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Effects of LP-805, a Newly Developed Vasodilator, on Myocardial Metabolism in Ischaemic Dog Hearts

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Abstract—The effects of LP-805, a newly developed vasodilator, on changes in the myocardial energy and carbohydrate metabolism induced by ischaemia were studied in open-chest anaesthetized dogs. Ischaemia was induced by ligating the left anterior descending coronary artery for 3 min. The myocardial energy stores were depleted, and the levels of glycolytic intermediates were altered 3 min after the onset of ischaemia. Energy change potential was decreased, and ([G6P]+[F6P])/[FDP] and [lactate]/[pyruvate] ratios were increased by ischaemia. These findings indicated that the myocardial metabolism was converted from an aerobic to an anaerobic type by ischaemia. LP-805 (10, 30, or 100 $\mu g\ kg^{-1}$) was injected intravenously 5 min before the onset of ischaemia. LP-805 prevented the myocardial energy depletion and alterations of myocardial carbohydrate metabolism due to ischaemia, indicating that it appeared to convert the anaerobic metabolism back to aerobic metabolism in the ischaemic myocardium. In conclusion, LP-805 may reduce the ischaemic influence on the myocardium.

We (Abiko et al 1979) have tried to evaluate the anti-anginal or anti-ischaemic effect of a drug by the use of an indicator of anaerobic metabolism in the ischaemic myocardium. Under these conditions, the ischaemia causes conversion of the myocardial metabolism from aerobic to anaerobic and a drug that reduces ischaemic injury can switch the metabolism back to aerobic. By this method, we examined how ischaemic injury proceeds in the myocardium. We have found that glyceryl trinitrate (Ichihara & Abiko 1975a), β-adrenoceptor blocking agents (Ichihara & Abiko 1977, 1987), and some calcium blockers (Ichihara et al 1989; Abe et al 1991) prevent anaerobic metabolism during ischaemia.

LP-805, 8-*tert*-butyl-6,7-dihydropyrrolo[3,2-*e*]-5-methylpyrazolo[1,5-*a*]pyrimidine-3-carbonitrile, is a newly synthesized vasodilator (Fig. 1), which dilates almost all arteries including cerebral and coronary arteries (Inazu et al 1991).

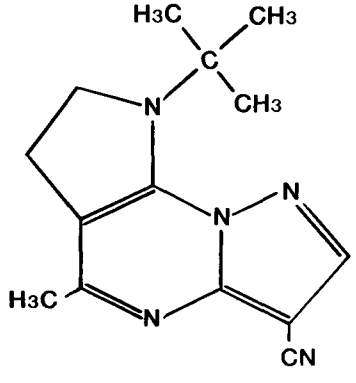

FIG. 1. Chemical structure of LP-805.

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Because LP-805 does not affect potassium-induced vasoconstriction, but prevents noradrenaline-induced vasoconstriction, it probably inhibits receptor-operated calcium mobilization inside the cell (Kishii et al 1991) not voltage-dependent calcium channels. LP-805 has also been shown to activate potassium channels, and to facilitate the release of endothelium-derived relaxing factor (EDRF) (Nakashima et al 1991). We (Ichihara et al 1991) have found that LP-805 attenuates the ischaemia-induced myocardial acidosis in dog heart, suggesting its beneficial effects on the ischaemic myocardium. The present study was undertaken to examine the effect of LP-805 on myocardial energy and carbohydrate metabolism in the ischaemic dog heart.

Materials and Methods

Animal preparation

Fifty-five healthy mongrel dogs of either sex, 8–15 kg, were anaesthetized with sodium pentobarbitone (30 $mg\ kg^{-1}$, i.v.), and endotracheally intubated and ventilated with a Harvard respirator. A left thoracotomy was performed between the fourth and fifth ribs to expose the left ventricular wall. After the heart was suspended in a pericardial cradle, the main trunk of the left anterior descending coronary artery (LAD) was dissected free from the adjacent tissue at a position distal to the first diagonal branch, and was loosely encircled with a 2-0 silk thread ligature. Ischaemia was initiated by ligating the LAD. Ischaemia of the myocardium was assessed by the appearance of visible cyanosis and by the elevation of the ST segment of the ECG recorded with a wire electrode attached on the surface of the left ventricular wall. Arterial blood pressures were measured via cannulae introduced into the left femoral artery. Coronary blood flow was measured by an electromagnetic flow probe positioned just proximal to the ligature.

After the control observations, saline (containing 0·1 M HCl) or LP-805 dissolved in saline containing 0·1 M HCl (10, 30, or 100 $\mu g\ kg^{-1}$) was injected intravenously. Five minutes later the ligature around the LAD was tied in about half the

animals receiving LP-805, and in half of those receiving saline. After 3 min of ischaemia, a sample of the myocardium was taken transmurally from the centre of the ischaemic area. An equivalent sample was taken from the control animals that had not had the ligature tied around the LAD. The samples were immediately pressed and frozen with clamps previously chilled in liquid nitrogen according to the method of Ichihara & Abiko (1975b). Although it took less than 5 s to remove and freeze a myocardial sample, the samples of non-ischaemic myocardium were taken from other groups of control animals to avoid a delay in sampling.

Biochemical analysis

The frozen sample of endocardial tissue was used to determine the levels of glucose-6-phosphate (G6P), fructose-6-phosphate (F6P), fructose-1, 6-diphosphate (FDP), pyruvate, lactate, adenosine triphosphate (ATP), adenosine diphosphate (ADP), adenosine monophosphate (AMP), and creatine phosphate in neutralized perchloric acid extracts, according to standard enzymatic procedures. The contents of metabolites in the myocardium are expressed in g wet weight, because ischaemia did not influence the water content of the myocardium in the present experiment. The energy charge potential (ECP) was calculated from the concentration of ATP, ADP, and AMP to estimate the myocardial energy state as ([ATP]+0·5[ADP])/([ATP]+[ADP]+[AMP]) (Atkinson & Walton 1967). The ratio of ([G6P]+[F6P])/[FDP] was calculated from the concentration of hexose phosphates to estimate the rate of glycolytic flux through the phosphofructokinase (PFK) reaction (Ichihara & Abiko 1982, 1987; Ichihara et al 1989; Abe et al 1991).

The ratio of lactate to pyruvate was also calculated as an index of the cytoplasmic redox state of the myocardial cell.

Statistical analysis

Haemodynamic data were evaluated by a paired Student's *t*-test, and biochemical data were analysed by one-way analysis of variance followed by Dunnett's *t*-test. *P* values $<0·05$ were considered significant.

Results

Haemodynamic changes

Haemodynamic data from the dogs whose LAD had been ligated for 3 min are illustrated in Fig. 2. Saline injection had no significant effect on systemic blood pressure or coronary flow, and 3 min post-LAD ligation, the control saline-treated hearts manifested significant reductions in blood pressure without reflex tachycardia, and showed significant elevation of the ST segment of epicardial ECG. A transient decrease in arterial blood pressures was observed in 30 and 100 $\mu g\ kg^{-1}$ of LP-805-treated groups, and a transient increase in heart rate due probably to reflex tachycardia, and a transient increase in coronary flow were also observed in the same groups. LP-805 (10 $\mu g\ kg^{-1}$), however, did not affect all haemodynamic parameters appreciably. In dogs pretreated with either dose of LP-805, LAD ligation resulted in decreases in blood pressures and ST segment elevation of epicardial ECG, similar to those in saline-treated dogs. The same responses of haemodynamics to LP-805 were obtained in the dog whose LAD was not ligated.

Energy metabolism

Changes in adenine nucleotide concentrations during ischaemia are shown in Table 1. Three minutes post-LAD ligation, ATP levels decreased significantly while ADP and AMP levels significantly increased. Overall total adenine nucleotide levels significantly decreased after the onset of ischaemia. LP-805-treated hearts reduced the depletion of both ATP and total adenine nucleotides, caused by ischaemia, but only at the highest dose (100 $\mu g\ kg^{-1}$) were these changes statistically significant compared with the saline controls.

To estimate the myocardial energy state, we calculated ECP from the levels of adenine nucleotides. As shown in Fig.

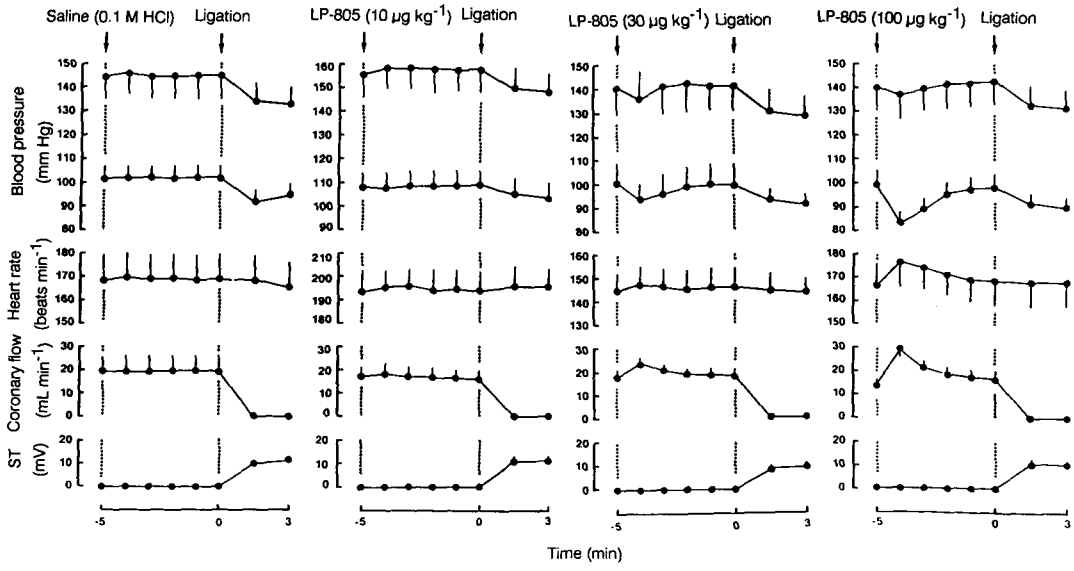

FIG. 2. The effects of LP-805 on haemodynamic parameters in dogs whose left anterior descending coronary artery (LAD) was ligated for 3 min. Saline containing 0·1 M HCl or LP-805 (10, 30, or 100 $\mu g\ kg^{-1}$) was injected intravenously 5 min before LAD ligation. Hearts were removed 3 min after LAD ligation. ST = difference in ST segment of the epicardial ECG between before and after LAD ligation.

Table 1. Changes in the levels of adenine nucleotides during ischaemia in saline- and LP-805-treated dogs.

	n	μmol (g wet wt)$^{-1}$ ATP	ADP	AMP	Total
Saline-treated					
Non-ischaemia	6	5·27±0·20	0·94±0·05	0·20±0·02	6·40±0·19
Ischaemia	9	3·63±0·23**	1·31±0·12*	0·26±0·02*	5·19±0·34**
LP-805-treated (10 μg kg^{-1})					
Non-ischaemia	6	5·33±0·12	0·76±0·04#	0·11±0·01##	6·20±0·11
Ischaemia	7	4·10±0·15**	1·23±0·08**	0·20±0·02**#	5·53±0·12**
LP-805-treated (30 μg kg^{-1})					
Non-ischaemia	6	5·69±0·13	0·94±0·08	0·12±0·01##	6·76±0·08
Ischaemia	6	4·09±0·13**	1·38±0·06**	0·23±0·01**	5·69±0·17**
LP-805-treated (100 μg kg^{-1})					
Non-ischaemia	8	5·19±0·14	0·86±0·03	0·23±0·03	6·28±0·15
Ischaemia	7	4·56±0·17*##	1·37±0·09**	0·18±0·01##	6·11±0·14##

Either saline containing 0·1 M HCl (saline-treated) or LP-805 (10, 30, or 100 μg kg^{-1}) was intravenously injected 5 min before LAD ligation. The myocardial samples were taken 3 min after the LAD ligation. Total = total adenine nucleotides. n = the number of observations. *P < 0·05, **P < 0·01 compared with non-ischaemia in each group. #P < 0·05, ##P < 0·01 compared with the corresponding value of saline-treated group.

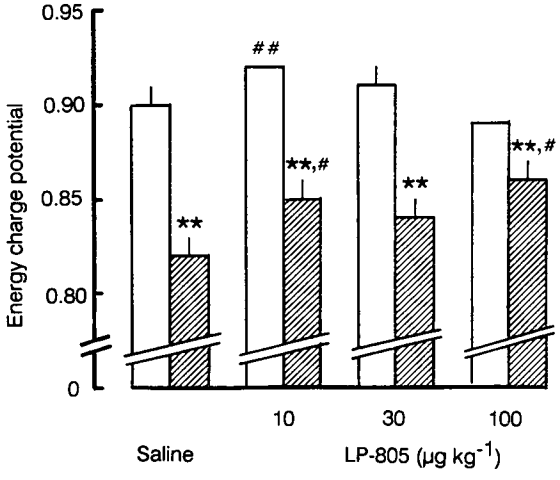

FIG. 3. Effect of LP-805 on changes in energy charge potential (ECP) during ischaemia. ECP was calculated from the levels of adenine nucleotides in the myocardium as ([ATP]+0·5[ADP])/([ATP]+[ADP]+[AMP]). Either saline containing 0·1 M HCl or LP-805 was injected intravenously 5 min before LAD ligation. Hearts were removed just before (non-ischaemia, □) or 3 min after LAD ligation (ischaemia, ■). **P < 0·01, compared with non-ischaemia in each group. #P < 0·05; ##P < 0·01, compared with ischaemia in the saline-treated group.

3, ECP decreased in ischaemic hearts, and pretreatment with LP-805 lessened the decrease in ECP caused by ischaemia; 3 min after ischaemia, ECP in LP-805 (10 and 100 μg kg^{-1})-treated hearts was significantly higher than that in saline-treated hearts.

Changes in the levels of creatine phosphate in ischaemic and non-ischaemic myocardium are shown in Table 2. Myocardial creatine phosphate concentrations were reduced significantly at 3 min post-LAD ligation in saline- and LP-805-treated hearts. Pretreatment with LP-805, however, reduced the depletion of creatine phosphate during ischaemia, but only at 30 μg kg^{-1} were creatine phosphate levels significantly higher than the saline-treated controls.

Table 2. Changes in the level of creatine phosphate during ischaemia in saline- and LP-805-treated dogs

	(n)	Creatine phosphate μmol (g wet wt)$^{-1}$
Saline-treated		
Non-ischaemia	6	5·87±0·47
Ischaemia	9	0·93±0·10**
LP-805-treated (10 μg kg^{-1})		
Non-ischaemia	6	6·11±0·53
Ischaemia	7	1·75±0·47**
LP-805-treated (30 μg kg^{-1})		
Non-ischaemia	6	5·49±0·40
Ischaemia	6	1·32±0·07**#
LP-805-treated (100 μg kg^{-1})		
Non-ischaemia	8	5·66±0·48
Ischaemia	7	1·99±0·60

n = the number of observations. **P < 0·01 compared with non-ischaemia in each group. #P < 0·05 compared with the corresponding value of saline-treated group.

Carbohydrate metabolism

Changes in the levels of hexose phosphates during ischaemia are summarized in Table 3. We determined the levels of G6P, F6P, and FDP in the myocardium with or without coronary artery ligation of LAD, and calculated the ratio of ([G6P]+[F6P])/[FDP] to assess the rate of glycolytic flux through the PFK reaction (Fig. 4). The levels of G6P and F6P increased in the ischaemic myocardium, whereas the level of FDP decreased; in total, the ratio of ([G6P]+[F6P])/[FDP] increased over ten times during ischaemia. LP-805 at either dose prevented the increase in the levels of G6P and F6P and the increase in the ratio of ([G6P]+[F6P])/[FDP] after 3 min of ischaemia.

The levels of lactate and pyruvate in the ischaemic and non-ischaemic hearts are shown in Table 4. Because ischaemia increased the lactate level, but did not change the pyruvate level, the ratio of [lactate]/[pyruvate] increased after 3 min of ischaemia (Fig. 5). This increase in the ratio of

Table 3. Changes in the levels of hexose phosphates during ischaemia in saline- and LP-805-treated dogs.

	n	μmol (g wet wt)$^{-1}$ G6P	F6P	FDP
Saline-treated				
Non-ischaemia	6	0·23±0·05	0·04±0·01	0·15±0·03
Ischaemia	9	1·67±0·21**	0·37±0·05**	0·10±0·01
LP-805-treated (10 μg kg^{-1})				
Non-ischaemia	6	0·15±0·03	0·02±0·01	0·09±0·02
Ischaemia	7	1·10±0·23**	0·24±0·05**	0·07±0·01
LP-805-treated (30 μg kg^{-1})				
Non-ischaemia	6	0·14±0·02	0·04±0·01	0·13±0·02
Ischaemia	6	1·09±0·13**#	0·24±0·03**	0·16±0·03
LP-805-treated (100 μg kg^{-1})				
Non-ischaemia	8	0·15±0·02	0·03±0·01	0·09±0·02
Ischaemia	7	0·80±0·18**##	0·16±0·04**##	0·08±0·01

(n) = the number of observations. **$P<0·01$ compared with non-ischaemia in each group. #$P<0·05$, ##$P<0·01$ compared with the corresponding value of saline-treated group.

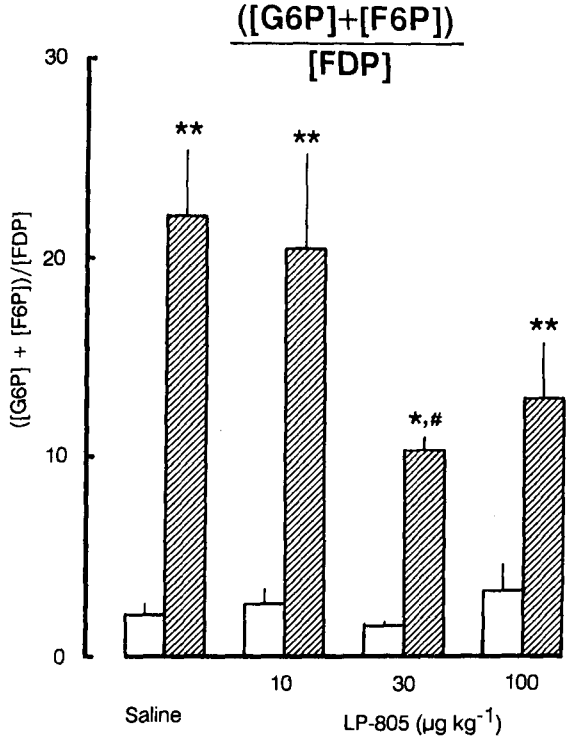

FIG. 4. Effect of LP-805 on the ratio ([G6P]+[F6P])/[FDP] in the ischaemic myocardium. Hearts were removed just before (non-ischaemia, □) or 3 min after LAD ligation (ischaemia, ■). *$P<0·05$; **$P<0·01$, compared with non-ischaemia in each group. #$P<0·05$, compared with ischaemia in the saline (0·1 M HCl)-treated group.

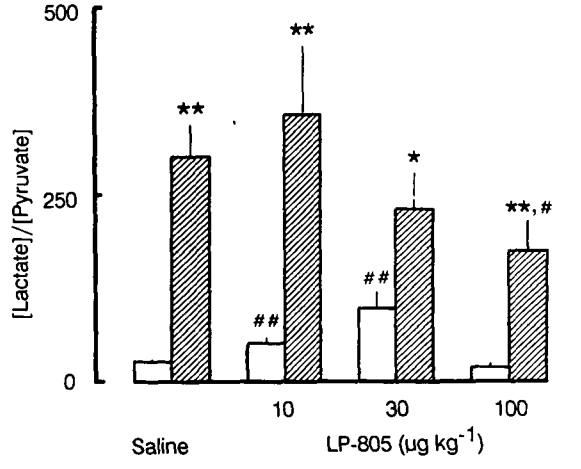

FIG. 5. The effect of LP-805 on the ratio of [lactate]/[pyruvate] in the ischaemic myocardium. Hearts were removed just before (non-ischaemia, □) or 3 min after LAD ligation (ischaemia, ■). *$P<0·05$, **$P<0·01$ compared with non-ischaemia in each group. #$P<0·05$, ##$P<0·01$ compared with ischaemia in the saline (0·1 M HCl)-treated group.

[lactate]/[pyruvate] was reduced by pretreatment with LP-805 at a dose of 30 and 100 μg kg^{-1}.

Discussion

We have examined the metabolic changes in the endocardial portion of the myocardium 3 min after induction of ischaemia (Winbury et al 1969). Ischaemic changes occurring within a few minutes after the onset of ischaemia are important for the patient with angina pectoris, since anginal attack occurs immediately after the coronary blood flow has been interrupted.

Myocardial ischaemia, initiated by ligating LAD, resulted in depletion of high energy phosphates with a switch from aerobic to anaerobic metabolism. Oxygen deficiency, therefore, accelerated the rate of glycolytic flux to produce ATP anaerobically. However, during ischaemia, in which the myocardium must be in anaerobic conditions, glycolysis was not stimulated (Ichihara & Abiko 1982). There may be a feedback mechanism in glycolysis during ischaemia, because accumulation of deleterious metabolic end products, such as lactate and hydrogen ions makes the ischaemic myocardial injury worse (Ichihara et al 1981). In fact, hydrogen ions inhibit the PFK reaction (Ui 1966). In the present experiment the ratio of ([G6P]+[F6P])/[FDP] increased more than ten times during ischaemia, indicating a negative cross-over point at the PFK reaction, and suggesting inhibition of

Table 4. Changes in the levels of pyruvate and lactate during ischaemia in saline- and LP-805-treated dogs.

	n	μmol (g wet wt)$^{-1}$ Pyruvate	Lactate
Saline-treated			
Non-ischaemia	6	0·04±0·01	1·12±0·16
Ischaemia	9	0·05±0·01	12·76±0·56**
LP-805-treated (10 μg kg^{-1})			
Non-ischaemia	6	0·02±0·01#	0·91±0·11
Ischaemia	7	0·04±0·01*	1·036±1·22**
LP-805-treated (30 μg kg^{-1})			
Non-ischaemia	6	0·02±0·00##	1·50±0·29
Ischaemia	6	0·05±0·01**	10·32±0·65**#
LP-805-treated (100 μg kg^{-1})			
Non-ischaemia	8	0·07±0·01	1·11±0·15
Ischaemia	7	0·06±0·01	8·46±1·14**##

n = the number of observations. *$P<0·05$, **$P<0·01$ compared with non-ischaemia in each group. #$P<0·05$, ##$P<0·01$ compared with the corresponding value of saline-treated group.

glycolytic flux at that step. This ratio does not necessarily reflect the rate of glycolytic flux, but it may reflect the degree of PFK inhibition during ischaemia.

In the present study, LP-805 attenuated the depletion of high energy phosphate stores and delayed the switching from aerobic and anaerobic metabolism in ischaemic myocardium. LP-805 reduced the increase in the ratio of ([G6P]+[F6P])/[FDP] caused by ischaemia. Since LP-805 reduces the ischaemic myocardial acidosis (Ichihara et al 1991), it may reverse an ischaemia-induced inhibition of anaerobic glycolysis, as mediated by the PFK reaction. These results suggest that LP-805 reduces the ischaemic influence on the myocardium although the LAD is ligated. We have demonstrated, however, that β-adrenoceptor blocking agents attenuate the metabolic derangement of the heart made ischaemic for 3 min, but not for 30 min (Hayase et al 1990). This suggests that the anti-ischaemic effects of drugs, may not truly protect the heart but just delay the ischaemic responses to LAD ligation.

LP-805 dilates vascular smooth muscle, including coronary artery (Inazu et al 1991). Coronary vasodilators do not always have a beneficial effect on the myocardial energy and carbohydrate metabolism. Dipyridamole, which is a potent coronary vasodilator, and nifedipine, which is a calcium channel blocker and has potent coronary dilatory effects, do not shift the ischaemic anaerobic metabolism to aerobic metabolism (Ichihara & Abiko 1975c; Ichihara et al 1979). On the other hand, propranolol, which is not expected to dilate the coronary artery, does appear to change the ischaemic anaerobic metabolism back to aerobic metabolism (Ichihara & Abiko 1977). Therefore, although LP-805 is a potent coronary vasodilator, it may have some direct cytoprotective effect on the myocardium during ischaemia. Increase in free Ca^{2+} in cytoplasm may influence several myocardial metabolic pathways. Because LP-805 prevents the intracellular Ca^{2+} accumulation induced by noradrenaline (Kishii et al 1991) in the smooth muscle, it may also affect the myocardial cell and can attenuate the changes in myocardial metabolism stimulated by noradrenaline via intracellular Ca^{2+}. During ischaemia, catecholamines are released from the sympathetic nerve endings located in the myocardium (Sakai & Abiko 1982). In addition, because LP-805 accelerates the release of EDRF from the endothelial cell (Nakashima et al 1991), the released EDRF may also contribute to the decrease in intracellular Ca^{2+} in the myocardial cell.

Increased LAD flow caused by LP-805 injection returned to its pre-injection level at the time when the coronary artery was ligated. Coronary flow, however, does not reflect peripheral blood flow through collateral vessels. Therefore, we cannot exclude the possibility that the cardioprotective effect of LP-805 is due to its vasodilatory action on the collateral vessels. Measurements of regional blood flow need to be made to clarify this.

In conclusion, pretreatment with LP-805 reduced the impairment of myocardial energy and carbohydrate metabolism in ischaemic dog hearts, suggesting that LP-805 is capable of improving the imbalance between oxygen supply and oxygen demand in the ischaemic myocardium.

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