Beneficial Effects of Diltiazem on the Ischemic Derangements of the Myocardial Metabolism Assessed by \(^{31}\)P-NMR in the Isolated Perfused Rat Heart

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Accepted June 3, 1985

Abstract—Using the isolated perfused heart preparations of the rat, effects of diltiazem, a calcium antagonist, on the ischemic derangements of the mechanical function and the energy metabolism of the ventricular myocardium were studied. The myocardial tissue levels of creatine phosphate (CP), ATP, inorganic phosphate (Pi) and pH were determined with \(^{31}\)P-NMR. Global ischemia was induced by cross-clamping of the aortic inflow line for 15 min, which resulted in a fall of CP, ATP and pH and a rise of Pi. The test hearts were perfused with diltiazem-containing solution (10\(^{-7}\), 10\(^{-6}\) and 10\(^{-5}\) M) for 12 min prior to the induction of the global ischemia. A significant dose-related decline of the myocardial mechanical function expressed as (left ventricular pressure) \(^{-}\) (heart rate) was observed in diltiazem treated hearts. In doses above 10\(^{-6}\) M, diltiazem delayed the onset of the fall of the myocardial CP and pH levels and the rise of Pi induced by ischemia, and there was an excellent correlation between the suppression of the myocardial mechanical function observed before induction of ischemia and the level of the myocardial CP and pH at the initial phase of ischemia, indicating that the improvement of the myocardial energy metabolism was due to the cardiodepressant effects of the compound.

Recently, the calcium channel blockers have emerged as a new group of pharmacological agents, expected to provide a significant advance in the treatment of angina pectoris, arrhythmias and hypertension. Moreover, many evidences indicate that they exert a beneficial effect on the ischemic myocardium (1–11). The beneficial effects have been ascribed to the ability of these agents to reduce myocardial contractile force, the left ventricular afterload, and the heart rate, as well as to the ability to improve the oxygen supply by the dilation of the collateral vessels (12, 13). However, the direct metabolic effect on the ischemic myocardium has not been excluded yet.

The present study was designed to clarify whether the beneficial effect of the calcium channel blocker on the ischemic myocardium was exerted by the direct metabolic effect at the cellular level or by the myocardial depressant effect. Experiments were performed in the isolated perfused rat heart preparation using \(^{31}\)P-NMR, a recently developed non-invasive technique for measurement of the cellular energy metabolism (14–18) that permitted a simultaneous measurement of the mechanical performance of the myocardium.

Materials and Methods
Experiments were performed in the isolated perfused heart preparation of the Wistar rat (Langendorff's method). Male Wistar rats weighing around 220 g were stunned by a blow on the head. Immediately
after opening the thorax, the hearts were rapidly excised and transferred to ice-chilled modified Krebs-Ringer bicarbonate solution to induce rapid cessation of the heart beat. The adherent connective tissue was removed and the ascending aorta was cannulated. Retrograde perfusion with a modified Krebs-Ringer bicarbonate solution from a reservoir 75 cm above the heart was begun immediately. The perfusion fluid contained NaCl (127.2 mM), KCl (4.7 mM), CaCl2 (2.5 mM), KH2PO4 (1.2 mM), and NaHCO3 (24.9 mM). It was oxygenated with 95% O2+5% CO2 gas by means of an oxygenating device as described by Neely et al. (19) to ensure PO2 values higher than 600 mmHg and kept at a temperature of 38°C. Sodium pyruvate (2 mM) and glucose (5.5 mM) were added to the perfusion fluid as substrates.

The coronary inflow was measured by means of an electromagnetic flowmeter probe (Statham, 1 mm i.d.) placed in the perfusate inflow-line coupled with an electromagnetic flowmeter (Statham 2201). The latex balloon was introduced into the left ventricle from the left atrium through the mitral valve. The left ventricular pressure was measured by a pressure transducer (Statham P50) connected to the balloon with a saline filled polyethylene tubing.

To monitor changes in adenosine triphosphate (ATP), creatine phosphate (CP) and intracellular pH, 31P-NMR spectra were obtained using a JEOL FX200 NMR spectrometer operating at 80.76 MHz in the Fourier transform mode equipped with a 15 mm probe. The spectral parameters were: 45° pulse, 0.5 sec intervals, 360 transients (3 min), 4K data points, 5000 Hz sweep width, line broadening of 10 Hz. The circumstances of the detector chamber were maintained at 38°C by means of an integral air flow system. The peak of CP was assigned a chemical shift of zero.

After a 10 min equilibration perfusion period, hearts were inserted into a 1.5 cm diameter glass NMR tube and placed in the magnetic field. Coronary effluent was evacuated from the NMR tube using a vacuum pump. Three control spectra (9 min) were collected from each heart. Following acquisition of the control spectra, perfusate was changed to diltiazem containing solution, and four spectra (12 min) were obtained during the pre-ischemic period. Then, ischemic arrest was initiated by cross clamping the aortic perfusate inflow line for 15 min, and five spectra were taken. At the end of the ischemic arrest, the aortic clamp was removed, and each heart was reperfused for 15 min to obtain five spectra.

The hearts were divided into four experimental groups: control (non-treated) hearts (n=6), diltiazem (10^-7 M)-treated hearts (n=6), diltiazem (10^-6 M)-treated hearts (n=6), and diltiazem (10^-5 M)-treated hearts (n=6). Diltiazem was generously provided by Tanabe Pharmaceutical Co., Ltd.

Data analysis: The distance between the peak of the intracellular inorganic phosphate (Pi) and that of the pH independent creatine phosphate (CP) was measured, and the intracellular pH was determined using the pH titration curve of Pi and CP solution at 38°C constructed in this laboratory.

Quantitative analysis was performed on the relative intensities of the β-ATP and CP peaks to the areas of an external standard, methylene diphosphonate (MDP). Areas under each peak were integrated with a digitizer (Graphic Tablet 9111A, Hewlett Packard) connected to a personal computer (HP85, Hewlett Packard). Data were expressed as percent changes from the values just before the drug treatment.

All data were determined to be significant at a P value less than 0.05 based on Student's t-test. In some cases Welch-Aspin’s method was used.

Results

Figure 1 shows typical spectra taken from the non-treated control and the 10^-6 M diltiazem treated heart before and after induction of the ischemia. The spectra taken after reperfusion are also depicted. In the control heart, intracellular Pi peak intensity increased markedly and moved toward the CP peak during the ischemia, indicating the fall of the intracellular pH. In the diltiazem treated heart, the onset of these changes in the intracellular pH was delayed. Figure 1 also shows that immediately after the
ischemia, CP peak intensity was markedly decreased in the control heart, while in the diltiazem treated heart, CP peak was well maintained. Figure 2 summarizes the changes in CP contents. It is evident from the figure that diltiazem significantly delayed the onset of breakdown of CP during the ischemic insult in a dose-related manner. The level of CP at the initial 3 min of ischemia was significantly higher in the preparations treated with 10^-6 M or more of diltiazem. After reperfusion, CP contents were almost completely recovered even in the control hearts. The rate of ATP depletion was slow as compared with that of CP contents. Diltiazem had no effects on the time course of ischemic changes in ATP contents (Fig. 3). The recovery of ATP after reperfusion was not as good as the recovery of CP.

Figure 4 summarizes the decreases in the myocardial intracellular pH induced by ischemia and the effects of diltiazem thereupon. As is evident from the figure, diltiazem also delayed the onset of the fall of intracellular pH in a dose-dependent manner. Here again the pH level at the initial 3 min of ischemia was significantly higher in the
hearts treated with $10^{-6}$ M or more of diltiazem. However, the rates of decline of the intracellular pH during the period of 6 to 15 min after induction of ischemia, at which all the hearts stopped beating, were not significantly different from each other, being $0.055\pm0.002$, $0.055\pm0.003$, $0.052\pm0.003$ and $0.065\pm0.001$ pH unit/min for the control, $10^{-7}$ M, $10^{-6}$ M and $10^{-5}$ M diltiazem treated hearts, respectively. Thus, there was no attenuation of the rate of decline of the intracellular pH during ischemia after treatment of the preparation with diltiazem.

In the present experiments, it was difficult to determine the intracellular Pi peak during the normal perfusion period due to the presence of a small amount of this substance. Therefore, we combined the three individual free induction decays (FIDs) and conducted the Fourier transformation of this composite FID. In the pretreatment period, the intracellular pHs were $7.08\pm0.02$ for the
control hearts and 7.03±0.03, 7.10±0.03 and 7.10±0.02 for the 10^{-7} M, 10^{-6} M and 10^{-5} M diltiazem-treated hearts, respectively. Addition of diltiazem resulted in no significant change in the intracellular pH, the values being 7.13±0.01 for the control and 7.11±0.03, 7.08±0.04 and 7.11±0.02 for the 10^{-7} M, 10^{-6} M and 10^{-5} M diltiazem-treated hearts, respectively.

The same procedure was conducted in the calculation of the CP and ATP content of the non-ischemic myocardium to clarify the effects of diltiazem on the high-energy phosphate compound contents of the normally-perfused preparations. No significant effects were found with diltiazem.

Figure 5 shows the typical effects of diltiazem on the coronary flow, the heart rate, the left ventricular pressure and its first derivative (dp/dt). The coronary flow was...
increased, while the heart rate, the left ventricular pressure and dp/dt were depressed. These changes were observed with 10^{-6} M or more of diltiazem. Figure 6 summarizes the effects of diltiazem on the coronary flow. The dose dependent increase in the coronary flow was observed just after treatment with diltiazem, but these effects were not sustained. As shown in Fig. 7, the myocardial performance as expressed by the heart rate multiplied by the left ventricular systolic pressure was depressed in a dose-dependent manner.

Figure 8 illustrates the relationship between the myocardial depressant effect observed just before initiation of the ischemia and the protective effect on the ischemic myocardial metabolism as reflected in the CP content and the intracellular pH levels during the initial three minutes period of ischemia. There was a linear relationship between the myocardial depressant effect and the CP or pH levels.

Discussion

Many papers have already been published pertaining to the beneficial effects of diltiazem, a calcium channel blocker, on the ischemic myocardium (1-11). The beneficial effects have been explained by (1) the direct metabolic effect, (2) the myocardial depressant effect, (3) the increase in the collateral flow, and (4) the peripheral vasodilating action (afterload reduction). In the present study, an attempt was made to clarify the mechanisms of the protective effects of diltiazem on the ischemic derangement of the myocardium using the isolated perfused heart preparations and measuring the intracellular concentrations of CP, ATP and Pi with \(^{31}\)P-NMR in combination with the measurements of the myocardial mechanical performance. As a measure of the ischemic derangement of the myocardial metabolism, the intracellular pH was also determined. The assessment of this parameter was impractical, if not impossible.
with previously available methods.

It was clearly demonstrated that diltiazem could delay the onset of breakdown of CP and the attenuation of the fall of the intracellular pH produced by diltiazem. Each point represents the mean±S.E.M. Open circles: control hearts (n=6), closed circles: 10^-7 M diltiazem (n=6), open triangles: 10^-6 M diltiazem (n=6), closed triangles: 10^-5 M diltiazem (n=6).

Abscissae: Myocardial work just before the induction of ischemia calculated as HR×LVP. The values just before the start of perfusion with diltiazem-containing Krebs-Ringer bicarbonate solution were taken as 100%.

Ordinates: Myocardial CP content or pH determined during the 3 min period after induction of ischemia. CP content was expressed as % of the values obtained during the 3 min period before the start of perfusion with diltiazem-containing Krebs-Ringer bicarbonate solution.

In the present study, it was demonstrated that diltiazem could retard the fall of the intracellular pH and the decline of CP content induced by ischemia. However, the retardation was produced only with 10^-6 M or more of diltiazem that produced a marked depression of the mechanical performance of the myocardium prior to induction of ischemia. Furthermore, the rates of decline of the myocardial pH in the control and the diltiazem-treated hearts were not different from each other. If diltiazem has direct metabolic effects, the rate of decline of the intracellular pH should be slowed down, as was demonstrated relative to the effects of low temperatures by Flaherty et al. (15). Thus, we may conclude that the beneficial effect of diltiazem on the ischemic heart was due primarily to a suppression of the myocardial mechanical activity produced by this agent. The fact that the linear correlations were observed between the depressant effect on the myocardial mechanical performance before the induction of ischemia and the level of CP and myocardial pH during the initial 3 min ischemic period is compatible with this interpretation.

In conclusion, the beneficial effects of
diltiazem, a calcium channel blocker, on the ischemic myocardial metabolism was ascribable to myocardial depressant effects and not to the direct metabolic action of this compound.

Acknowledgements: The authors wish to express their thanks to Miss R. Nakagawa and Miss M. Sato for their help in preparing the manuscript. A part of the expenses of this work was defrayed by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture of Japan.

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