Ischemia-Reperfusion Induced Elevation of Diastolic Tension in the Isolated Guinea Pig Heart and the Effects of Calcium Antagonists

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SUMMARY

Characteristics of the temporal elevation of diastolic tension, produced by ischemia-reperfusion in isolated and paced Langendorff’s hearts of guinea pigs, were studied. The elevation of diastolic tension corresponded with an elevation of left ventricular end diastolic pressure after a short ischemic period in the isovolumic heart. These phenomena were thought to be a result of incomplete relaxation. The degree of the elevation of diastolic tension depended upon the duration of ischemic period (3–10 min). This elevation was reproducible in one preparation; nearly the same changes were obtained in a second trial after 35 min of reperfusion when the ischemic period was within 5 min. An increment in the pacing rate to 150% of the first trial value doubled the elevation of diastolic tension by the second 5 min ischemia. Inhibition of glycolytic flux by iode acetic acid augmented the elevation after 3 min of ischemia. In addition, 5 min of ischemia with iode acetic acid caused contracture and recovery was slight. On the other hand, either lowering the Ca²⁺ concentration in the perfusing solution to a half the normal value, or treatment with Ca²⁺ antagonists (such as diltiazem), reduced the elevation of diastolic tension significantly. Diltiazem also suppressed the increment in elevation produced by a high pacing rate.

It can be concluded that the temporal elevation of diastolic tension during reperfusion reflects the ischemic failure of the heart. This change is presumably due to intracellular Ca²⁺ overload or accumulation. In addition, since ischemic changes were reproducible in this preparation, it is a useful model for estimating the effects of drugs on the ischemic heart.
THE ischemia results in impairment of the heart, including a decrease in contractility and in oxidative phosphorylation, an accumulation of lactic acid and a lowering of tissue pH.\(^1,2\) When the ischemic period is short, these changes remain in a functionally reversible state. However, impairment of the ischemic heart after reperfusion or reoxygenation is observed in many experimental models.\(^1,2\)

It is also recognized that reconstruction of the blood flow sometimes causes impairment of the mechanical function of the heart.\(^3\) Reperfusion-induced changes depend partly on the degree of the damage caused by prior ischemia, which should involve factors such as the duration of ischemia, temperature, metabolic requirements, and energy supply. In addition, ischemia results in an increase in membrane permeability, and there is increasing evidence that restoration of coronary flow produces a cellular calcium accumulation or calcium overload, which is claimed to augment ischemic damage to the myocardium.\(^4\)–\(^6\)

Although there are many studies of the ischemic heart, few studies deal with reproducible changes in one preparation.\(^7\) We studied the elevation of diastolic tension after temporal and global ischemia in the isolated guinea pig heart and found that it was reproducible in the same preparation. We characterized this phenomenon and studied the effects of Ca\(^{2+}\) antagonists in this preparation.

**Methods**

1. Preparations

Male guinea pigs (Hartley strain) weighing 225 to 350 Gm were stunned and bled. The heart was isolated and perfused by Langendorff’s method. Modified Locke-Ringer’s solution (NaCl: 154 mM, KCl: 5.6 mM, CaCl\(_2\): 2.2 mM, MgCl\(_2\): 2.1 mM, NaHCO\(_3\): 5.9 mM, glucose: 11 mM, pH: 7.0), containing 2% defibrinated rabbit blood and aerated with 95% O\(_2\) and 5% CO\(_2\), was used as a perfusion solution. The temperature of the solution and the perfusing pressure were 35°C and 30 cm H\(_2\)O, respectively. The heart was paced with rectangular pulses (10 v, 5 msec) through electrodes attached to the ventricles (Nihon Kohden, MSE-3). The pacing rate was 240–280 times/min. The contractile force was measured with a strain gauge transducer, which was connected to the apex cordis with thread. An initial dia-
systolic tension of 2 Gm was applied to the heart.

In another experiment, the isovolumic heart was prepared by placing a balloon in the left ventricle from the left atria through the mitral valve; the heart was perfused as mentioned above. The balloon was filled with saline (0.1–0.2 ml) and its internal pressure was measured by a pressure transducer (Century Technology Company, CP-01) and denoted as left ventricular pressure (LVP). The first derivative of LVP (dp/dt) was obtained with a differentiator (Nihon Kohden, S-5151, time constant 2 msec).

2. Procedure

The experiment was started after developed tension (systolic tension-diastolic tension) and diastolic tension of the heart equilibrated. Global ischemia was induced by a short cessation of perfusion for 3 to 10 min. Although the post-ischemic elevation of diastolic tension depended on the duration of ischemic period, we used mostly 5 min of ischemia because of the reproducibility. A 35 min interval was allowed between the first and the second ischemic periods in each experiment. We studied the effects of various treatments by comparing the second trial with the initial control trial for the same preparation. In the experiment studying the influence of heart rate, the pacing rate was increased to 150% of the first trial value, both 10 min before and during the second trial. When the effect of extracellular Ca²⁺ concentration was examined, the Ca²⁺ concentration of the perfusion solution was lowered by 50% 20 min before the second trial. In the experiment examining the influence of glycolysis inhibition, the perfusion solution was changed to one containing 0.1 mM iode acetic acid (IAA) and 5 mM sodium acetate 10 min before the second trial. This concentration of IAA was selected to inhibit glycolysis specifically.⁷

In order to see negative inotropic actions of Ca²⁺ antagonists (diltiazem, verapamil, and nifedipine), drug doses were infused into the aortic cannula for 5 min. Each drug was administered cumulatively to the preparation. In the reperfusion study, drugs were infused into the preparation for 5 min before the second trial.

In the isovolumic heart, the duration of ischemic period was 5 min and changes in LVP were observed during and following 5 min of ischemia.

3. Drugs

Diltiazem and verapamil were dissolved in physiological saline (1 mg/ml) and nifedipine was dissolved in propylene glycol (1 mg/ml). The infusion volume was adjusted to 0.33 ml/min by dilution with Locke-Ringer's solution. In the case of nifedipine the perfusion apparatus was intercepted
of light. The vehicles did not effect developed tension.

RESULTS

Fig. 1 represents a typical experimental record, showing the changes in contractile force of the heart during and after ischemia. Developed tension started to decline soon after cessation of coronary perfusion and the heart failed to follow the ventricular pacing within 2 min. Although no tension development was observed afterwards, irregular beats were seen during ischemia in some cases. When perfusion was re-established after 5 min of ischemia, irregular beats appeared immediately and the heart began to follow the pacing about 10 sec later. Developed tension recovered gradually, with a concomitant increase in diastolic tension. The elevation of diastolic tension attained a peak between 1 and 2 min after reperfusion, and returned to pre-ischemic levels within 3 and 4 min. Similar changes in developed tension and diastolic tension were observed in the second ischemic trial.

A record of an experiment using an isovolumic heart preparation is shown in Fig. 2. Under the same experimental conditions as Fig. 1, LVP
began to decline soon after the cessation of perfusion and contraction ceased about 2 min later. Reperfusion after 5 min of ischemia caused the elevation of left ventricular end diastolic pressure (LVEDP), along with a recovery of LVP. As shown in the chart record, the typical diastolic phase, observed before ischemia, became incomplete after reperfusion. In addition, the time courses of the changes in LVP and LVEDP were quite similar to those of developed tension in Fig. 1.

1. Influence of the duration of the ischemic period

Fig. 3 shows the elevation of diastolic tension and recovery of developed tension after various durations of ischemia.

![Fig. 3](image-url)

**Fig. 3.** Influences of the duration of the ischemic period on the reperfusion-induced elevation of diastolic tension (upper panel) and recovery of developed tension, measured 3 min after the start of reperfusion (lower panel). The elevations of diastolic tension are shown as differences between peak values of the post-ischemic period and pre-ischemic values. The recovery of developed tension 3 min after the start of reperfusion is expressed as the percent of pre-ischemic control values (shown in Table I). □: first trial, □: second trial. Bars indicate the SEM (N=6).

**Table I. Control Values of Developed Tension in Guinea Pig Langendorff's Heart**

<table>
<thead>
<tr>
<th>Ischemic period</th>
<th>Developed tension (Gm)</th>
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<tbody>
<tr>
<td></td>
<td>Before 1st trial</td>
</tr>
<tr>
<td>3 min</td>
<td>3.25±0.23</td>
</tr>
<tr>
<td>5 min</td>
<td>3.74±0.20</td>
</tr>
<tr>
<td>10 min</td>
<td>2.97±0.11</td>
</tr>
</tbody>
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Each value is a mean±SEM of 6 experiments.
tension following 3, 5, or 10 min of ischemia in the first and the second trial. Table I summarizes the pre-ischemic control values of developed tension in these experiments. No significant differences were observed between control values for the first and second trials. As shown in Fig. 3, a longer period of ischemia caused a larger elevation of diastolic tension. The elevation of diastolic tension in the second trial was similar to that of the first trial after 3 and 5 min of ischemia, but it tended to decrease after 10 min of ischemia (0.1 > p > 0.05). On the other hand, the degree of recovery of developed tension following the first and the second ischemia was identical.

2. Influence of heart rate

Fig. 4 shows a record of the experiment in which the pacing rate in the second trial was increased to 150% of that of the first trial. When the pacing rate was increased (from 256.7±8.0 to 385.0±12.0 times/min), developed tension decreased to 82.7±1.7% (mean±SEM). The reperfusion-induced elevation of diastolic tension was augmented significantly under this condition and the recovery of developed tension 3 min after reperfusion was also significantly smaller than for the first trial. Thus an increase in heart rate impaired the ischemic heart.

3. Influence of Ca\(^{2+}\) concentration in the perfusion solution

Fig. 5 shows an example of an experiment studying the influence of Ca\(^{2+}\) concentration in the perfusion solution. A low Ca\(^{2+}\) perfusion solution (a half of the normal Ca\(^{2+}\) concentration) caused a decrease in developed tension to about 75% of the value obtained with a normal perfusion solution. Under this condition, the elevation of diastolic tension following reperfusion was significantly reduced (0.76±0.13 Gm to 0.31±0.07 Gm, N=6, p<0.01),
while the percent recovery of developed tension after reperfusion was almost the same as that of the first trial (90.2±2.9% to 91.6±2.2%). Therefore, a lower Ca²⁺ concentration in the perfusion solution reduced the severity of reperfusion-induced heart failure.

4. Influence of glycolysis blockade

Fig. 6 shows records from the experiments in which glycolysis was inhibited by IAA. When perfusion was stopped in the presence of IAA, the developed tension diminished quickly. The contractions ceased within 1 min without the appearance of irregular beats. As shown in Fig. 6A, tension began to rise 3 min after cessation of perfusion, finally resulting in a contracture.
Reperfusion after 5 min of ischemia produced little recovery of the developed tension under this condition and the diastolic tension continued to rise, even after 10 min. At this time, the heart was stiff. By contrast, developed tension recovered when the heart was reperfused after 3 min of ischemia, but the degree of recovery of developed tension was smaller than in the first trial (Fig. 6B). The elevation of diastolic tension in this group was augmented significantly in the second trial.

5. Effects of Ca\textsuperscript{2+} antagonists

(1) Effects of Ca\textsuperscript{2+} antagonists on developed tension

Dose-response curves for the effects of diltiazem, verapamil, and nifedipine on developed tension are shown in Fig. 7. All 3 drugs exhibited dose-related, negative inotropic actions and relative potencies of diltiazem, verapamil, and nifedipine were approximately 1, 30, and 30, respectively. The doses which exhibited equipotent, negative inotropic actions were chosen for each drug for further experiments.

(2) Effects of Ca\textsuperscript{2+} antagonists on the reperfusion-induced elevation
of diastolic tension

Fig. 8 shows the effect of diltiazem on the reperfusion-induced elevation of diastolic tension. Diltiazem decreased developed tension before ischemia, but the heart could follow the pacing rate for a longer period during ischemia. Reperfusion-induced elevation of diastolic tension was significantly reduced by diltiazem. Similar results were obtained with verapamil and nifedipine (Fig. 9A). Fig. 9A also shows the recovery of developed tension, 3 min after start of reperfusion. Diltiazem and nifedipine did not affect the recovery of developed tension, but the depression of contraction persisted with verapamil.

Fig. 9B shows the influence of rapid pacing on the elevation of diastolic tension with or without diltiazem (30 μg/min). As mentioned in section 2, rapid pacing augmented the elevation of diastolic tension and the reduced contraction persisted 3 min after the start of reperfusion. Diltiazem pre-
vented the augmentation of diastolic tension and the reduction in recovery of developed tension after reperfusion.

**DISCUSSION**

The ischemic heart requires a reconstruction of blood flow patterns to recover from failure. However, it is also recognized that reperfusion sometimes impairs the ischemic heart. Since the observations of impairment of the ischemic heart due to reperfusion by Jennings et al., many experimental studies have been performed, including studies of papillary muscle and/or trabecular muscle of cats and guinea pigs. These studies showed that reperfusion sometimes causes dysfunction of the heart, which is termed incomplete relaxation.

Trautwein and Dudel observed a transient prolongation of tension duration and the plateau of the cardiac action potential when the heart muscle was recovering from hypoxia. Henry et al. found that an elevation of LVEDP was correlated intimately with calcium accumulation in mitochondria in the ischemic isovolumic heart of the rabbit. We also found an elevation of diastolic tension after temporal ischemia in the isolated guinea pig heart. This elevation of diastolic tension was augmented by a longer ischemic period or a high pacing rate (larger metabolic requirement), which cause impairment of the ischemic heart. Furthermore, depressed energy production caused by inhibition of glycolysis with IAA led to an increment of the elevation of diastolic tension or contracture. Therefore, we considered the elevation of diastolic tension to be one of the manifestations of ischemic heart failure. The cause of this response is probably an incomplete relaxation, because the diastolic phase disappeared during the elevation of LVEDP in the isovolumic heart and the time course of the elevation of diastolic tension corresponded to that of the elevation of LVEDP.

The ischemia-reperfusion-induced elevation of diastolic tension was reversible as long as the ischemic period did not exceed 5 min. In addition, it was reproducible in the same preparation. From these results, this change should reflect mild functional damage. It was confirmed by an electron microscopic study, which revealed no histological abnormalities in this preparation (data was not shown).

In our model, the changes due to ischemia were reproducible in the same preparation. Therefore, this model seems to be useful for estimating the effects of drugs on ischemic heart damage. We studied the effects of a low Ca\(^{2+}\) perfusion solution and Ca\(^{2+}\) antagonists on the ischemic heart and found that these treatments diminished the elevation of diastolic tension after
temporal ischemia. Therefore, our results suggest that Ca\(^{2+}\) antagonists such as diltiazem protect the heart from ischemic failure through both a negative inotropic action and a suppression of intracellular Ca\(^{2+}\) accumulation or Ca\(^{2+}\) overload, due to their Ca\(^{2+}\) antagonistic action. Diltiazem,\(^{15,16}\) verapamil,\(^{17}\) and nifedipine\(^{18-20}\) all show negative inotropic actions. The doses which we used were selected to exhibit the same negative inotropic potency. However, in comparison with other studies, potency of verapamil was stronger. This was probably due to the pacing rate.\(^{21,22}\) We also recognized that the negative inotropic action of verapamil was longer lasting than for diltiazem and nifedipine.

**REFERENCES**

Arzneim-Forsch 12: 549, 1962


