>20</td>
<td>3 X 10⁻⁴</td>
</tr>
<tr>
<td>4</td>
<td>0</td>
<td>&gt;22</td>
<td>5 X 10⁻⁴</td>
</tr>
<tr>
<td>5</td>
<td>2</td>
<td>&gt;15</td>
<td>5 X 10⁻⁴</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Perfusion fluid</th>
<th>No. of hearts</th>
<th>Mean K (mg./L) Before and after fibrillation</th>
<th>During fibrillation</th>
<th>Per cent increase in K concentration During fibrillation</th>
</tr>
</thead>
<tbody>
<tr>
<td>50 per cent of normal K</td>
<td>5</td>
<td>112.5</td>
<td>121.9</td>
<td>8.0</td>
</tr>
<tr>
<td>2 X normal Ca</td>
<td>5</td>
<td>203.1</td>
<td>207.9</td>
<td>2.3</td>
</tr>
</tbody>
</table>
was taken. The potassium content was estimated using a Beckmann flame photometer (table 6). Whether the fibrillation was produced by perfusing the heart with a solution containing a low potassium or a high calcium concentration, there was always a higher potassium concentration in the perfusate during fibrillation. The increase was considerably higher in the hearts perfused with a low potassium concentration, since under these conditions the exit of potassium was greatly facilitated.

**Discussion**

The study of ventricular fibrillation in the isolated rabbit heart has revealed that it is dependent on the ionic composition of the Ringer's solution perfusate and is a reversible phenomenon. When hearts are perfused with a solution containing only 25 per cent of the normal content of potassium ions (normal being 5.6 mM/L), they soon pass into fibrillation without electric stimulation and remain fibrillating until the potassium concentration is raised. They then revert to normal rhythm. When the potassium concentration is near to that of other ions at normal, fibrillation occurs only as the result of electric stimulation. The fibrillation so produced can be arrested and normal rhythm restored by the addition of adenosine triphosphate to the perfusate.

These observations indicate that electric stimulation is not essential for the production of fibrillation. Its appearance depends rather on disturbances of metabolism which may develop independently and can be reversed by raising the external concentration of potassium, by lowering to zero the external concentration of calcium or by the addition of adenosine triphosphate.

The relation of potassium ions to fibrillation follows a smooth curve. When the concentration of these ions outside the cell is low, fibrillation occurs easily; when the concentration is high, fibrillation occurs rarely or not at all. During contraction potassium passes out of the cell by diffusion, through the depolarized and permeable cell membrane. Between contractions the potassium must be carried back into the cell by a process which requires energy. Fibrillation occurs readily when external potassium is low and it is therefore difficult to push potassium back into the cell. This explanation gains support from the production of fibrillation by cooling one point on the surface of the ventricle, so that the chemical processes which push potassium back into the cell proceed very slowly.

The relation of calcium ions to fibrillation is undoubtedly more complex, as is shown by the shape of the curve relating calcium concentration to the proportion of hearts fibrillating. The curve suggests that calcium plays a double role. Fibrillation never occurs in the absence of calcium, and then, as the concentration rises, it occurs more readily in proportion to the concentration. This holds true for the range 0 to 0.275 mM/L, and again at the concentration of 4.4 mM/L., but in the range 0.55 to 2.2 mM/L. there appears to be a second effect which reduces the occurrence of fibrillation. One possibility is that in this range calcium assists in maintaining the stability of the cell membrane. The bottom of this range is close to a concentration of 0.3 mM/L., below which point the excitability of nerve rises.

The action of the substances which arrested fibrillation and restored normal rhythm only in low calcium solutions can probably be attributed to the removal of calcium from the solution. These substances were inosine triphosphate, adenosine monophosphate and Versene. The explanation is almost certainly true for Versene, since in the concentration in which it was used it removed all calcium ions from the perfusing solution.

Adenosine triphosphate was more effective in arresting fibrillation because it was able to do this whether the calcium concentration was low or high. Thus 0.16 mM/L abolished fibrillation in the presence of 2 or 4 mM/L. calcium. Its ability to arrest fibrillation and the ability of dinitrophenol to prolong it may well be related phenomena. Dinitrophenol depresses the action of the sodium pump, that is, the mechanism which expels the sodium from the cell between contractions and to which may be linked the potassium pump for driving
potassium back into the cell. Dinitrophenol, moreover, prevents the energy provided by oxidative processes from being used to replenish the cell's own store of adenosine triphosphate by phosphorylation. We might then say that ATP arrested fibrillation by providing more energy for forcing back the potassium between the contractions. The difficulty of this view is that ATP is not believed able to enter the cell and that when present externally it cannot supply energy. By what means, then, does ATP arrest fibrillation, if it does not remove all calcium from the perfusing solution and if it does not supply energy to the cell? It would seem that it must be able to enter the membrane even if it does not enter the cell itself, and that it then supplies energy which can arrest fibrillation. It is conceivable that its power to combine with calcium may play a role and that it reduces the fibrillatory action of this ion. Further experimental observations are required.

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Ventricular Fibrillation and Ion Transport
A. K. ARMITAGE, J. H. BURN and A. J. GUNNING

*Circ Res.* 1957;5:98-104
doi: 10.1161/01.RES.5.1.98

*Circulation Research* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7330. Online ISSN: 1524-4371

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