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The Effect of Pimobendan on Myocardial Mechanical Function and Metabolism in Dogs: Comparison with Dobutamine

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Abstract—The effect of pimobendan, a newly developed cardiotonic agent, on myocardial mechanical function and energy metabolism has been examined in the dog heart, and compared with that of dobutamine. Either saline, vehicle for pimobendan, dobutamine (0·3 and 1 $\mu g\ kg^{-1}$), or pimobendan (0·3 and 1 $mg\ kg^{-1}$) was injected intravenously. Dobutamine and pimobendan both increased the first derivative of left ventricular pressure and percent segment shortening, indicating their positive inotropic action. After 2 min of dobutamine injection, or after 20 min of pimobendan injection, the myocardium was removed, and used for determination of the tissue levels of metabolites of energy and carbohydrate metabolism. In general, all metabolic parameters measured were not changed by either dobutamine or pimobendan injection. In animals with aortic constriction for 10 months, dobutamine and pimobendan injections did not alter the myocardial energy and carbohydrate metabolism. Although dobutamine and pimobendan increased the cardiac mechanical function, they did not disturb the myocardial energy and carbohydrate metabolism.

Cardiac glycosides and many positive inotropic agents acting via inhibition of cardiac phosphodiesterases are available for increasing contractile force in heart failure patients (Farah et al 1984). Pimobendan, a newly developed cardiotonic drug, has little inhibitory effect on cyclic AMP phosphodiesterase (Berger et al 1985). Jaquet & Heilmeyer (1987) and Fujino et al (1988) have reported that sensitivity of actomyosin to calcium ions is increased by pimobendan. Whatever a positive inotropic mechanism is, increase in the cardiac mechanical function will increase the myocardial energy demand. The increase in the energy demand may be harmful to the failing heart. We (Abiko & Ichihara 1978) have demonstrated that ouabain does not modify the changes in the myocardial energy metabolism during ischaemia. Although there are many reports on the cardiac mechanical function of a cardiotonic agent, it is not fully understood whether the myocardial energy status is influenced by this kind of drug. The present study, therefore, was undertaken to examine whether pimobendan alters myocardial energy status, and to compare the effect of pimobendan with that of dobutamine.

Materials and Methods

Forty-five healthy mongrel dogs of either sex were anaesthetized with sodium pentobarbitone (30 $mg\ kg^{-1}$, i.v.). After endotracheal intubation, respiration was controlled by a respirator with room air. Left thoracotomy between the fourth and fifth ribs was performed, and the left ventricle was exposed and suspended in a pericardial cradle. To measure left ventricular end-diastolic pressure (LVEDP) and the first derivative of left ventricular pressure (LVdP/dT), a polyethylene tubing connected to a pressure transducer was inserted into the left ventricular chamber through the cardiac apex.

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The left anterior descending coronary artery (LAD) was dissected free, distal to its site of origin, and a magnetic flow probe was positioned around the artery to measure LAD flow. A pair of ultrasonic crystals was implanted in a circumferential plane at the LAD region. The two crystals of each pair were separated by about 1 cm. The value of percent segment shortening (%SS) was calculated as

$$((DL-SL)/DL)\times 100$$

where DL is the diastolic segment length, SL is systolic segment length. Arterial blood pressure was measured via a cannula introduced from the left femoral artery to a point near the aortic arch. Heart rate was monitored from the electrocardiogram reference lead on the limb.

Either saline, vehicle for pimobendan, dobutamine (0·3 and 1 $\mu g\ kg^{-1}$), or pimobendan (0·3 and 1 $mg\ kg^{-1}$) was injected intravenously over 30 s. After 2 min of dobutamine injection, or after 20 min of pimobendan injection, the left ventricular myocardial sample was removed, and frozen quickly with freezing clamps previously chilled in liquid nitrogen. The levels of adenosine triphosphate (ATP), adenosine diphosphate (ADP), adenosine monophosphate (AMP), creatine phosphate, glucose 6 phosphate (G6P), fructose 6 phosphate (F6P), fructose 1,6 diphosphate (FDP), pyruvate and lactate were determined in neutralized perchloric acid extract according to the standard enzymatic procedure (Bergmeyer 1974). To estimate energy status in the myocardial cell, energy charge potential (ECP) was calculated as ([ATP]+0·5[ADP])/([ATP]+[ADP]+[AMP]) (Atkinson & Walton 1967). The ratios of ([G6P]+[F6P])/[FDP] and [lactate]/[pyruvate] were calculated to examine the effect of each drug on carbohydrate metabolism.

In experiments on failing hearts, the ascending aorta had been occluded in dogs for about 10 months before the experiment. After 10 months of aortic constriction, the inner diameter of the proximal portion of the aorta to the constrictor was 5·6 ± 0·4 mm, whereas that of the distal

portion was 4·4±0·3 mm. Twenty min after vehicle or pimobendan (1 mg kg^{-1}) was injected intravenously, the myocardium was removed and used for determination of the tissue metabolites as mentioned above.

Animals without failing hearts were divided into 6 groups; saline- (control for dobutamine; 7 dogs), vehicle- (saline-+polyethyleneglycol 200; 1:1; 6 dogs, control for pimobendan), dobutamine (0·3 $\mu g\ kg^{-1}$; 6 dogs)-, dobutamine (1 $\mu g\ kg^{-1}$; 6 dogs)-, pimobendan (0·3 mg kg^{-1}; 6 dogs)-, and pimobendan (1 mg kg^{-1}; 6 dogs)-treated groups. In addition, dogs with failing hearts were divided into 2 groups; vehicle-(3 dogs) and pimobendan (1 mg kg^{-1}; 5 dogs)-treated groups.

All values are expressed as means±standard error of the mean. Data were analysed by one-way analysis of variance followed by Dunnett's *t*-test. *P* values less than 0·05 were considered significant.

Results

Cardiac mechanical function

Fig. 1 shows the haemodynamic changes in the saline- and dobutamine-treated groups. In the saline-treated group, all haemodynamic parameters measured were unchanged throughout the experiment. In the dobutamine (0·3 $\mu g\ kg^{-1}$)-treated group, systolic blood pressure, LVdP/dT and %SS were increased, and diastolic blood pressure, heart rate, coronary flow and LVEDP were not changed by dobutamine injection. In the dobutamine (1 $\mu g\ kg^{-1}$)-treated group, all parameters increased after the drug injection.

Haemodynamic changes in the vehicle- and pimobendan-treated groups are shown in Fig. 2. In the vehicle-treated group, all parameters were unchanged except for decreased heart rate. In the pimobendan-treated groups, systolic blood

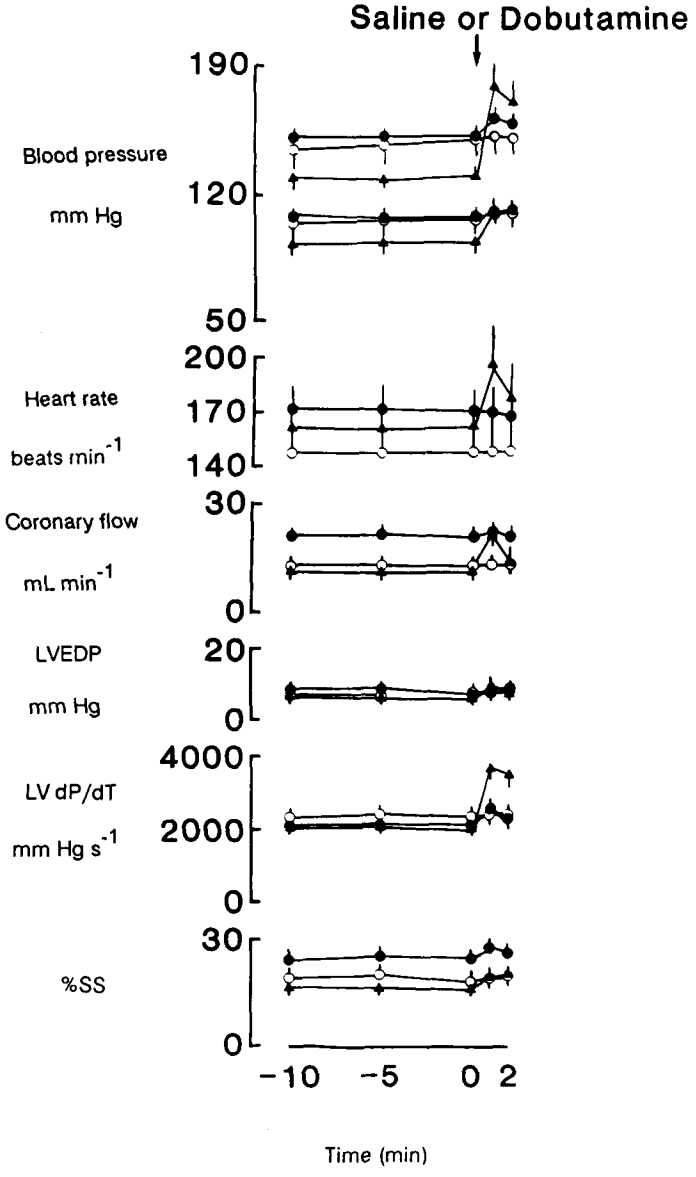

FIG. 1. Haemodynamic changes caused by dobutamine injection in normal heart. Saline (○), 0·3 $\mu g\ kg^{-1}$ dobutamine (●), 1 $\mu g\ kg^{-1}$ of dobutamine (▲). Hearts were removed 2 min after the drug injection. BP = systolic and diastolic blood pressures, HR = heart rate, CF = coronary flow, LVEDP = left ventricular end diastolic pressure, LVdP/dT = the first derivative of left ventricular pressure, and %SS = percent shortening.

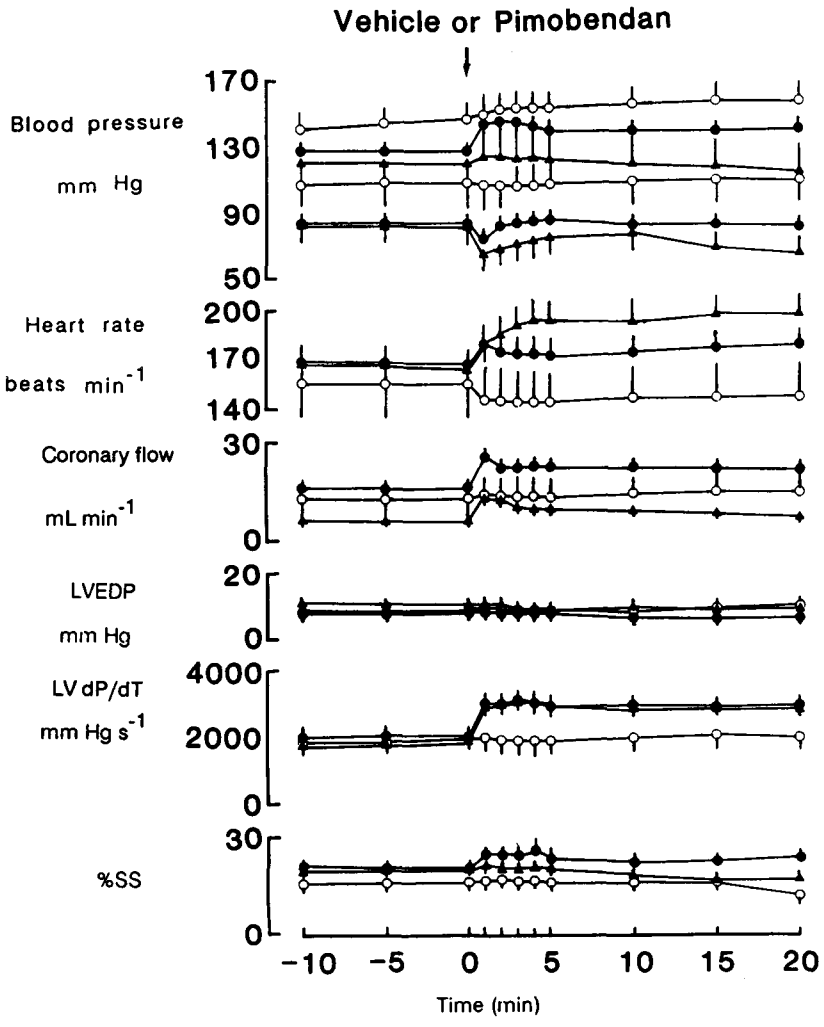

FIG. 2. Haemodynamic changes caused by pimobendan injection in normal heart. Vehicle (○), 0·3 mg kg^{-1} of pimobendan (●), 1 mg kg^{-1} pimobendan (▲). Hearts were removed 20 min after the drug injection.

pressure, heart rate, coronary flow, LVdP/dT and %SS were increased, and diastolic blood pressure was decreased by the injection of pimobendan. These changes were greater in the 1 mg kg^{-1} group than those in the 0·3 mg kg^{-1} group. LVEDP was unchanged or was slightly decreased by pimobendan.

Myocardial metabolism

Tissue levels of ATP, ADP, and AMP are summarized in Table 1. There were no significant changes in ATP and AMP levels between saline- and either dose of dobutamine-treated groups, whereas the level of ADP slightly but significantly increased after dobutamine injection. Pimobendan did not alter the levels of ATP, ADP or AMP. Fig. 3 (left panel) illustrates ECP values calculated with adenine nucleotide levels. Neither dobutamine nor pimobendan changed ECP values significantly. CrP level was not modified by dobutamine and pimobendan (Table 2).

Table 1. Effects of dobutamine and pimobendan on the levels of adenine nucleotides in the myocardium.

Treatment	n	ATP	ADP	AMP
			μmol (g wet wt)$^{-1}$	
Saline	7	5·10±0·15	0·78±0·02	0·12±0·01
Dobutamine (0·3 μg kg^{-1})	6	5·33±0·08	0·94±0·03**	0·11±0·01
Dobutamine (1 μg kg^{-1})	6	5·14±0·06	0·88±0·12*	0·12±0·01
Vehicle	6	5·53±0·11	0·82±0·04	0·13±0·01
Pimobendan (0·3 mg kg^{-1})	6	5·20±0·12	0·88±0·04	0·14±0·01
Pimobendan (1 mg kg^{-1})	6	5·06±0·19	0·82±0·03	0·11±0·01

The myocardial samples were taken 2 min after saline or dobutamine injection, or 20 min after vehicle or pimobendan injection. Vehicle was a mixture of saline and polyethylene glycol 200 (1:1). (n)=the number of observations. *$P<0·05$, **$P<0·01$ compared with the value of the saline-treated group.

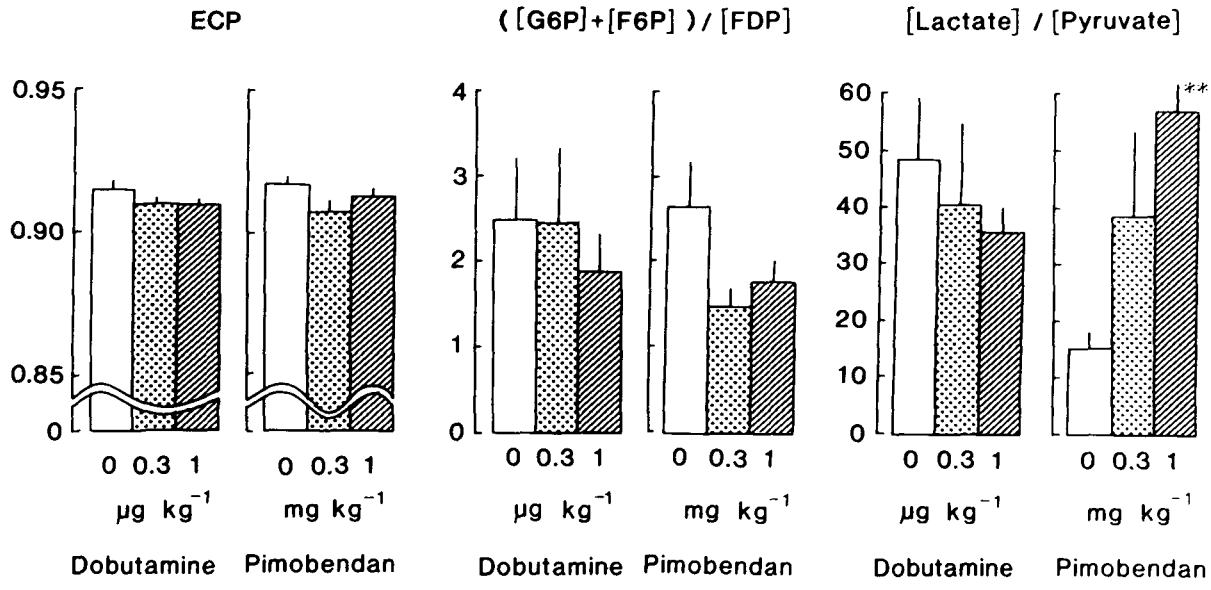

FIG. 3. Effects of dobutamine and pimobendan on ECP, ([G6P]+[F6P])/[FDP], and [lactate]/[pyruvate]. Saline [0, □), vehicle (0, □), 0·3 μg kg^{-1} of dobutamine (0·3, ▨), 1 μg kg^{-1} of dobutamine (1, ■), 0·3 mg kg^{-1} of pimobendan (1, ▨), 1 mg kg^{-1} of pimobendan (1, ■). $^{**}P<0.01$ compared with the value of the vehicle-treated group.

Table 2. Effects of dobutamine and pimobendan on the level of CrP in the myocardium.

Treatment	n	CrP μmol (g wet wt)$^{-1}$
Saline	7	6·60±0·37
Dobutamine (0·3 μg kg^{-1})	6	5·43±0·43
Dobutamine (1 μg kg^{-1})	6	5·89±0·68
Vehicle	6	6·92±0·45
Pimobendan (0·3 mg kg^{-1})	6	5·72±0·59
Pimobendan (1 mg kg^{-1})	6	5·76±0·50

n = the number of observations.

Table 3 shows the levels of G6P, F6P, and FDP. In the dobutamine-treated groups, 0·3 μg kg^{-1} of dobutamine increased the level of G6P significantly. In the pimobendan-treated groups, both 0·3 and 1 mg kg^{-1} of pimobendan significantly increased the level of FDP, and 1 mg kg^{-1} of the drug increased the level of F6P. The ratio ([G6P]+[F6P])/[FDP] is shown in Fig. 3 (middle panel). The ratio ([G6P]+[F6P])/[FDP] was not changed by the smaller dose of dobutamine (0·3 μg kg^{-1}), while it was decreased by the larger dose of dobutamine (1 μg kg^{-1}). The ([G6P]+[F6P])/[FDP] ratio in both pimobendan-treated groups appeared to decrease as compared with that in the vehicle-treated group. The differences in ([G6P]+[F6P])/[FDP] observed were not significant as compared with the saline- or vehicle-treated groups.

Table 4 shows the levels of lactate and pyruvate. Dobutamine injection did not change the levels of lactate and pyruvate. The level of lactate in the pimobendan (1 mg kg^{-1})-treated group was significantly higher than that in the vehicle-treated group. The changes in [lactate]/[pyruvate] ratio after drug injection are shown in Fig. 3 (right panel). Dobutamine slightly decreased the [lactate]/[pyruvate] ratio. Because of decreased ratio of [lactate]/[pyruvate] in the vehicle-treated group, pimobendan injection appeared to increase the [lactate]/[pyruvate] ratio. The difference in the ratio between vehicle- and 1 mg kg^{-1} pimobendan-treated groups was significant.

Heart failure experiments

In an additional experiment with failing hearts, 5 animals out of 8 were injected i.v. with 1 mg kg^{-1} pimobendan, and 3 were injected with vehicle. The LVdP/dT of the failing hearts increased after pimobendan injection as did that of the normal hearts (Fig. 4). The increase in LVdP/dT in the failing

Table 3. Effects of dobutamine and pimobendan on the levels of hexose phosphates in the myocardium.

Treatment	n	G6P	F6P μmol (g wet wt)$^{-1}$	FDP
Saline	7	0·17±0·02	0·02±0·01	0·11±0·02
Dobutamine (0·3 μg kg^{-1})	6	0·25±0·04**	0·04±0·01	0·17±0·04
Dobutamine (1 μg kg^{-1})	6	0·27±0·05	0·03±0·01	0·19±0·04
Vehicle	6	0·14±0·03	0·02±0·01	0·08±0·03
Pimobendan (0·3 mg kg^{-1})	6	0·21±0·03	0·02±0·01	0·17±0·03*
Pimobendan (1 mg kg^{-1})	6	0·23±0·02	0·04±0·01**	0·17±0·03*

n = the number of observations. $^{*}P<0.05$, $^{**}P<0.01$ compared with the values of saline-treated or vehicle-treated groups.

Table 4. Effects of dobutamine and pimobendan on the levels of pyruvate and lactate in the myocardium.

Treatment	n	Pyruvate μmol (g wet wt)$^{-1}$	Lactate
Saline	7	0·02±0·01	0·99±0·16
Dobutamine (0·3 μg kg^{-1})	6	0·02±0·01	0·87±0·36
Dobutamine (1 μg kg^{-1})	6	0·03±0·01	1·13±0·19
Vehicle	6	0·06±0·01	0·78±0·13
Pimobendan (0·3 mg kg^{-1})	6	0·04±0·01	1·11±0·19
Pimobendan (1 mg kg^{-1})	6	0·03±0·01	1·86±0·29**

n = the number of observations. **$P<0·01$ compared with the value of the vehicle-treated group.

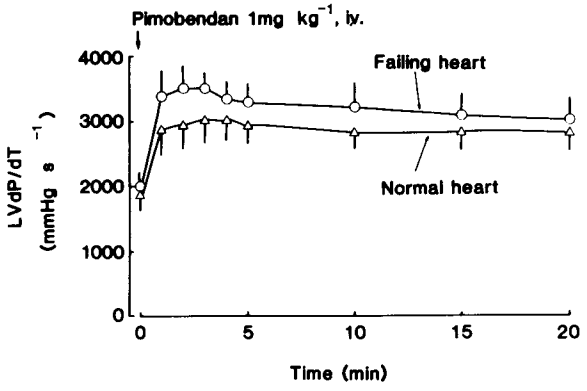

FIG. 4. Effects of pimobendan on LVdP/dT in the normal and failing hearts. The failing heart was produced by aortic constriction for 10 months. LVdP/dT values of the normal heart were as shown in Fig. 2. Although all values obtained after pimobendan injection were significantly different from those obtained before the injection, symbols indicating significance are not shown, for simplicity.

Table 5. Effects of pimobendan (1 mg kg^{-1}) on the tissue levels of myocardial metabolites in the failing heart.

Metabolites	Vehicle μmol (g wet wt)$^{-1}$	Pimobendan
ATP	5·07±0·12	5·10±0·15
ADP	1·01±0·13	0·99±0·07
AMP	0·16±0·03	0·13±0·02
CrP	5·34±1·89	4·76±0·70*
G6P	0·30±0·07	0·26±0·03
F6P	0·04±0·01	0·05±0·01
FDP	0·25±0·04	0·25±0·06
Pyruvate	0·06±0·01	0·05±0·01
Lactate	2·56±0·64	1·28±0·35

The aorta was constricted with an occluder for 10 months, resulting in reduction of the diameter of the aorta from 5·6±0·4 to 4·4±0·3 mm. Three dogs were injected with vehicle and 5 dogs with pimobendan (1 mg kg^{-1}). *$P<0·05$ compared with the value of the vehicle-treated group.

hearts tended to be greater than that in the normal hearts. Tissue levels of the myocardial metabolites measured are summarized in Table 5. Pimobendan significantly decreased the creatinine phosphate level as compared with the vehicle-treated group. The levels of the other metabolites in the pimobendan-treated group did not differ from those in the vehicle-treated group. Values of ECP, ([G6P]+[F6P])/[FDP] and [lactate]/[pyruvate] are shown in Fig. 5. All these values were not significantly altered by the pimobendan injection.

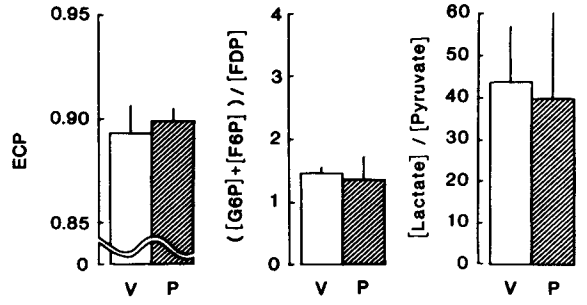

FIG. 5. Effects of pimobendan on ECP, ([G6P]+[F6P])/[FDP], [lactate]/[pyruvate] in failing hearts. Hearts were removed 20 min after vehicle (V, □) or 1 mg kg^{-1} of pimobendan (P, ■).

Discussion

In the present study, pimobendan revealed pronounced and long lasting positive inotropic effects in the dog heart. Dobutamine also increased LVdP/dT, indicating its positive inotropic action. However, the increased LVdP/dT caused by dobutamine injection began to decrease within a few minutes. Increase in systolic blood pressure after dobutamine and pimobendan injection is probably due to their positive inotropic effects. Pimobendan decreased diastolic blood pressure and increased coronary flow. Verdouw et al (1986) reported that pimobendan caused a marked vasodilation in the pig; thus changes in the diastolic pressure and coronary flow can be explained by arterial vasodilation. A positive inotropic action of a drug may potentiate the increase in coronary flow, since 1 μg kg^{-1} dobutamine also increased the coronary flow.

To determine the myocardial energy status after the positive inotropic effects had been kept at the maximum, the heart samples were taken 2 min after dobutamine injection, and 20 min after pimobendan injection. Lack of significant changes in ECP value after either dobutamine or pimobendan injection suggest that neither drug caused energy alteration although both increased cardiac contraction. The ratio of ([G6P]+[F6P])/[FDP] may indicate glycolytic flux through its rate-limiting step, phosphofructokinase reaction (Ichihara & Abiko 1982, 1987). The decrease in ([G6P]+[F6P])/[FDP] by pimobendan may indicate the acceleration of glycolytic flux. Glycolysis may partly supply contractile energy for the heart when pimobendan is administered. It is not easy to explain why the [lactate]/[pyruvate] ratio was decreased by vehicle injection. Vehicle decreased heart rate (Fig. 2). Decreased heart rate may reduce cardiac work load and myocardial energy demand, resulting in an improvement of myocardial redox state. In the failing heart experiment, however, the [lactate]/[pyruvate] ratio was about 44 in the vehicle-treated heart (Fig. 5). Although there was a significant difference in [lactate]/[pyruvate] ratio between vehicle- and pimobendan (1 mg kg^{-1})-treated groups, it was assumed that the redox state in the myocardial cell did not shift so much, since the [lactate]/[pyruvate] ratio in the normal heart is variable in the range of 10 to 100 (Yoshida et al 1990).

There was no significant difference in mechanical and metabolic function between the normal and the failing heart. Aortic constriction for 10 months reduced its diameter by

only 22%, and the heart failure may not be severe enough to produce alterations of mechanical and metabolic function. Treatment with pimobendan increased LVdP/dT with significant reduction of creatinine phosphate level. When the mechanical function of the myocardium increases, the tissue level of creatinine phosphate decreases (Ichihara & Abiko 1984). Pimobendan may decrease the creatinine phosphate level through the increase in cardiac contraction.

In conclusion, although pimobendan can maintain the increased cardiac mechanical function for a longer period than dobutamine, it does not cause significant alteration of myocardial energy status.

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