EFFECTS OF PROPRANOLOL AND DILTIAZEM ON THE RATE OF HIGH-ENERGY PHOSPHATE METABOLISM IN REPERFUSED RAT HEARTS

—— ^{31}P-NMR SATURATION TRANSFER STUDY ——

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The relationships between pressure rate product (PRP) and flux (PCr→ATP) or flux (Pi→ATP) were studied in isolated perfused rat hearts by the saturation transfer method using ^{31}P-NMR. The effects of propranolol and diltiazem on phosphate metabolism were also studied. After a 40 min preischemic period, the hearts were subjected to a 15 min period of ischemia, followed by 60 min of reperfusion. Propranolol (0.4−1.2 μM) or diltiazem (3.0−6.0 μM) was infused for 30 min before ischemia and reinfused after reperfusion for 60 min. The flux (PCr→ATP)/PRP ratio at reperfusion did not differ from that at preischemia. This value was also not affected by propranolol or diltiazem treatment. However, the flux (Pi→ATP)/PRP ratio at reperfusion was significantly less than that at preischemia. Moreover, this value was significantly improved by propranolol or diltiazem treatment. This study demonstrates that 1) flux (PCr→ATP) has a good correlation with cardiac performance, 2) stunned myocardium needs less ATP turnover for survival of its depressed contractile activity, and 3) flux (Pi→ATP) can limit recovery of postischemic performance. Protective effects of propranolol and diltiazem are exerted on the flux (Pi→ATP), i.e. ATP derived from glycolytic flux, in the reperfused heart.

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HIGH-ENERGY phosphate metabolism plays an important role in myocardial function, and its production by oxidative phosphorylation is directed toward contractile activity[1,2] Michael et al[3] have reported that the ATP level is decreased in ischemic heart. Braunwald and Kloner[4] have also reported that a mechanical dysfunction in stunned myocardium resulted from an inability to generate sufficient ATP to support a normal workload. However, Ambrosio et al[5] recently showed, a discrepancy between ATP levels and pressure rate products (PRP) using nuclear magnetic resonance techniques (^{31}P-NMR). Furthermore, Neely and Grotyohann[6] could find no relation between tissue ATP levels and postischemic recovery. Thus, it is still unclear whether the degree of decrease in the ATP level is an index of the severity of myocardial dysfunction. It is now clear that measurements of ATP levels can not substitute for direct measurement irreversible ischemic injury, such as ultrastructural changes or ischemic contrac-

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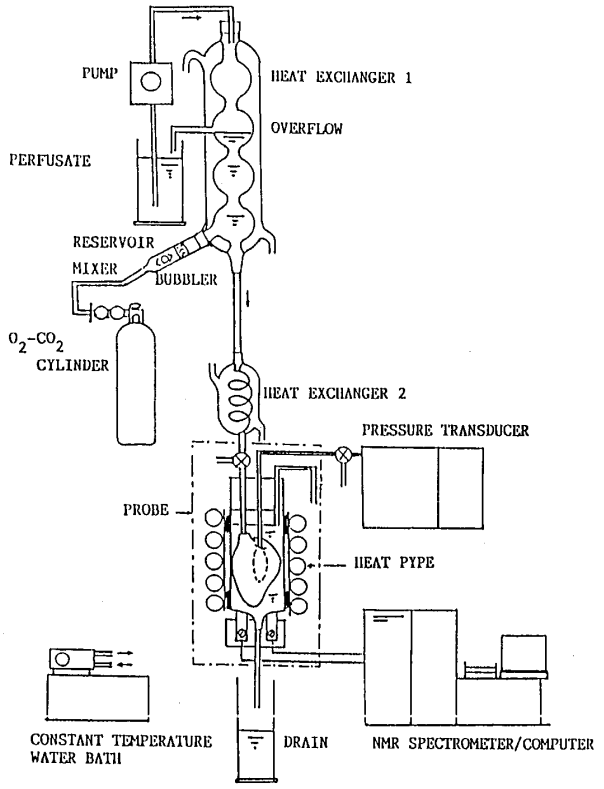

Fig.1. Block diagram of the ^{31}P-NMR and Langendorff perfusion apparatus.

tures.

Recently, the ^{31}P-NMR technique of magnetization transfer has been used to define the rate of ATP synthesis in isolated hearts by oxidative phosphorylation and from creatine phosphate (PCr) via the creatine kinase reaction[7,8] In myocardium, energy production matches energy utilization, so the tissue ATP level remains constant, or nearly so, despite large changes in ATP turnover and cardiac performance[9] In contrast, increases in wall stress and inotropic stimulation decrease the myocardial level of creatine phosphate. Therefore, it is important to study the rate of highenergy phosphate metabolism in association with cardiac work. Bittl et al[7] and Neubauer et al[8] reported that flux (PCr→ATP), which reflected the creatine kinase reaction, correlated with cardiac performance.

In the normal heart, 90% of the ATP is formed by oxidative phosphorylation while 10% is produced via glycolysis[10] However, there has been no study of both flux (PCr→ATP) and flux (Pi→ATP) in the reperfused heart model. In the present study, we investigated the exchange rates of flux (PCr→ATP) and flux (Pi→ATP) in ischemia/reperfused isolated rat hearts. We also observed the relation between these rates and PRP during infusion of propranolol or diltiazem.

MATERIALS AND METHODS

1. *Isolated perfused rat hearts*

Male Wistar rats, weighing 250−400g, were anesthetized with pentobarbital (30 mg/kg, i.p.) and thoracotomies were performed. The hearts were rapidly excised and immediately immersed in an ice-cold buffer solution. The aorta was dissected free, and mounted onto a cannula, which was attached to a perfusion apparatus (Fig. 1). Retrograde perfusion of the heart was begun in the Langendorff mode under a constant perfusion pressure of 100 cm H_2O with a modified Krebs-Henseleit buffer containing (mM/L): NaCl 118.0, KCl 4.7, CaCl 2.5, $MgSO_4$ 1.2, KH_2PO_4 0.3, $NaHCO_3$ 25.0, Na_2EDTA 0.5, and glucose 10. The perfusate buffer was equilibrated with 95% O_2 and 5% CO_2. All experiments were performed at 37 °C.

2. *Experimental protocol*

Fig. 2 shows an experimental schedule. After an equilibrium period (about 30 min), the hearts were adjusted to a PRP of 18.0×10^3 mmHg-beats/min by infusing water into the latex balloon. Total normothermic (37 °C) ischemia was induced for 15 min and reperfusion followed for 60 min. The control group (n=7) was perfused with a modified Krebs-Henseleit buffer solution. Both propranolol (n=6) and diltiazem (n=7) groups were infused with propranolol or diltiazem, respectively, into the perfusion line for 30 min during the preischemic period and for 60 min during the reperfusion period. The dosages of propranolol (0.4−1.2 μM) and diltiazem (3.0−6.0 μM) were based on the PRP values in the preischemic period, which were adjusted to 9.0×10^3 mmHg-beats/min. Cardiac performance indices, such as development pressure (DP), beating rate (BR), and PRP, were measured 3 times; at 40 and 15 min before the start of ischemia and at 45 min after reperfusion. Since a complete saturation transfer measurement could be ac-

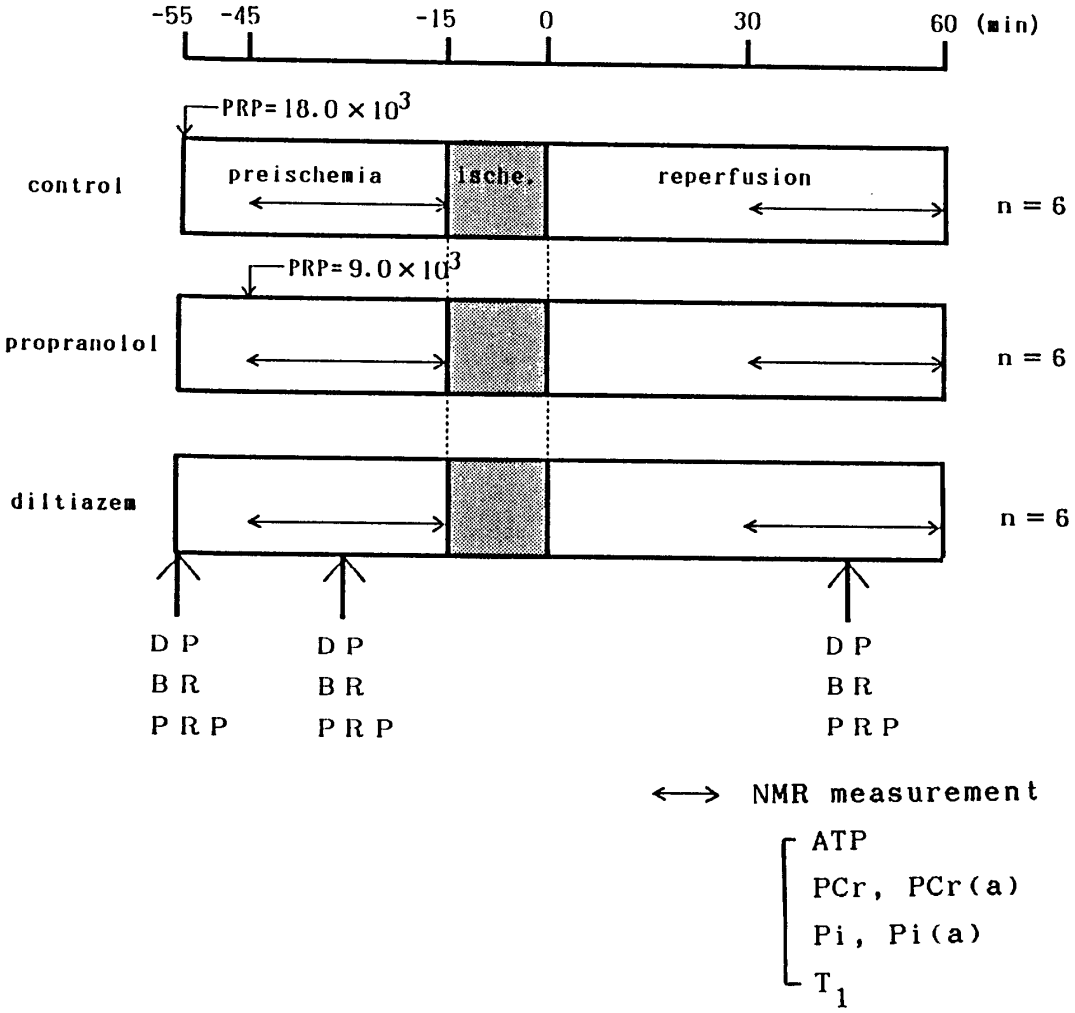

Fig.2. Schema of the experimental protocol. Hearts were subjected to 15 min of ischemia, followed by 60 min of reperfusion. A complete saturation transfer measurement was acquired at 30 min. These measurements were carried out twice; once in the preischemic period and again in the reperfused period. DP: development pressure, BR: beating rate, PRP: pressure rate product, T_1: longitudinal relaxation rate.

quired in 30 min, saturation transfer measurements were carried out twice: once during the pre-ischemic period and again during the reperfusion period (from 30 min to 60 min). Two hearts that developed irreversible ventricular fibrillation (one each from the control, and diltiazem-treated groups) were excluded from this study. Therefore, the final study consisted of three groups of six hearts each.

3. *Measurement of cardiac performance*

A water-filled latex balloon was inserted into the left ventricle through the left atrium-via the mitral valve. The balloon was connected to a pressure transducer (Nihon Kohden, Japan) via a small-bore polyethylene tube for continuous measurement of the left ventricular-development pressure (LVEDP) and beating rate, in order to observe changes in the pressure rate product.

4. *^{31}P-NMR spectra*

^{31}P-NMR spectral of perfused hearts were obtained using the JEOL GSX 270 WBNMR spectrometer (JEOL, Japan) operating at 109.16 MHz. The probe consisted of a vertically-mounted Helmholtz coil (17 mm in diameter) tuned to the phosphorus frequency. Spectra were recorded using 45 °C pulses and a 1 sec repetition time, and accumulated

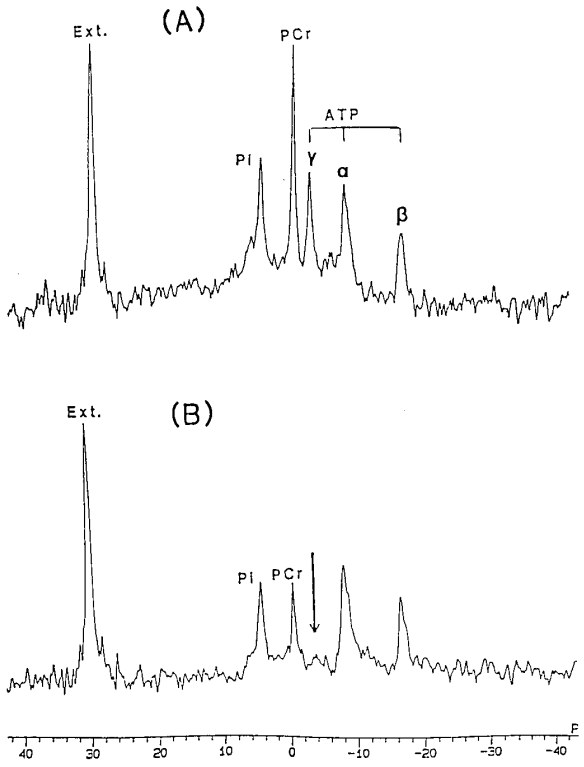

Fig.3. (A) represents ^{31}P-NMR spectra of isolated perfused rat heart collected under isometric pressure at 100 cm H_2O perfusion pressure. Spectra were accumulated using 200 transients with a 45 °C pulse and a 1.0 sec interpulse delay. Ext.: external standard peak.
(B) represents the selective saturating irradiation at the γ-phosphate resonance of ATP. The arrow indicates the position of the saturating radiofrequency and the γ-P of ATP which was made invisible.

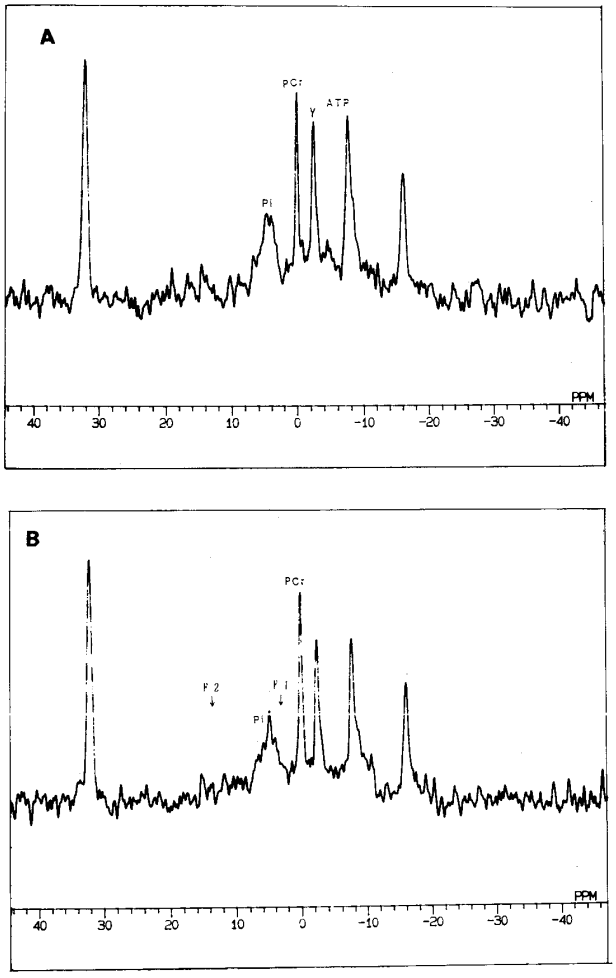

Fig.4. Selective saturation irradiation was placed at the γ-P resonance of ATP (Fig. 3-B). Carrier frequency was placed at a symmetrical position against the PCr signal (f_1) or Pi (f_2).

200 times to obtain steady-state concentrations of ATP, PCr, and Pi. Quantification was carried out by comparison of the peak height of interest with that of the external standard (Hexamethylphosphoric triamide: 100 mM) within the radiofrequency coil Peak height value of the external standard was defined as 100 U. Fig. 3-A shows a typical ^{31}P-NMR spectra obtained in this study. Immediately thereafter, 200 additional scans were again accumulated with selective irradiation applied at the γ-phosphate of ATP. The intensity of the creatine phosphate [PCr (a)] and Pi [Pi (a)] signals were then measured (Fig. 3-B). Separate studies showed that the narrow-band pulse directly attenuated the magnetization of PCr and Pi by less than 5% when the carrier frequency was placed at a symmetrical position against the PCr of Pi signal (Fig. 4).

5. *Saturation transfer measurement*

We used the saturation transfer method to test for creatine kinase activity in the perfused hearts. Saturation transfer is a pulse-labeling technique with analogies to radioactive pulse techniques. In the NMR saturation-transfer method, the pulse label is phosphate, which is temporarily made invisible in NMR or "masked" through saturation. NMR saturation is the equalization of the population of spins in their two energy levels. The mask has a half-life, much like a radioisotope. With NMR saturation, the decay of the mask, or invisibility, occurs with a time constant of the apparent T_1 relaxation

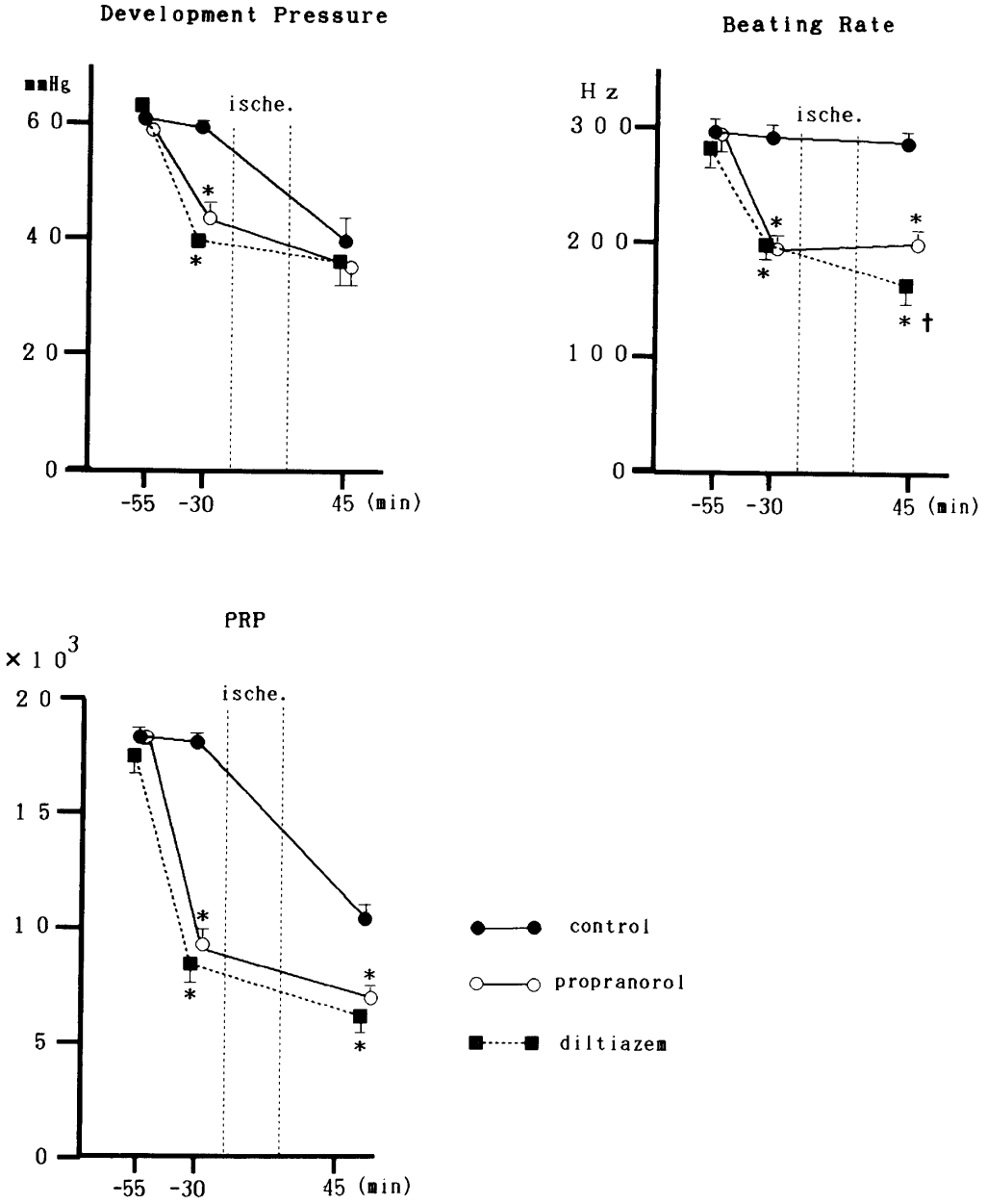

Fig.5. Time course of changes in cardiac performance. Each point represents the mean±S.E.. (●): control group (n=6), (○): propranolol group (n=6), (■): diltiazem group (n=6). *denotes statistically different from the control group ($p<0.05$). +denotes statistically different from the propranolol group ($p<0.05$).

value. Either PCr or the phosphate (γ -P) of ATP is made invisible. In the absence of any reaction, no other phosphate resonances in the ^{31}P spectrum of the heart are modified in any way. If a reaction such as the creatine kinase reaction is occurring at a rate substantially faster than 1/apparent T_1, interconversion of the invisible label will occur. When PCr is made invisible, it will result in a partial invisibility of the γ -P of ATP, and conversely, the invisibility γ -P of ATP will result in a partial invisibility of PCr. Rate constants (κ) for the forward and reverse reactions may potentially be derived from NMR data[11]

In this study, saturation recovery experiments consisted of a 90° pulse in the presence of selective irradiation of the γ -P peak of ATP, followed by a variable time delay (0.3, 0.6 1.2, 2.4, and 4.8 sec) and a 90 °C observation pulse (Fig. 3-A). This cycle was repeated 50 times. T_1 was calculated

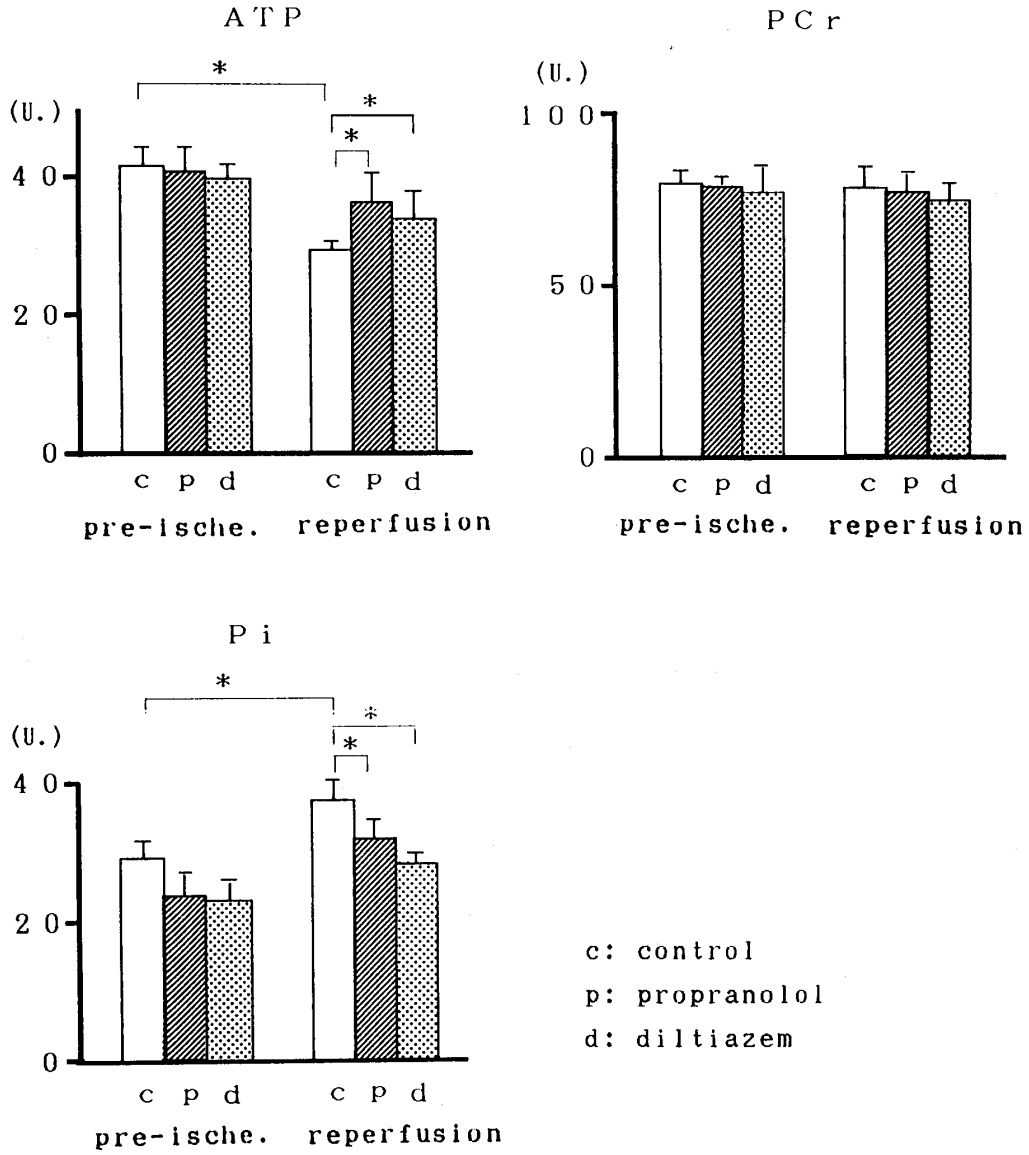

Fig.6. ^{31}P-NMR spectra were collected from perfused rat hearts. Spectra were quantified by the peak height method comparing the resonance peak of interest to the external standard peak. Results are expressed as mean±S.E.. *denotes statistically different ($p<0.05$). c: control, p: propranolol group, d: diltiazem group. preische: preischemic period. reperfusion: reperfused period.

with T_1 analyzing software (JEOL). A complete saturation transfer measurement was acquired in 30 min.

Magnetization transfer was used to study the pseudo-first order rate constants (κ_1 and κ_2) and the forward fluxes of the Pi to ATP exchange reaction (Pi to γ-P of ATP) and the creatine kinase reaction in the direction of PCr to γ-P of ATP, respectively. The theoretical principles underlying this technique have been described previously[12,13] Briefly, two equations were used to determine κ:

$$M^+/M^o=(1+\kappa T_1)^{-1} \quad \cdots\cdots (1)$$

$$1/\tau=1/T_1+\kappa \quad \cdots\cdots (2)$$

where M^+ is the magnetization intensity of the Pi or PCr resonance in the presence of selective irradiation of the γ-phosphate resonance of ATP, and M^o is the magnetization intensity of these resonances in the presence of a control irradiation. $1/T_1$ represents the intrinsic longitudinal relaxation rate (in the absence of chemical exchange) and $1/\tau$ is the measured or apparent longitudinal relaxation rate. The pseudo-first-order con-

TABLE I LONGITUDINAL RELAXATION RATES (T_1)

		preischemia	*reperfusion*
control	*PCr*	*2.1±0.1 sec.*	*2.1±0.2 sec.*
	Pi	*0.8±0.1*	*0.7±0.2*
propranolol	*PCr*	*2.0±0.2*	*2.0±0.2*
	Pi	*0.7±0.2*	*0.7±0.1*
diltiazem	*PCr*	*2.0±0.1*	*1.8±0.2*
	Pi	*0.9±0.1*	*0.9±0.1*

TABLE II FLUX (PCr→ATP)

	preischemia (U/sec)	*reperfusion (U/sec)*
control	*27.9±1.5*	*23.0±2.4*
propranolol	*22.4±1.4**	*18.8±3.8*
diltiazem	*18.7±1.8**	*16.7±1.2**

*Results are expressed as mean±S.E.. *denotes statistically different from the control group ($p<0.05$)*

stants of κ_1 (κ Pi→γ-ATP) and κ_2 (κ PCr→γ-ATP) were calculated using the following equations:

$$\kappa_1\,(\kappa\,\mathrm{Pi}\rightarrow\text{-ATP}) = \Delta M_{Pi}/M^{o}{}_{Pi} \times 1/\tau_{Pi} \cdots\cdots (3)$$

$$\kappa_2\,(\kappa\,\mathrm{PCr}\rightarrow\text{-ATP}) = \Delta M_{PCr}/M^{o}{}_{PCr} \times 1/\tau_{PCr} \cdots (4)$$

These equations are derived from equations 1 and 2. The exchange fluxes for the PCr→ATP and Pi→ATP reactions were then calculated by the equation:

$$\mathrm{Flux} = \kappa_1[\mathrm{Pi}] \text{ or } \kappa_2[\mathrm{PCr}] \cdots\cdots (5)$$

using the corresponding concentrations with the appropriate $\Delta M/M^o$ measurement.

6. *Statistical analysis*

All data are presented as the mean±SE. The one-way ANOVA was used to test for significant differences among the data of the three groups. The paired t-test was used to test for significant differences between the data of the preischemia and reperfusion periods. The relationship between PRP and flux rate was tested with linear regression analysis. $p<0.05$ was considered to be statistically significant.

TABLE III FLUX (PI→ATP)

	preischemia (U/sec)	*reperfusion (U/sec)*
control	*10.5±1.1*	*4.5±1.6#*
propranolol	*5.3±1.1**	*5.4±1.1*
diltiazem	*8.1±0.8**	*6.9±0.5**

*Results are expressed as mean±S.E.. *denotes statistically different from the control group ($p<0.05$) and # denotes statistically different from preischemic period ($p<0.05$).*

RESULTS

1. *Cardiac performance*

Fig. 5 shows the results of cardiac performance. Development pressure was significantly decreased in the preischemic period after treatment with propranolol or diltiazem (both $p<0.05$). In the reperfused period, there was no significant difference among the three groups. Beating rate was significantly decreased in the preischemic period after treatment with propranolol or diltiazem (both $p<0.05$). The PRP of the control group was significantly decreased during reperfusion (from $18.1\pm0.1\times10^3$ to $11.2\pm0.5\times10^3$) mmHg-beat/min, $p<0.05$). After treatment with propranolol or diltiazem, PRPs were significantly decreased during the preischemic and reperfused periods. However, there was no difference between the two periods.

2. *Phosphometabolites in pre-ischemic and reperfused hearts*

The levels of phosphorous metabolites in preischemic and reperfused hearts are shown in Fig. 6. There were no significant differences between the ATP levels during the preischemic period of the control group and the treated groups. Treatment with propranolol or diltiazem significantly increased ATP levels compared with those of the control group in the reperfused period (both $p<0.05$). However, we could find no significant change in PCr levels in the preischemic and reperfused periods for each group. The Pi level of the control group during the reperfused period was significant higher than that in the preischemic period ($p<0.05$). On the other hand, treatment with propranolol or diltiazem significantly decreased Pi levels

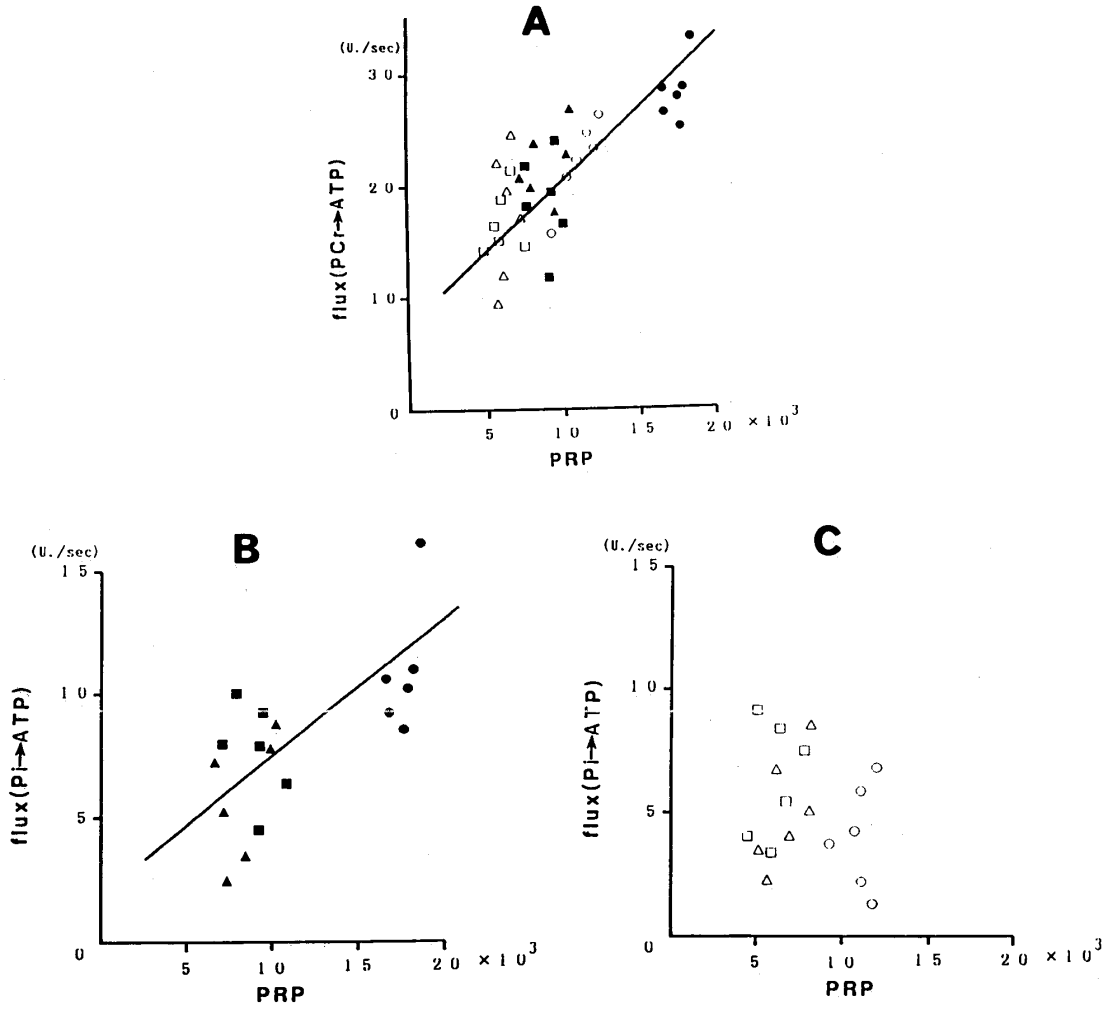

Fig.7. (A) represents the relation between flux (PCr→ATP) and PRP, (B) represents the relation between flux (Pi→ATP) and PRP in the preischemic period, and (C) represents the relation between flux (Pi→ATP) and PRP in the reperfused period. ●: preischemic period of control, ○: reperfused period of control, ▲: preischemic period of propranolol group, △: reperfused period of propranolol group, ■: preischemic period of diltiazem group, □: reperfused period of diltiazem group.

in the reperfused period (both vs. control group, both $p<0.05$).

3. *T_1 (longitudinal relaxation rates)*

The data of T_1 are summarized in Table I. At preischemia, there were no significant differences among the values of T_1 for PCr and in the three groups. Similarly, there were no significant differences among the values of T_1 for PCr and Pi at reperfusion in the three groups.

4. *Flux (PCr→ATP) and flux (Pi→ATP)*

Table II shows the data of flux (PCr→ATP). Flux (PCr→ATP) during reperfusion showed no significant difference from that at preischemia in the control group. Treatment with propranolol or diltiazem significantly decreased flux (PCr→ATP) at preischemia ($p<0.05$). Flux (PCr→ATP) for diltiazem treatment during the reperfusion period was significantly lower than that of the control (both $p<0.05$).

Table III shows data of flux (Pi→ATP). Flux(Pi→ATP) during the reperfusion period was significantly less than that during preischemia in the control group ($p<0.05$). Treatment with propranolol or diltiazem significantly decreased flux (Pi→ATP) in the preischemic period. However, while treatment with diltiazem significantly increased flux (Pi→ATP) during the reperfused period

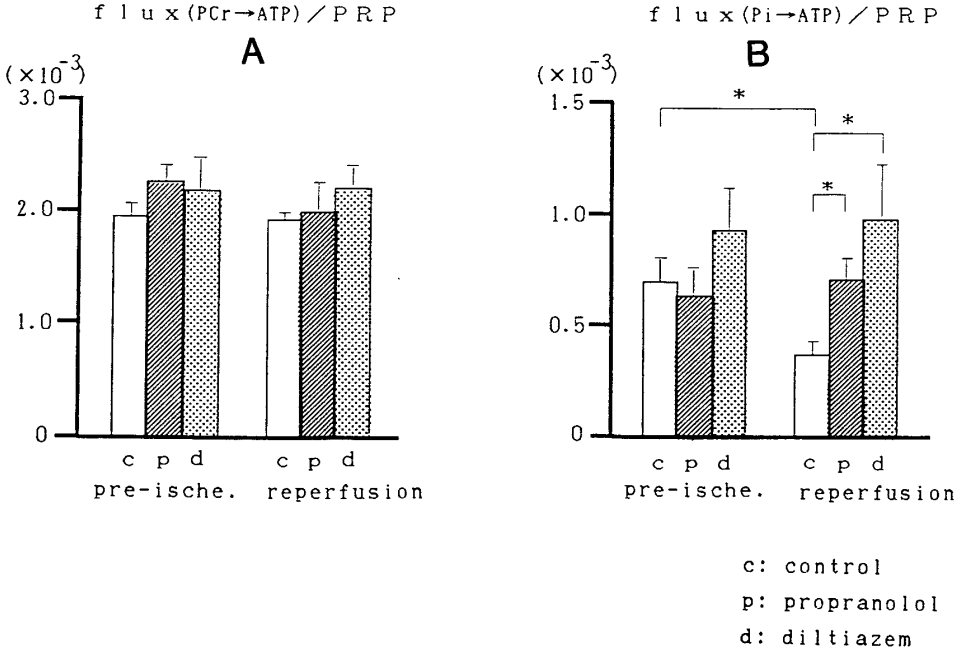

Fig.8. (A) shows flux (PCr→ATP)/PRP ratio and (B) shows flux (Pi→ATP)/PRP ratio. Each bar represents the mean±S.E.. *denotes a statistically significant difference ($p<0.05$). c: control, p: propranolol group, d: diltiazem group.

($p<0.05$), treatment with propranolol did not.

5. *Relation between PRP and flux (PCr→ATP) or flux (Pi→ATP)*

Since flux (PCr→ATP) and flux (Pi→ATP) change to various degrees in cardiac work, we investigated the relationship between PRP and flux (PCr→ATP) or flux (Pi→ATP). The relation between flux (PCr→ATP) and PRP showed a close positive linear correlation ($Y=1.13\times10^{-3}$ X $+8.5$, $r=0.76$, $p<0.05$) (Fig. 7-A). Flux (Pi→ATP) has a strong correlation with PRP during the preischemic period ($Y=0.44\times10^{-3}$ X $+3.6$, $r=0.70$, $p<0.05$) (Fig. 7-B). However, since the value of flux (Pi→ATP) for the control group was lower in proportion to PRP during the reperfusion period, there was no linear correlation (Fig. 7-C).

There were no significant differences among the flux (PCr→ATP)/PRP ratios in the three groups during both preischemic and reperfused periods (Fig. 8-A). Flux (Pi→ATP)/PRP ratios also showed no significant differences among the three groups during the preischemic period. In contrast, the flux (Pi→ATP)/PRP ratio during the reperfused period was significantly lower than that in the preischemic period for the control group. Pretreatment with propranolol or diltiazem significantly increased flux (Pi→ATP)/PRP ratios during the reperfused period (both vs. control, both $p<0.05$) (Fig. 8-B). There was no significant difference between the flux (Pi→ATP)/PRP ratios of the propranolol and diltiazem groups.

DISCUSSION

The amount of high energy phosphates, ATP plus PCr, at the steady-state presumably reflects the overall balance between metabolic supply and contractile utilization. Mitochondrial oxidative phosphorylation synthesizes most of the ATP in the heart, and the creatine kinase reaction resynthesizes the ATP that breaks down during cardiac contraction. As ATP consumed creatine kinase catalyzes the transfer of a high-energy phosphate group from creatine to ADP to insure a constant supply of ATP

$$CPr+ADP \rightleftharpoons creatine+ATP \rightleftharpoons ADP+Pi$$

Creatine kinase resynthesizes ATP so rapidly that direct measurement of the reaction rates in the heart is not possible using

conventional techniques.

This limitation has been partially overcome with the relatively recent expansion of NMR spectroscopoy to studies of intact cells and tissues[7,8,11,12] The magnetization transfer techniques of NMR enable us to study the kinetics of enzymes in situ. ^{31}P-NMR spectroscopy yields repetitive measurements of phosphorus-containing compounds. Using magnetization transfer, we have directly investigated the intracellular kinetics of creatine kinase and ATP synthesis reactions in the isolated heart. Furthemore, it is possible that the glycolytic enzymes, glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and 3-phosphoglycerate kinase (PGK), can together catalyze Pi→ATP exchange through the phosphorylated intermediate 3-PGP, and contribute to the NMR- measured Pi→ATP rate![12,13] Therefore, in the present study, the saturation transfer technique has been used to examine the forward flux of the Pi to ATP exchange reaction [flux Pi→ATP)] and the creatine kinase reaction in the conversion of PCr to ATP [flux (PCr→ATP)].

Recovery of postischemic function may be limited by energy production, energy utilization, or energy transfer. We observed a close correlation between cardiac performance and flux (PCr→ATP). Neubauer et al[8] reported that flux (PCr→ATP) correlated to cardiac performance for each degree of myocardial injury. Furthermore, Bittl et al[7] have demonstrated that in isolated rabbit heart which had been reperfused after 10−60 min of ischemia, mitochondrial creatine kinase activity decreases in proportion to the duration of ischemia and that recovery of postischemic function correlated well with mitochondrial creatine kinase activity. Perry et al[14] also showed that in rabbit hearts at different developmental stages, mitochondrial creatine kinase activity correlated with creatine kinase reaction velocity. These data suggest that mitochondrial creatine kinase plays a role in energy transfer, and that energy transfer plays an important role in the recovery of postischemic function. However, Neubauer et al[8] found a close correlation between energy transfer and performance in postischemic myocardium, whereas they observed no decrease of mitochondrial creatine kinase activity after 20, 40, or 60 min of ischemia. Finally, Neubauer et al. have concluded that flux (PCr→ATP) does not limit the recovery of function in mildly or severely injured postischemic myocardium.

In this study, propranolol and diltiazem significantly decreased the flux (PCr→ATP) and cardiac performance (PRP), and there was no significant difference in the flux (PCr→ATP)/PRP ratio among the three groups in the preischemic period. Furthermore, the flux (PCr→ATP)/PRP ratio in the reperfused period was not influenced by treatment with propranolol or diltiazem. This result suggests that, in postischemic myocardium, flux (PCr→ATP) decreased only in proportion to cardiac performance. In other words, stunned myocardium requires less ATP turnover to ensure survival of its depressed contractile activity. Therefore, we concluded that flux (PCr→ATP) does not limit the amount of high-energy phosphate available for contraction during the postischemic period. Postischemic function may also be limited by ATP utilization by the myofibril. Greenfield and Swain[15] found that postischemic ADP levels decreased, and concluded that ADP limits the myofibrillar creatine kinase reaction velocity in postischemic myocardium. Postischemic dysfunction was therefore attributed to the limitation of energy utilization at myofibrils caused by a decreased CK mm (MM isozyme of creatine kinase) activity and by a restricted substrate (ADP) availability. We did not measure myofibrillar CK mm activity and ADP levels, but it is possible that energy utilization by myofibril is limited by ADP availability.

The flux (Pi→ATP) reflects the rate of ATP production through the glycolytic pathway generated by the activation of both glycogenolysis and glycolysis. We measured the unidirectional exchange flux (Pi→ATP) in the preischemic and periods. While there was a close correlation between cardiac performance and flux (Pi→ATP) during the preischemic period, but there was no similar correlation between cardiac performance and flux (Pi→ATP) during the reperfusion period, i.e. the flux (Pi→ATP)/PRP ratio decreased significantly during the postischemic period. On the other hand, treatment with diltiazem or propranolol significantly increased the flux (Pi→ATP)/PRP ratio and

decreased the Pi level during the postischemic period. Therefore, we suspect that flux (Pi→ATP) can limit the recovery of postischemic cardiac performance.

Since GAPDH and PGK, the key regulatory enzyme in glycolysis, are the rate-determining enzymes in the Pi→ATP exchange reaction[16] reduction of flux (Pi→ATP) is interpreted as a reflection of decreased glycolytic flux during the postischemic period (stunned myocardium). This result corresponds well to previous reports that mitochondrial ATP production and glycolysis are disturbed by ischemia/reperfusion, and that treatment with propranolol or diltiazem can protect against reperfusion injury of the myocardium[17,18] Glycolytically-produced ATP is thought to be preferentially used for pumping sodium, potassium, and calcium ions across the sarcolemma[19] Therefore, we would argue that the decreased flux (Pi→ATP) may reduce the ability of the Na^+/K^+-ATPase and ATP-dependent Ca^{2+} pump activity, which cause Ca^{2+} extrusion, and may then induce a Ca^{2+} overload in postischemic myocardium. Decreased subsarcolemmal ATP may help to open ATP-sensitive potassium channels, thereby inhibiting contraction in reperfused myocardium[20]

Pi levels significantly increased during the postischemic period. There is considerable evidence that increased concentrations of Pi inhibit the contractile force by decreasing the number of cross-bridges in the force-generating state[21] The actin-myosin ATPase activity decreased in association with the increased Pi levels, suggesting that Pi inhibits the fundamental force-generating reaction. Calcium overloading-induced decrease in the Ca^{2+} sensitivity of the myofibrils[22] and free radical mediated damage[23] are two possible primary causes of myocardial stunning. An associated decrease in the Ca^{2+} affinity of troponin has been proposed to account for the decrease in myofilament sensitivity[24] A decrease in the Ca^{2+} affinity of troponin is substantiated not only by the shift in the Ca^{2+}-tension relation, but also by the increased rate of the relaxation of skinned cardiac muscle at high Pi levels[21]

It is still unclear why propranolol or diltiazem are effective against reperfusion injury. Many mechanisms may be involved, including energy preservation arising either as a direct consequence of a reduction in afterload or diminished contractility, and a reduction in Ca^{2+} overload upon reperfusion[25,26] Since our present experimental design does not answer this question, additional experiments are needed. However, in most previous studies, protection by calcium antagonists was associated with depression of cardiac performance prior to, and also during, the early phase of ischemia[27] Since verapamil is ineffective when given after the onset of ischemia, this pathological calcium accumulation may occur by some means other than a slow channe[28] Therefore, we suspect that the basis for this effect appears to be largely related to inhibition of the contractility, heart rate, and afterload due to pretreatment with propranolol or diltiazem during the period of ischemia. Consequently, pretreatment with propranolol or diltiazem significantly preserved high-energy phosphates during the period of ischemia.

In conclusion, coupling between flux (PCr→ATP) and PRP was maintained during the pre-ischemic and postischemic periods with and without propranolol or diltiazem treatment. Flux (PCr→ATP) does not limit the recovery or function in the postischemic myocardium. On the other hand, flux (Pi→ATP)/PRP ratio is depressed by ischemia/reperfusion, and the depressed flux (Pi→ATP)/PRP ratio is restored by pretreatment with propranolol or diltiazem. We concluded that propranolol and diltiazem exert a protective effect on the rate of production of glycolytic ATP in ischemia/reperfused myocardium.

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