Different Effects of Acute Ischemia and Anoxia on the Canine Myocardium

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Summary

We investigated differences between ischemic and anoxic myocardium with respect to early mechanical and metabolic changes. Ischemia and anoxia were induced in the area perfused by the distal left anterior descending artery in 32 mongrel dogs. Since both the ischemia and the anoxia in this preparation resulted in very little change in global cardiac hemodynamics, indirect mechanical and metabolic effects on the involved myocardium were minimal. However, regional anoxia caused a later development of a myocardial systolic bulge than did regional ischemia (44.8±13.6 vs 26.8±9.9 sec). Myocardial ATP content was reduced to the same level 5 min after the onset of ischemia and anoxia. Anoxia with high K+ did not result in an earlier myocardial systolic bulge time, but myocardial ATP was maintained at a higher level than during ischemia. Anoxia with low pH also did not affect the time for development of a myocardial systolic bulge.

We concluded that neither acidosis nor hyperpotassemia are more causally related to the earlier development of a myocardial systolic bulge during regional ischemia than during regional anoxia. Also the absolute value of myocardial ATP content is unlikely to be causally related to the determination of myocardial contraction, as reflected by the development of a myocardial systolic bulge.

Additional Indexing Words:
ATP Lactate Coronary perfusion Myocardial contraction

The difference between ischemia and anoxia is the absence of washout of anaerobically produced metabolites in ischemia. In the early phase of ischemia, accumulation of the metabolites, such as H+ and K+ ions and lactate,
have been speculated to be the cause of a difference in the mechanical response of the myocardium to ischemia and anoxia. However, the effect of each metabolite upon early mechanical changes during anoxia has not been carefully tested under in vivo conditions.

For an evaluation of the effects of various metabolites upon regional myocardial function and metabolism during anoxia, it is important not to alter global ventricular function during the experimental interventions, since function and metabolism in the region under study may be dependent on global ventricular function. In the present study we examined the effects of regional myocardial ischemia and anoxia in a canine preparation in the absence of prominent changes in global ventricular function and we showed that neither low pH nor a high K⁺ level in interstitial fluid was the cause of an earlier deterioration of myocardial contraction in ischemic myocardium as compared to that in anoxic myocardium.

Methods

Thirty-two mongrel dogs of both sexes weighing 10.5 to 29 Kg were anesthetized with intravenous Nembutal in a dose of 25-35 mg/Kg. Under artificial ventilation with a Harvard respirator, arterial blood PO₂, PCO₂ and pH were maintained between 100 and 150 torr, at approximately 30 torr, and at approximately 7.4, respectively.

A left thoracotomy was performed, the pericardium was opened, and the left anterior descending artery (LAD) was dissected distal to the first diagonal branch. A pair of small ultrasonic crystals was implanted subendocardially in the region of the left ventricle which became cyanotic and akinetic during transient occlusion of the dissected artery. The left subclavian artery was isolated and prepared for cannulation. A section of silicon tubing containing an electromagnetic flow probe and a three-way stop cock was connected to the subclavian artery at one end and to the dissected part of the LAD at the other end. The peripheral LAD perfusing pressure through this tubing was about 10% less than aortic pressure, but the peak flow rate during reactive hyperemia, induced by 15 sec occlusion of the tubing, was more than 200% of the control flow rate.

Aortic and left ventricular pressures (AoP, LVP) were measured through catheters introduced into the femoral artery and the apex of the left ventricle, respectively. Aortic blood flow was measured at the root of the aorta by an electromagnetic flowmeter. A tubing pump (Cole-Parmer Instrument Co.) was used for regional anoxic perfusion through the three-way stop cock of the by-pass circuit (Fig. 1).
Before and after cannulation of the LAD with the tip of the by-pass circuit, records of the hemodynamic variables were obtained and the course of development of the systolic bulge induced by ischemia was determined. After hemodynamics were stabilized, the three-way stop cock was turned to stop arterial blood flow and to start the infusion of the anoxic solution, which was pumped into the LAD at the same flow rate as the LAD blood flow in the control period. After 5 min of the infusion of anoxic solution, the center of the anoxic myocardium was biopsied by using a cylindrical knife mounted on an electric drill for the measurement of high energy phosphate, lactate and pyruvate. High energy phosphates were measured with high performance liquid chromatography. Lactate and pyruvate were quantified by an enzymatic method. They were expressed by μmol/g wet weight of myocardium.

According to the experimental interventions employed the animals were divided into the following 3 groups:

Group I (8 dogs subjected to regional myocardial ischemia): The bypass circuit was not used and the LAD was occluded distal to the first diagonal branch for 5 min.

Group II (8 dogs subjected to regional myocardial anoxia): The peripheral LAD area was perfused for 5 min with normal Krebs-Henseleit solution saturated with a gas mixture of 95% N₂ and 5% CO₂.

Group III (16 dogs subjected to regional myocardial anoxia and either high K⁺ or low pH): The modified anoxic Krebs-Henseleit solution was perfused for 5 min.
Subgroup i (7 dogs in the high K+ group): The content of K+ in the anoxic solution was increased to 10 mEq/L with KCl.

Subgroup ii (9 dogs in the low pH group): The pH of the anoxic solution was decreased to 6.8 with lowered NaHCO₃ or with modification of gas used for deoxygenation to 25% CO₂ and 75% N₂.

The use of a 10 mEq/L K+ solution was based on the finding of Hill and Gettes\(^\text{10}\) that the interstitial K+ concentration in ischemic myocardium increases to 10 mEq/L 5 min after coronary occlusion in the swine. The use of the pH 6.8 solution was based on the finding of Ichihara et al\(^\text{11}\) that the pH in the ischemic myocardium decreases to 6.8 5 min after coronary occlusion in the dog.

The interval between the start of anoxia or ischemia and the completion of the systolic bulge of the involved myocardium was adopted as an index of the change in regional myocardial contraction. The definition of the systolic bulge in this study was the prolongation of the segment length from the end-diastolic length throughout the systolic period. The systolic period was defined as the period from the tip of the R wave in lead II of the ECG to the peak of negative LVdP/dt. Since LVP was measured through a catheter, the time lag due to the catheter was corrected by subtracting the time between the tip of the R wave in the ECG and the onset of the steep rise of LVP. The time of onset of anoxia was also corrected by subtracting the time lag for the passage of the anoxic solution from the three-way stopcock to the tip of the tubing inserted into the LAD.

The difference between ischemic and anoxic effects upon the myocardium was observed by comparing groups I to II. In a comparison between groups II and III, the effects of high K+ and low pH which might affect the myocardial contraction in group I but not in group II were estimated in the absence of oxygen supply.

Statistical analyses consisted of paired and unpaired t-tests and the Cochran-Cox equation. A "p" value less than 0.05 was considered to be statistically significant.

Results

1. Effects of regional ischemia and anoxia upon global hemodynamics

Fig. 2 shows a hemodynamic tracing obtained from an animal in group II. In this particular animal, perfusion of the LAD after insertion of the cannula from the subclavian artery did not result in complete return to the control state in hemodynamics (L-control vs P-control in Fig. 2). However, the mean values for the hemodynamic variables were not statistically different.
Fig. 2. An example of the hemodynamic tracings obtained in a group II animal. L-control: control for ischemia (ligation), L-30sec: 30 sec after ischemia, P-control: control for anoxia (perfusion).

Table I. Hemodynamic Data Before and After LAD Cannulation

<table>
<thead>
<tr>
<th></th>
<th>Heart Rate (beat/min)</th>
<th>Mean AoP (mmHg)</th>
<th>LVEDP (mmHg)</th>
<th>AoF (ml/beat)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before</td>
<td>160.0 ± 22.4 NS</td>
<td>103.4 ± 20.1 NS</td>
<td>4.8 ± 3.5 NS</td>
<td>7.4 ± 2.0 NS</td>
</tr>
<tr>
<td>After</td>
<td>158.0 ± 19.4</td>
<td>100.6 ± 18.5</td>
<td>5.9 ± 4.0</td>
<td>7.3 ± 2.4</td>
</tr>
</tbody>
</table>

(mean ± SD)

Table II. Effects of Segmental Ischemia and Anoxia upon Hemodynamics

<table>
<thead>
<tr>
<th>Group</th>
<th>HR (beat/min)</th>
<th>mAoP (mmHg)</th>
<th>LVEDP (mmHg)</th>
<th>AoF (ml/beat)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>before</td>
<td>before</td>
<td>before</td>
<td>before</td>
</tr>
<tr>
<td></td>
<td>5 min</td>
<td>5 min</td>
<td>5 min</td>
<td>5 min</td>
</tr>
<tr>
<td>I</td>
<td>Ischemia</td>
<td>108 ± 29</td>
<td>6.3 ± 1.6</td>
<td>7.5 ± 3.6</td>
</tr>
<tr>
<td>n=8</td>
<td></td>
<td>175 ± 31</td>
<td>± 16</td>
<td>±1.7</td>
</tr>
<tr>
<td>II</td>
<td>Anoxia</td>
<td>193 ± 22</td>
<td>106 ± 4.2</td>
<td>9.5 ± 4.5</td>
</tr>
<tr>
<td>n=8</td>
<td></td>
<td>161 ± 23</td>
<td>± 20</td>
<td>±3.4</td>
</tr>
<tr>
<td>i</td>
<td>High K</td>
<td>190 ± 27</td>
<td>106 ± 4.9</td>
<td>7.9 ± 2.8</td>
</tr>
<tr>
<td>n=7</td>
<td></td>
<td>161 ± 23</td>
<td>± 23</td>
<td>±0.6</td>
</tr>
<tr>
<td>III</td>
<td>Low pH</td>
<td>194 ± 22</td>
<td>106 ± 4.7</td>
<td>6.4 ± 2.8</td>
</tr>
<tr>
<td>n=9</td>
<td></td>
<td>154 ± 18</td>
<td>± 25</td>
<td>±2.8</td>
</tr>
</tbody>
</table>

(mean ± SD)
before and after the completion of the by-pass circuit (Table I).

Neither 5 min of regional ischemia nor anoxia changed the heart rate (HR) and the mean aortic pressure (mAoP) in all 3 groups. Left ventricular end-diastolic pressure (LVEDP) was increased above the control level in all 3 groups. This increase, which was the same percentage in all groups, was probably due to preloading of the ventricle with Krebs-Henseleit solution. Stroke volume (AoF) was unchanged in group I, but increased in groups II and III. This was also probably due to an increase preload. The double product was not changed significantly in any group (Table II).

2. Changes in regional myocardial segment length by ischemia and anoxia (Table III)

In group I, the systolic bulge was completed 26.3±3.5 sec after the LAD occlusion, followed by changes in % systolic shortening (after completion of the systolic bulge, this value became negative) and end-diastolic length of ischemic myocardium for about 25 sec. The bulge formation time during ischemia was highly variable but the differences among the 3 groups were not statistically significant. Those times were consistently shorter than the ones noted during anoxia. These data suggested that neither high K⁺ nor low pH were the cause of the earlier systolic bulge in ischemia than in anoxia.

3. Changes in myocardial nucleotides, lactate and pyruvate induced by ischemia and anoxia (Table IV)

In 18 of the animals used in this study, a myocardial biopsy specimen, obtained simultaneously from an uninvolved area of the ventricle when ischemic or anoxic myocardium, was biopsied. The results of the chemical analyses are shown in Table IV. Either 5 min of ischemia or anoxia reduced myocardial ATP content significantly in comparison to the normal area. The myocardial ATP content in group III i was higher than that in groups I, II and III ii. This suggests that a high interstitial K⁺ concentration preserves the myocardial ATP in the absence of oxygen supply.

![Table III. Metabolic Changes Induced by Ischemia and Anoxia](image-url)
Both ischemia and anoxia increased the lactate content of myocardium, but the washout effect resulted in less lactate content in the anoxia groups (II, III i and III ii) than in the ischemia group (I). Although ATP was preserved in group III i, lactate was not significantly lower than in group II. By contrast, the decrease in ATP in group III ii was almost the same as in group II; however, the lactate level was significantly lower than in group II.

**DISCUSSION**

**I. Effects of the changes in global cardiac hemodynamics upon regional myocardium**

With a regional reduction in coronary blood flow, the amount of tension exerted on the ischemic myocardium should be dependent on the size of the ischemic region. If a large region of myocardium ceased to contract, ventricular pressure would decline markedly and the tension exerted on the ischemic myocardium would become minimal. In the present study the mechanical load reduction was expected to protect the ischemic myocardium\(^5\) and an in vitro study revealed that an increase in isometric tension in the anoxic myocardium resulted in an increase in high energy phosphate utilization.\(^6\) Irreversible myocardial injury was believed to result from ATP reduction in the myocardium.\(^12\) Therefore, a preparation in which a regional disturbance in myocardial contraction does not induce global hemodynamic changes is needed for investigating the relation between regional myocardial contraction and metabolic change.

The coronary anatomy of the mongrel dog is variable and an occlusion of the LAD distal to the first diagonal branch does not always result in the...
same hemodynamic changes. In the present study, visible collaterals from the circumflex to the LAD were prominent in a few cases and LAD occlusion did not result in a myocardial systolic bulge. These cases were eliminated from the study.

In our study, the increase in LVEDP was statistically significant. Although the percent changes in LVEDP was not significantly different among the 3 groups, the absolute values for LVEDP in groups II and III were higher than those in group I. An increase in AoF in groups II and III was also noted. Both of these changes might be expected to cause greater deterioration of mechanical function and depletion of high energy phosphate stores in groups II and III than in group I. However, the myocardial ATP content was not significantly higher in group I than in groups II and III. Moreover, in group III i, the myocardial ATP content was higher than in groups I, II and III ii. The high concentration of K+ in the anoxic myocardium was considered to preserve ATP.

2. Factors which promote the development of a myocardial systolic bulge

The pattern of systolic bulge development was not different with anoxia or ischemia, but the time required for complete formation of the systolic bulge was a little longer with anoxia. Braasch et al reported that coronary occlusion led to a rapid depletion of creatine phosphate and subsequent diminution in tissue ATP, resulting in cessation of contraction of the ischemic myocardium. In their experiment, ATP started to decline 15 sec after coronary occlusion, but not significantly. By contrast, we could not find a statistically significant decrease in ATP content when the ischemic myocardium was biopsied 40 sec after coronary occlusion. There are several papers reporting that the onset of the cessation of contraction of the ischemic myocardium cannot be related to a depletion of ATP.

In our study, lack of data on creatine phosphate left the question of the importance of high energy phosphate stores unresolved. Further studies on creatine phosphate combined with functional compartmentation of ATP are indicated.

The washout effect of anoxia in preventing acidosis and the accumulation of K+ has been proposed as an important difference between ischemia and anoxia. Acidosis in the myocardium is well known as a depressant of the contractile state. We simulated the acidosis of ischemia by perfusing with an anoxic solution which had a low pH. This method should directly lower the pH of interstitial fluid but only indirectly lower the pH of intracellular fluid. The manner used to reduce the pH of the perfusate was investigated for its effects on myocardial contraction. Low pH perfusate prepared by the
addition of CO₂ has been reported as a more potent myocardial depressant than perfusate of low pH prepared by removal of HCO₃⁻. In our study, lowering the pH of the perfusate was done by adding CO₂ in 3 cases and by removing HCO₃⁻ in 3 cases, but no difference in the development of a systolic bulge was noted. Although the work of Steenbergen et al. suggests that perfusion with a low pH solution might affect myocardial contraction, we did not find an earlier development of the systolic bulge. The reason for a lower myocardial lactate in group III than in group II was thought to be a blocking effect of acidosis on glycolysis.

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