Mechanisms of Abnormal Myocardial Relaxation Induced by Ischemia: Comparison of Low Flow Ischemia and Hypoxia in Isolated Rabbit Heart

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To investigate the mechanisms of altered myocardial diastolic stiffness and relaxation induced by myocardial oxygen deficit, we compared the hemodynamic effects of low flow ischemia (myocardial infarction model) and hypoxia in which the coronary flow was not reduced (angina pectoris model) in isolated retrograde perfused rabbit hearts.

A 15-min hypoxia induced a significant increase in the left ventricular end diastolic pressure (LVEDP 10 ± 3 to 21 ± 7 mmHg, $p < 0.05$), P_o (-1 ± 6 to 12 ± 5 mmHg, $p < 0.01$) and T_B (41 ± 8 to 54 ± 10 ms, $p < 0.05$), where P_o and T_B are the asymptote and the time constant of the isovolumetric left ventricular pressure decline ($P = P_o + A \cdot e^{-t/T_B}$), respectively. There was a close linear correlation between the delta P_o and delta LVEDP ($t = 0.98$, $p < 0.01$), and between delta T_B and delta LVEDP ($r = 0.73$, $p < 0.05$). Low flow ischemia increased P_o (0 ± 4 to 2 ± 3 mmHg, $p < 0.05$) and T_B (43 ± 4 to 50 ± 11 mmHg, NS).

When DPI 201-106, a cardiotonic agent, was given to the hypoxia model by increasing the Ca^{++} sensitivity of contractile proteins, further increases were observed in LVEDP (to 25 ± 8 mmHg, $p < 0.05$) and P_o (to 15 ± 7 mmHg, $p < 0.05$). DPI also increased LVEDP (7 ± 2 to 9 ± 4 mmHg, NS) and P_o (to 4 ± 4 mmHg, $p < 0.05$) in the ischemia model. However, T_B was not altered by DPI.

Conclusions: 1) P_o reflects the LVEDP. 2) The discrepancies between the changes of T and P_o induced by DPI indicate different mechanisms underlying changes in LV diastolic stiffness and relaxation. 3) Both hypoxia and ischemia induced abnormalities in myocardial diastolic stiffness and relaxation.

INCREASED left ventricular diastolic chamber stiffness during angina pectoris in man has been observed repeatedly^{1,2} and this phenomenon

has been reproduced in anesthetized open chest dogs with severe two vessel coronary arterial stenoses, with the heart paced at twice the resting rate for a few minutes³

Increased myocardial stiffness with slow relaxation also has been induced by hypoxia in the isolated rabbit heart, but global low flow ischemia, with or without pacing tachycardia in the same experimental model, failed to increase

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Fig.1. Effects of hypoxia and Ca^{++} agonist.

Oxygen content of perfusate was reduced to 32% of the control. LV systolic pressure fell abruptly and LVEDP rose gradually following hypoxia and stabilized at 15 min after the beginning of hypoxia. Then 10^{-6} M DPI was infused and increased to 3×10^{-6} M and 10^{-5} M every 10 min. CPP: coronary perfusion pressure, LVP: left ventricular pressure.

the left ventricular diastolic chamber stiffness⁴; similar findings were made in experiments using canine hearts^{5,6}.

To explain these differences, we postulated the possible mechanisms as follows: 1) Left ventricular chamber stiffness increases when the myocardial O_2 demand exceeds the supply. 2) This change is usually masked in ischemic (reduced coronary flow) preparations, perhaps because of the reduced turgor or tone of the coronary vascular bed, marked reduction in the systolic work (and therefore myocardial O_2 requirements), and local accumulation of hydrogen ions and metabolites following acute severe reduction of the coronary flow⁴.

Although the diastolic abnormalities induced by ischemia were considered to be the consequence of abnormal intracellular Ca^{++} availability secondary to an oxygen deficit^{3,4,7} the precise mechanism, especially the relationship between slow relaxation and increased diastolic myocardial stiffness, has not yet been ascertained.

The purpose of this study was to investigate the mechanism of altered myocardial diastolic stiffness and relaxation induced by a myocardial oxygen deficit using two different types of ischemia. Isolated rabbit hearts were perfused at a low coronary flow rate (low flow ischemia,

analogous to myocardial infarction) and the normal flow rate of a buffer with a reduced O_2 content (hypoxia, analogous to effort angina pectoris). We also attempted to elucidate the role of Ca^{++} in increased left ventricular diastolic chamber stiffness, i.e., myocardial stiffness and slow relaxation, in these different types of ischemia.

MATERIAL AND METHOD

Surgical and perfusion techniques.

The experimental method used was fundamentally the same as that of previously reported studies⁴. Briefly, rabbits were decapitated and the hearts quickly removed from the thorax and placed in a water-jacketed constant temperature chamber (37°C). The coronary arteries were perfused retrogradely by a constant flow pump at a rate of 30 ml/min through a cannula inserted into the aortic root.

The perfusate consisted of modified Krebs-Henseleit buffer: 118 mM. NaCl, 4.7 mM. KCl, 2.0 mM. CaCl_2 , 1.2 mM. KH_2PO_4 , 1.2 mM. MgSO_4 , 25 mM. NaHCO_3 , 0.4 mM. NaEDTA, 5.5 mM. Glucose and 1.0 mM. lactic acid. The perfusate was equilibrated with a 5% CO_2 -95% O_2 gas mixture and pO_2 was above 500 mmHg.

Fig.2. Effects of ischemia and Ca^{++} agonist.

Coronary flow was reduced to 33% of the control. LV systolic pressure, LVEDP and coronary perfusion pressure fell abruptly and stabilized quickly. DPI was infused in the same manner as hypoxia.

A collapsed latex balloon, slightly larger than the left ventricular chamber of a rabbit, was inserted into the left ventricle via a left atrial incision. The balloon was then filled with bubble-free saline and attached to a Statham P23 DI pressure transducer (Statham Instruments, Inc., Calif) via 15 cm long polyethylene tubing with an inner diameter of 1.08 mm.

Aortic pressure, which in this model is the coronary perfusion pressure, was measured via a Y-shaped connector attached to the infusion cannula using another Statham P23 DI pressure transducer.

A bipolar pacemaker electrode was inserted into the right ventricle via a right atrial incision and the heart was paced at a constant rate of 3.0 Hz by an electric stimulator S-7272B (Nihon Kodon Co., Tokyo).

Signals were amplified by a Nihon Kodon Polygraph System KM-6000 (Nihon Kodon Co., Tokyo) and stored on magnetic tape (Sony Data Recorder DFR-3515, Sony Magnescale Inc., Tokyo). The stored data were analyzed by a DEC LSI 11/2 microcomputer system (Digital Equipment Co., Massachusetts). Using this system, the peak left ventricular systolic pressure, left ventricular end-diastolic pressure, left ventricular dp/dt , dp/dt/p , $(-)\text{dp/dt}$ and the time constant of the left ventricular pressure fall (Weiss's method, $P = A \cdot e^{-t/T_w}$ ⁹ and best fit

method, $P = P_o + A \cdot e^{-t/T_B}$ ¹⁰ were obtained.

Coronary vascular resistance (CVR) was calculated as the mean coronary perfusion pressure (CCP)/coronary flow (CF) and expressed as $\text{mmHg} \times \text{min/ml}$.

Before starting each experiment, the heart was perfused for 30 min at a coronary flow rate of 30 ml/min and paced at a heart rate of 3.0 Hz to allow the performance to stabilize. At the beginning of each experiment the balloon volume, i.e., the isovolumic left ventricular volume, was adjusted so that the left ventricular end-diastolic pressure was kept constant at 10 mmHg throughout the experiment.

Experimental Protocol:

1. Effects of hypoxia at constant coronary flow ($n = 6$)

After the heart parameters were stabilized, the perfusate was switched to a Krebs-Henseleit buffer that had the same composition as the control perfusate but was equilibrated with a 5% CO_2 –20% O_2 –75% N_2 gas mixture. Hemodynamic measurements were performed 15 min, 25 min, 35 min and 45 min after the beginning of hypoxia.

2. Effects of hypoxia and Ca^{++} ($n = 6$)

After 15 min of hypoxia when hemodynamic parameters were stable, an infusion of 10^{-6}M DPI 201–106 was started. Hemodynamic mea-

Fig.3. Effect of hypoxia and Ca^{++} agonist on time constant and asymptote of left ventricular isovolumic pressure decline.

T_w : time constant calculated by Weiss's method, T_b : time constant calculated by best fit method, P_o : asymptote calculated by best fit method, C: control, H: effects of 15 min of hypoxia, D: effects of hypoxia and 10^{-6} M DPI.

Fig.4. Relationships between delta LVEDP and delta P_o induced by hypoxia, ischemia and DPI.

A close linear correlation was observed between delta LVEDP and delta P_o following hypoxia ($Y = 0.99X - 1.3$, $r = 0.98$, $p < 0.01$). The same relationships were observed in the effect of 3×10^{-6} M DPI on both hypoxia ($Y = 1.08 + 1.5$, $r = 0.93$) and ischemia ($Y = 1.03X + 0.69$, $r = 0.80$) and all changed values were within the 95% confidence area of the regression line. ●, ○, □ indicates changes induced by hypoxia, hypoxia and 3×10^{-6} M DPI and ischemia and 3×10^{-6} M DPI, respectively.

measurements were performed 10 min after the infusion was started and then DPI was increased to 3×10^{-6} M and 10^{-5} M every 10 min.

3. Effects of low flow ischemia ($n = 8$)

Coronary flow was reduced from 30 ml/min to 10 ml/min and hemodynamic parameters were measured at 15 min, 25 min, 35 min and 45 min after the beginning of ischemia.

4. Effects of low flow ischemia and Ca^{++} ($n = 8$)

After 15 min of ischemia when the hemodynamic parameters were stable, infusion of 10^{-6} M DPI 201-106 was started. Hemodynamic measurements were performed 10 min after the infusion was started and then DPI was increased to 3×10^{-6} M and 10^{-5} M every 10 min.

All values are expressed as the mean $\pm$ SD. Significance tests were performed by the Student's t test for paired data to compare the effects of ischemia or hypoxia against the control. Other comparisons were performed using the Student's t test for unpaired data.

RESULTS

1. Effects of hypoxia at constant coronary flow

Hypoxia in which the oxygen content of the perfusate was reduced from 524 ± 17 (equilibrated by 5% CO_2 -95% O_2) to 166 ± 10 mmHg, (equilibrated by 5% CO_2 -20% O_2 -75% N_2) induced an abrupt drop in the peak systolic pressure, LV dp/dt and coronary perfusion pressure.

Fig. 5. Effect of ischemia and Ca^{++} agonist on time constant and asymptote of left ventricular isovolumic pressure decline.

C: control, I: effects of 15 min of ischemia, D: effects of ischemia and 10^{-6} M DPI.

Left ventricular end diastolic pressure rose gradually during the hypoxia. However, all changes stabilized 5 min after the onset of hypoxia and were sustained for another 10 min (Fig. 1).

At the end of 15 min of hypoxia, significant reductions were observed in the peak systolic pressure (101 ± 8 to 67 ± 7 mmHg, $p < 0.01$), LV dp/dt (1631 ± 285 to 999 ± 204 mmHg/s, $p < 0.01$) and LV (-) dp/dt (1218 ± 167 to 616 ± 71 mmHg/s, $p < 0.01$). LVEDP (10 ± 3 to 21 ± 7 mmHg, $p < 0.05$), Weiss's T (T_W 44 ± 7 to 94 ± 16 ms, $p < 0.01$), best fit T (T_B 41 ± 8 to 54 ± 10 ms, $p < 0.01$) and asymptote P_o (-1 ± 6 to 12 ± 5 mmHg, $p < 0.01$) were increased by hypoxia. There were no significant changes in the coronary perfusion pressure and coronary vascular resistance.

2. Effects of hypoxia and Ca^{++}

DPI 201–106 increased the coronary perfusion pressure and LVEDP in a dose-dependent manner and at a dosage of 10^{-5} M myocardial contracture developed (Fig. 1).

When 10^{-6} M DPI was infused for 10 min in a hypoxic heart, further increments were observed in LVEDP (from 21 ± 7 to 25 ± 8 mmHg, $p < 0.05$), T_W (94 ± 16 to 113 ± 20 ms, $p < 0.05$) and P_o (12 ± 5 to 15 ± 7 mmHg, $p < 0.05$). The peak systolic pressure, coronary perfusion pressure, coronary vascular resistance, LV (-) dp/dt, and T_B remained unchanged (Fig. 3).

3. Effects of low flow ischemia

Following reduction of the coronary perfusion rate, peak systolic pressure, LVEDP, LV dp/dt, LV (-)dp/dt and coronary perfusion pressure

dropped abruptly and these parameters were stable during the 15 min of low flow ischemia (Fig. 2).

At the end of 15 min of low flow ischemia, there was a significant decrease in the peak systolic pressure (105 ± 8 to 52 ± 6 mmHg, $p < 0.01$), left ventricular end diastolic pressure (11 ± 1 to 7 ± 2 mmHg, $p < 0.01$), LV dp/dt (1587 ± 197 to 833 ± 74 mmHg/s, $p < 0.05$), LV (-)dp/dt (1231 ± 159 to 573 ± 107 mmHg/s, $p < 0.05$) and coronary perfusion pressure (71 ± 12 to 28 ± 8 mmHg, $p < 0.01$). Significant increases were observed in T_W (45 ± 9 to 61 ± 20 ms, $p < 0.05$) and P_o (0 ± 4 to 2 ± 3 mmHg, $p < 0.05$).

4. Effects of low flow ischemia and Ca^{++}

DPI 201–106 increased LVEDP and the coronary perfusion pressure in a dose-dependent manner in ischemic rabbit hearts. However, compared to the changes observed in the hypoxic rabbit hearts the magnitude of the changes was very small.

10^{-6} M DPI induced significant changes only in T_W (61 ± 20 to 72 ± 26 ms, $p < 0.05$) and P_o (2 ± 3 to 4 ± 4 mmHg, $p < 0.05$). No significant changes were observed in any other parameters (Fig. 5).

5. Relationships between myocardial relaxation and diastolic stiffness

There was a very close linear correlation between delta P_o and delta LVEDP following hypoxia. The fact that the same relationships were observed in the effects of 3×10^{-6} M DPI on both hypoxia and ischemia suggest that there is a common mechanism in increased myocardial diastolic stiffness due to myocardial oxygen

deficit and DPI (Fig. 4).

A close linear relationship was observed between Δ LVEDP and ΔT_B ($Y = 0.97X + 3.5$, $r = 0.73$, $p < 0.05$) following hypoxia, but such a relationship was not observed when DPI was added to hypoxia.

DISCUSSION

In our previous study in which hypoxia (anoxia) was induced by switching the perfusate from buffer equilibrated with 95% O₂–5% CO₂ to 95% N₂–5% CO₂ (PO₂ < 40 mmHg), rapid and progressive deterioration in both the systolic and diastolic functions developed and myocardial contracture occurred.⁴ We tried to induce mild hypoxia instead of anoxia to obtain stable hemodynamic conditions and to make a comparison with the effects of ischemia in the present study. The O₂ content of the buffer was reduced to 32% of control by equilibrating the buffer with a 20% O₂–5% CO₂–70% N₂ gas mixture and stable hemodynamic conditions were achieved. On the other hand, the coronary flow was reduced from 30 ml/min to 10 ml/min (33%) with a fully oxygenated buffer to induce an equivalent ischemia. So, the oxygen supply was identical in these two experimental models.

Two min of anoxia turned the lactate extraction rate to negative in the previous study, but the same period of low flow ischemia (30 ml/min to 12 ml/min) only decreased this rate and failed to turn it to negative, indicating that hypoxia caused more severe myocardial damage than ischemia.⁴ In the present study, the left ventricular diastolic pressure, i.e., the preload to the ventricle, dropped following ischemia because of decreased coronary vascular tones. As a result, the already decreased systolic function secondary to the reduced oxygen supply dropped further. In contrast, the lesser drops in the peak systolic pressure and LV dp/dt in the hypoxic model suggest a worsened oxygen supply-demand balance. These findings do not contradict the previous study and might be important in explaining the differences in myocardial diastolic abnormalities between ischemia (infarction model) and hypoxia (angina pectoris model).

In the early diastole, a special protein in the sarcoplasmic reticulum (SR), phospholamban, plays a central role in the accumulation and storage of Ca⁺⁺, thus actively transporting Ca⁺⁺ from the cytoplasm into the lumen of SR. No relationship between these biochemical processes

and the time constant of the left ventricular isovolumic pressure decline T has yet been determined; however, evidence suggests that T reflects the rate of Ca⁺⁺ uptake by SR.⁸ Therefore, this parameter has been used as an indicator of myocardial relaxation.

Classically, the left ventricular isovolumic pressure decline curve was assumed to be a monoexponential curve with 0 mmHg asymptote (T_W)⁹ and was always reported to be prolonged during ischemia in both clinical² and experimental studies.^{3,4,6} However, there is no theoretical background for the assumption that the asymptote is constantly 0 mmHg. For this reason calculation of T_W without considering variable Po, which possibly contains erroneous information, and thus simultaneous calculation of the time constant T_B and asymptote Po by the best fit method was proposed.¹⁰ However, there are conflicting studies concerning T_B and asymptote Po.

In their first study where T_B and Po were calculated by the best fit method, Thompson and co-workers¹⁰ showed T_B always to be shorter than T_W, and both T_B and T_W were prolonged during pacing induced angina pectoris. Asymptote Po, which was always negative, was lower in the ischemic group than the non ischemic one, and increased during pacing induced angina pectoris. However, the amount of the increased value was smallest in the severest ischemic group compared to the mild or moderate group.

According to a study by Carroll and co-workers¹¹ with ischemia induced by exercise in patients, LVEDP and Po increased significantly but T_B was reduced. Yellin and co-workers¹² determined the asymptote by measuring the pressure decline after the mitral annulus was occluded at the volume of the left ventricular end-systole in the canine heart, and concluded that there was little actual difference between T_W and T in the concept of a true asymptote.

These results evoked confusion about the physiological meaning of Po and T_B and raised questions about obtaining T_B using such a complex method. However, T_W is affected by the left ventricular end-diastolic volume¹³ as well as the end-systolic volume,⁹ which is determined by the extent of systolic shortening and considered to represent the stored potential energy for elastic recoil. T_B and Po also apparently can be affected by the left ventricular volume. One of the reasons why our results were different from others is that we used an

isovolumetric heart model. In the present study, T_W and T_B were prolonged both by ischemia and hypoxia and the amount of change was greater in T_W . This difference must have been based on the difference of the asymptote, because in monoexponential fitting, the pressure decline curve must fall to a constant asymptote 0 mmHg beyond the actual asymptote P_o . The calculated T_W becomes greater than T_B .

There was a close linear correlation between delta LVEDP and delta P_o induced by hypoxia. LVEDP could be used as an index of myocardial diastolic stiffness because the heart was contracting isovolumetrically in the present study. Therefore, P_o could be a good indicator of myocardial stiffness. And if the above reasoning is correct, the results observed in the present study, that P_o consistently increases following ischemia and ischemia with DPI, have directly proven the concept that myocardial diastolic stiffness increases even in an ischemic model, although a collapsed coronary vascular bed secondary to the reduced coronary flow masked the effects.⁴ Furthermore, these findings indicated that in cases when the end-diastolic pressures, in other words the end point of relaxation, were changed by intervention, a combination of P_o and T_B was used in the discussion on myocardial relaxation and stiffness.

DPI 201-106 (DPI) is a new cardiotonic agent that increases the sensitivity of contractile elements to Ca^{++} and is quite different from other inotropic agents mediated by increased intracellular cyclic AMP or Na^+-K^+ exchange inhibition.¹⁴ In our preliminary study using the same experimental preparation, $10^{-6}M$ DPI increased LV dp/dp/p by 14% and decreased the coronary vascular resistance and left ventricular end-diastolic pressure by 10% and 23%, respectively. Time constant T calculated by either method was not altered at this dosage.

In both hypoxic and ischemic conditions DPI acted in a different manner from the aerobic condition. P_o , which was already increased secondary to ischemia or hypoxia, was increased further. These facts suggest that the increased Ca^{++} affinity to contractile elements by DPI result in an increased number of crossbridges in the systole; however, in the diastole by disturbed Ca^{++} sequestration from contractile protein, which is an energy consuming process affected by an insufficient O_2 supply, a part of Ca^{++} still remains on the contractile proteins and tension is maintained throughout the period. As a result,

LVEDP and P_o are increased by DPI and the same mechanisms are believed to explain the increased diastolic stiffness following ischemia and hypoxia.

Following this theory, in a previous study separated the diastolic abnormalities into two parts and considered that each was potentially an independent aspect of the process. One was a slow relaxation reflected by an increase in T which might reflect an impaired Ca^{++} sequestration to SR from cytoplasm. The other was an incomplete relaxation due to failure of the systolic tension to be completely dissipated by the end-diastole because Ca^{++} could not separate from the contractile proteins.⁴

Weisfeldt and co-workers¹⁵ showed that myocardial relaxation was completed by 3.5T after a peak LV(-)dp/dt in the normal canine heart and in cases when the relaxation time was prolonged and was not completed by the beginning of the next systole, an elevation of LVEDP occurred. In the present study, we showed a close linear correlation between delta LVEDP and delta T_B following hypoxia. We had already shown the same relationship between T_W and LVEDP. These findings do not necessarily contradict the hypothesis that elevated LVEDP induced by ischemia is not due to an increased myocardial stiffness but to an impaired myocardial relaxation. However, their hypothesis cannot explain the exclusive increase of LVEDP with unaltered T_B in conditions like DPI under hypoxia. As cited above, because the separation of Ca^{++} from contractile proteins and Ca^{++} sequestration from cytoplasm are energy requiring processes, it is not surprising to see a simultaneous deterioration in the myocardial relaxation and stiffness following myocardial oxygen deficit. The same phenomenon might be observed when cytosolic Ca^{++} increases secondarily to the impaired SR function to take up Ca^{++} . On the other hand, if SR function is not altered and only the separation of Ca^{++} from contractile proteins is impaired, an increased myocardial diastolic stiffness without any changes in the myocardial relaxation ensues, as shown in the present study. Myocardial diastolic abnormalities may be governed by separate mechanisms.

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