Effects of Adenosinetriphosphate and Potassium Chloride on Ventricular Fibrillation Induced by Lack of Substrates

by Mario Penna, M.D., Alejandro Illanes, M.D., and Marcos Pupkin, M.D.

In previous papers from this laboratory, it has been shown that isolated guinea pig and cat hearts perfused with Tyrode solution without substrates undergo a decrease in rate, a decrease in amplitude of the R wave, a lengthening of the P-R interval, ventricular premature contractions, A-V dissociation, and, finally, ventricular fibrillation. The ventricular fibrillation, induced by the lack of substrates both in isolated guinea pig and cat hearts, does not revert spontaneously to normal or idioventricular rhythm. In guinea pig hearts, either glucose or butyrate reversed ventricular fibrillation induced by this procedure to normal or idioventricular rhythm. In contradistinction, the ventricular fibrillation of the isolated cat heart is not reversed by perfusion with Tyrode solution containing glucose, fructose, galactose, butyrate, or pyruvate, nor is the contractile force increased by glucose and butyrate in the isolated hypodynamic cat heart.

The perfusion of the isolated heart, according to the Langendorff technique by a glucose-free Tyrode solution, washes several substrates from the heart, among which adenosinetriphosphate (ATP) and potassium seem very important. It is probable that, under the experimental conditions in which ventricular fibrillation is produced in the isolated heart perfused with a substrate-free solution, the restoration of intracellular potassium is rather difficult since the presence of glucose or other substrates is required to supply the energy necessary for the re-entry of potassium into the cell. Observing this, we studied the effects of potassium and an energy-rich phosphorylated substance like ATP upon ventricular fibrillation induced by a lack of substrates.

Methods

The experiments were performed on isolated guinea pig and cat hearts of both sexes perfused according to a modified Langendorff procedure, which has been previously described. The hearts were perfused with oxygenated Tyrode solution without glucose at a temperature of 37°C. The electrolyte composition of the solution was the same as mentioned in previous papers (in mM/L): NaCl, 138; KCl, 2.7; CaCl₂, 1.8; MgCl₂, 0.1; NaHCO₃, 11.9; and NaH₂PO₄, 0.37.

The electrocardiogram was registered in a model 3D Grass electroencephalograph and three bipolar leads were taken simultaneously (fig. 1). The hearts were perfused from the beginning with oxygenated Tyrode solution without glucose until five minutes after the establishment of ventricular fibrillation. At this time, the perfusing fluid was changed to: (a) Tyrode solution containing ATP (Nutritional Biochemistry Corporation, crystalline disodium salt), 10 mg./L.; (b) Tyrode solution with potassium chloride, 5.4 mM/L. (instead of the 2.7 mM/L. of the original solution). In some experiments performed on isolated cat hearts, potassium chloride and ATP in the above mentioned concentration were added simultaneously to the Tyrode solution with or without glucose (1 Gm./L.).

Results

The results are summarized in table 1.

EXPERIMENTS ON THE ISOLATED GUINEA PIG HEARTS

The perfusion of isolated guinea pig hearts with a substrate-free Tyrode solution induces a decrease in rate, a decrease in amplitude of the R wave, a lengthening of the P-R interval, ventricular premature contractions, A-V dissociation, insignificant changes of the Q-T interval, and, finally, ventricular fibrillation with regular atrial rhythm. Ventricular
VENTRICULAR FIBRILLATION

ISOLATED GUINEA PIG HEART * 550 g

RA/LA —

RA/Ap —

LA/Ap —

2mV 0' 90' 100' 110' 115'

Tyrode Sol. without Glucose

FIGURE 1

RA/LA=bipolar lead from right to left atrium; RA/Ap=bipolar lead from right atrium to apex; LA/Ap=bipolar lead from left atrium to apex; standardization=2 mv.

The figures represent the time when records were taken expressed in minutes after starting the experiment. At 105 minutes, 200 mg. potassium chloride per L. were added to the Tyrode solution without glucose, so that concentration increased from 2.7 mM/L. in the original Tyrode solution to 5.4 mM/L.

fibrillation appeared within 30 to 100 minutes after perfusion started, averaging 60 minutes.

EFFECT OF ADDING ATP TO THE PERFUSION SOLUTION AFTER ESTABLISHING VENTRICULAR FIBRILLATION IN THE ISOLATED GUINEA PIG HEART

Five minutes after ventricular fibrillation was present in seven isolated guinea pig hearts, ATP (10 mg./L.) was added to the perfusion solution. In only two of the seven cases was ventricular fibrillation reversed. However, in neither of these two cases was a sinus rhythm re-established. In one of these two hearts, paroxysmal ventricular tachycardia appeared. Atrioventricular dissociation with a slow ventricular rhythm appeared in the other heart. In the other five cases, ventricular fibrillation persisted after perfusing with ATP.

EFFECTS OF ADDING POTASSIUM TO THE PERFUSION SOLUTION AFTER ESTABLISHING VENTRICULAR FIBRILLATION IN THE ISOLATED GUINEA PIG HEART

Once ventricular fibrillation was established in guinea pig hearts, potassium chloride (200 mg./L.) was added to the perfusion solution in such a way that the total potassium concentration reached 5.4 mM/L. In eight hearts, the addition of potassium chloride reversed ventricular fibrillation. The effects of potassium on the atrial rate, P-R and Q-T intervals, and amplitude of the R wave appear in table 2. As can be observed, even though potassium reversed ventricular fibrillation, this ion does not significantly change the aforementioned values of the electrocardiogram relative to the values found in the minutes preceding ventricular fibrillation. In four of the eight cases, a regular sinus rhythm was re-established. An example appears in figure 1. In three hearts, A-V dissociation with idioventricular rhythm occurred and in one case ventricular tachycardia was observed.

EXPERIMENTS ON THE ISOLATED CAT HEART

As in the isolated guinea pig heart, the perfusion of the isolated cat heart with the Tyrode solution without substrates induced a decrease in the atrial rate, a decrease in amplitude of the R wave, a lengthening of the P-R interval, ventricular premature contractions, A-V dissociation, and finally, ventricular fibrillation within 45 to 155 minutes, averaging 95 minutes.

EFFECTS OF ADDING ATP TO THE PERFUSION SOLUTION AFTER ESTABLISHING VENTRICULAR FIBRILLATION IN THE ISOLATED CAT HEART

After five minutes of ventricular fibrillation, the perfusion fluid was changed to a Tyrode solution containing ATP (10 mg./L.). In four hearts, ATP was present in a solution containing glucose, and in the other four cases, the solution contained no glucose. In

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TABLE 1
Reversal of Ventricular Fibrillation Induced by Lack of Substrates in Isolated Hearts Observed After Adding Glucose, Potassium, or Adenosine-triphosphate*

<table>
<thead>
<tr>
<th>Tyrode solution</th>
<th>Guinea pig hearts</th>
<th>Cat hearts</th>
</tr>
</thead>
<tbody>
<tr>
<td>Without glucose + ATP</td>
<td>2/7</td>
<td>0/4</td>
</tr>
<tr>
<td>Without glucose + K</td>
<td>8/8</td>
<td>3/7</td>
</tr>
<tr>
<td>Without glucose + K + ATP</td>
<td>no experiment</td>
<td>2/4</td>
</tr>
<tr>
<td>With glucose</td>
<td>5/5§</td>
<td>0/9</td>
</tr>
<tr>
<td>With glucose + ATP</td>
<td>no experiment</td>
<td>0/4</td>
</tr>
<tr>
<td>With glucose + K</td>
<td>no experiment</td>
<td>7/7</td>
</tr>
</tbody>
</table>

*Perfusion five minutes after onset of fibrillation.

†ATP, disodium salt 10 mg./L.

§5.4 mM/L. instead of 2.7 mM/L. of the normal Tyrode solution.

none of the eight hearts was reversal of ventricular fibrillation accomplished (table 1).

EFFECTS OF ADDING POTASSIUM CHLORIDE TO THE PERFUSION SOLUTION AFTER ESTABLISHING VENTRICULAR FIBRILLATION IN THE ISOLATED CAT HEART

In seven cases in which ventricular fibrillation had been present for five minutes, the perfusing solution was changed to a Tyrode solution enriched with potassium chloride so that the concentration reached 5.4 mM/L. In only three hearts was ventricular fibrillation terminated, reverting to a normal sinus rhythm and an A-V dissociation in the other case.

Four fibrillating hearts were perfused with a Tyrode solution containing both ATP and potassium chloride in the concentrations already mentioned. In two cases, ventricular fibrillation was terminated, reverting to a sinus rhythm in one case and to ventricular tachycardia in the other.

Seven fibrillating cat hearts were perfused with a Tyrode solution containing glucose (1 Gm./L.) and potassium chloride in the concentration already mentioned (5.4 mM/L.). In seven cases, ventricular fibrillation stopped. In two cases, a normal sinus rhythm was re-established; in three cases, a ventricular tachycardia was observed. In the other two cases, A-V dissociation with a slow ventricular rhythm occurred.

Table 3 includes the following values after the onset of fibrillation: atrial rate, P-R and Q-T intervals, and amplitude of the R wave (whenever this measurement could be determined). As can be observed in table 1, Tyrode solution enriched in potassium terminated ventricular fibrillation in all cases when glucose was present in the medium. However, the atrial rate, P-R interval, and amplitude of the R wave did not return to control values with cessation of fibrillation.

Discussion

Ventricular fibrillation induced by the lack of substrates may be related to a decrease in the cellular potassium and perhaps to a decrease in cellular ATP. The lack of substrates decreases the formation of energy-rich phosphate bonds and makes rather difficult the re-entry of potassium into the cell after every contraction. Of these conditions, the lack of potassium seems to be more important because, in the absence of glucose, recovery following potassium is observed in all cases in guinea pigs, whereas recovery following ATP is infrequent.

These facts lead us to believe that prolonged perfusion of the isolated heart with substrate-free perfusate produces potassium loss in the myocardial fibers. This loss is probably due to the difficulty in re-establishing intracellular potassium when there are no substrates available to supply the energy necessary for returning potassium into the cell.

The effect of increasing potassium concentration upon ventricular fibrillation induced by lack of substrates may be related to a decrease in the cellular potassium and perhaps to a decrease in cellular ATP. The lack of substrates decreases the formation of energy-rich phosphate bonds and makes rather difficult the re-entry of potassium into the cell after every contraction. Of these conditions, the lack of potassium seems to be more important because, in the absence of glucose, recovery following potassium is observed in all cases in guinea pigs, whereas recovery following ATP is infrequent.

These facts lead us to believe that prolonged perfusion of the isolated heart with substrate-free perfusate produces potassium loss in the myocardial fibers. This loss is probably due to the difficulty in re-establishing intracellular potassium when there are no substrates available to supply the energy necessary for returning potassium into the cell.

The effect of increasing potassium concentration upon ventricular fibrillation induced by lack of substrates is in agreement with the antagonistic effect of the increase in potassium upon ventricular fibrillation induced by the following procedures in the isolated rabbit heart: high frequency electrical stimulation and calcium chloride injection. The antifibrillatory effect of potassium observed both in the experimentally induced atrial fibrillation of the dog heart-lung preparation and of the isolated rabbit atrium can be added to the list of similarities.
TABLE 2

Electrocardiographic Changes of Eight Isolated Guinea Pig Hearts Perfused with Tyrode Solution without Glucose

<table>
<thead>
<tr>
<th>Perfusing time</th>
<th>Atrial rate (per minute) Mean ± S.E.</th>
<th>P-R interval (seconds) Mean ± S.E.</th>
<th>Q-T interval (seconds) Mean ± S.E.</th>
<th>R amplitude (mv.) Mean ± S.E.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial</td>
<td>220.7 ± 11.49</td>
<td>0.07 ± 0.02</td>
<td>0.23 ± 0.02</td>
<td>4.54 ± 1.96</td>
</tr>
<tr>
<td>Before ventricular fibrillation 10'</td>
<td>160 ± 18.20</td>
<td>0.09 ± 0.02*</td>
<td>0.22 ± 0.015</td>
<td>2.46 ± 0.90</td>
</tr>
<tr>
<td>After reversal of fibrillation by addition of KCL 10'</td>
<td>104 ± 19.37</td>
<td>0.11 ± 0.04*</td>
<td>0.21 ± 0.04</td>
<td>1.40 ± 0.67</td>
</tr>
<tr>
<td>After reversal of fibrillation by addition of KCL 20'</td>
<td>150 ± 7.70</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*In hearts without A-V dissociation.

TABLE 3

Electrocardiographic Changes of Seven Isolated Cat Hearts Perfused with Tyrode Solution without Glucose

<table>
<thead>
<tr>
<th>Perfusing time</th>
<th>Atrial rate (per minute) Mean ± S.E.</th>
<th>P-R interval (seconds) Mean ± S.E.</th>
<th>Q-T interval (seconds) Mean ± S.E.</th>
<th>R amplitude (mv.) Mean ± S.E.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial</td>
<td>148 ± 5.9</td>
<td>0.10 ± 0.06</td>
<td>0.17 ± 0.01</td>
<td>2.68 ± 0.57</td>
</tr>
<tr>
<td>10' before ventricular fibrillation</td>
<td>118 ± 8.5</td>
<td>0.12 ± 0.01*</td>
<td>0.19 ± 0.10</td>
<td>1.7 ± 0.27</td>
</tr>
<tr>
<td>10' after reversal of fibrillation by addition of KCL and glucose</td>
<td>115 ± 11.46</td>
<td>0.11 ± 0.03*</td>
<td>0.16 ± 0.03</td>
<td>1.85 ± 0.8</td>
</tr>
</tbody>
</table>

*In hearts without A-V dissociation.

ATP arrested ventricular fibrillation in only two of seven cases in guinea pig hearts and in none of the eight cat hearts. ATP is not believed able to enter the cell and, when present externally, cannot supply the energy. Therefore, the infrequent reversals might be due only to the calcium chelating properties of this substance. These results are not in agreement with those reported by Armitage et al. on the electrically induced ventricular fibrillation of the isolated rabbit heart. In fact, these authors observed constant reversal of ventricular fibrillation. This difference might be explained on the basis of the higher ATP concentration used by them and also in the fact that the fibrillation was induced by a different procedure in a different species.

The reversal of ventricular fibrillation in the isolated cat heart was only accomplished when potassium chloride concentration was increased and glucose was added to the perfusion solution. As shown in table 1, glucose alone did not reverse ventricular fibrillation in any of the nine cases tried, and potassium chloride reversed it in three of seven cases.

The fact that glucose addition did not reverse ventricular fibrillation in the isolated cat heart and, on the other hand, as shown by Garb et al., does not increase the contractile force of the isolated hypodynamic cat heart, is not an indication that the isolated cat heart does not use glucose. Furthermore, when the isolated cat heart is perfused from the beginning with Tyrode solution containing glucose (1 Gm./L.) as shown by Torres of this laboratory, ventricular fibrillation does not appear within three hours. On the other hand, the isolated hypodynamic cat papillary muscle at 27 C, but not at 37 C, shows an important increase in contractile force when glucose is added to the medium. These facts afford some understanding of the constant reversal of ventricular fibrillation induced by the combination of glucose and increasing potassium concentrations in the present experiments.

The longer time required by the cat heart to fibrillate can be related to the species dif-
ferences between guinea pig and cat hearts. In the cat hearts, the effect of washing out potassium, substrates, and energy-rich phosphate bonds may be more important than in guinea pig hearts in reaching an irreversible state.

**Summary**

The perfusion of the isolated guinea pig or cat heart with a substrate-free Tyrode solution induces a decrease in rate, a lengthening of the P-R interval, a decreased amplitude of the R wave, ventricular premature contractions, A-V dissociation, insignificant changes of the Q-T interval, and, finally, ventricular fibrillation with a regular atrial rhythm. In isolated hearts of both species, the reversal of ventricular fibrillation induced by lack of metabolites was attempted as follows: in guinea pig hearts, increasing the potassium chloride concentration of the Tyrode solution without glucose to 5.4 mM/L reversed ventricular fibrillation in all cases. Adenosinetriphosphate (ATP) reversed this arrhythmia in only two of seven cases. However, in the isolated cat heart, potassium chloride in the same concentration (5.4 mM/L.) reversed ventricular fibrillation only in three out of seven cases. ATP did not reverse ventricular fibrillation in any cases, but potassium given with ATP reversed this arrhythmia in two out of four cases. In the cat heart, the reversal of ventricular fibrillation in all cases was accomplished only by increasing potassium chloride concentration to 5.4 mM/L. and adding glucose (1 Gm./L.).

**References**

3. **Torres, S.** Effects de la ausencia de metabolitos en el liquido de perfusión sobre el electrogramma del corazón aislado del gato. Tesis de Licenciatura Facultad de Medicina, Universidad de Chile, 1957.
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