J Anesth (1996) 10:269–275

Journal of
Anesthesia

Amrinone improves postischemic myocardial metabolism in the rat heart-lung preparation

MASAKI KUME, SATOSHI KASHIMOTO, ATSUSI FURUYA, and TERUO KUMAZAWA

Department of Anesthesiology, Yamanashi Medical University, Shimokato 1110, Tamaho-cho, Nakakoma, Yamanashi 409-38, Japan

Abstract: Amrinone, a phosphodiesterase inhibitor, is a nonglycosidic noncatecholamine with both vasodilator and positive inotropic effects. We were interested in assessing the effect of amrinone on postischemic cardiac performance in the isolated heart-lung preparation. Twenty-four male Wistar-ST rats were used. They were randomly divided into three groups. Amrinone, 10 $\mu g \cdot ml^{-1}$ or 100 $\mu g \cdot ml^{-1}$ was administered 8 min after the start of perfusion except in the control group. Ten minutes after the start of perfusion, all hearts were made globally ischemic for 8 min. Subsequently, the preparations were reperfused for 10 min. At the end of the experimental period, the hearts were freeze-clamped, and then myocardial high-energy phosphates, lactate, pyruvate, and glycogen were measured. Hemodynamic parameters in all groups decreased significantly during ischemia. However, there were no significant differences among the groups. The myocardial ATP level in the 100 $\mu g \cdot ml^{-1}$ group was significantly higher than that in the control group. Adenosine diphosphate (ADP) and adenosine monophosphate (AMP) levels in the 100 $\mu g \cdot ml^{-1}$ group were significantly lower than those in the control group. Myocardial lactate, pyruvate, and glycogen levels were not significantly different among the groups. This result suggests that amrinone improves postischemic myocardial metabolism. Although we could not measure coronary flow, amrinone might increase coronary flow with direct coronary vasodilation which would have increased the myocardial ATP and energy charge levels.

Key words: Amrinone, Myocardial metabolism, Ischemia

Introduction

Amrinone, a phosphodiesterase inhibitor, is a nonglycosidic noncatecholamine with both vasodilator and positive inotropic effects. Amrinone may be useful to treat the clinical patients with poor left heart function after cardiopulmonary bypass and patients with heart failure associated with acute myocardial infarction. In previous studies, amrinone was reported to improve cardiac performance in patients undergoing cardiac surgery [1–4]. In those studies, the effects of amrinone were modified by autonomic sympathetic and parasympathetic reflexes. As the isolated heart-lung preparation eliminates any confounding neurohumoral effects of in vivo studies, it is interesting to assess the effect of amrinone on postischemic cardiac performance and metabolism in this model.

Materials and methods

This study was approved by the animal care committee of Yamanashi Medical University. The techniques used were almost identical to those used in earlier studies [5,6]. Twenty-four male Wistar-ST rats weighing 300–320 g were used. They were randomly divided into three groups (each group $n = 8$) as follows. (1) Control group which received no drugs. (2) 10 $\mu g \cdot ml^{-1}$ group, which received 10 $\mu g \cdot ml^{-1}$ of amrinone. (3) 100 $\mu g \cdot ml^{-1}$ group, which received 100 $\mu g \cdot ml^{-1}$ of amrinone. All rats were anesthetized with isoflurane during the preparation. Tracheotomy was performed, and intermittent positive pressure ventilation was instituted with room air. Immediately, the chest was opened and flooded with ice-cold saline and the heart arrested. Cannulae were inserted into the aorta and the superior and inferior venae cavae. The cannula in the superior vena cava was used for monitoring right atrial pressure.

The heart-lung preparation was perfused with a solution (total volume 25 ml) containing red blood cells collected from another rat as well as Krebs-Ringer bicarbonate buffer, with a hematocrit and pH of 25% and 7.4, respectively. The concentrations (mM) of the buffer

Address correspondence to: M. Kume
Received for publication on December 12, 1995; accepted on April 30, 1996

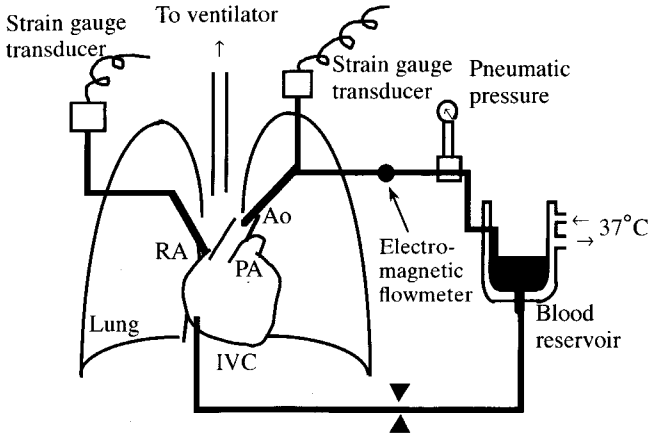

Fig. 1. Perfusate blood coming from the aorta (*Ao*) was passed through a pneumatic resistance, collected in a reservoir kept at 37°C, and then returned to the inferior vena cava (*IVC*). *RA*, right atrium; *PA*, pulmonary artery

constituents were: NaCl 127, KCl 5.1, $CaCl_2$ 2.2, KH_2PO_4 1.3, $MgSO_4$ 2.6, $NaHCO_3$ 15, glucose 5.5, and heparin. The blood pumped out from the heart was passed through a pneumatic resistance and was collected in a reservoir kept at 37°C and then returned to the inferior vena cava. In this model, no other organs except the heart and lung were perfused, cardiac output was determined by the inflow as long as the heart did not fail, and mean arterial pressure was regulated by the pneumatic resistance (Fig. 1).

After all the cannulae were inserted completely, the cooling heart was warmed with saline and kept at 37°C during the experiment. As soon as the heart had been warmed, it beat spontaneously. Pneumatic resistance and the inflow to the inferior vena cava from the reservoir were increased by degree, and all hearts were perfused initially with a cardiac output of 30 ml·min^{-1} and mean arterial pressure of 70 mmHg.

Heart rate was recorded with a bioelectric amplifier (AB-621G, Nihonkohden, Tokyo, Japan) and cardiac output was measured with an electromagnetic blood flow meter (MFV-1200, Nihonkohden). Arterial pressure and right atrial pressure were measured with transducers (TP101T and LPU-0.1A) and carrier amplifiers (AP-621G, Nihonkohden). The rates of left ventricular tension development (LV d*P*/d*t*) were calculated from aortic blood pressure obtained electronically [7].

Eight minutes after the start of perfusion, either amrinone 250 μg + saline 0.45 ml or amrinone 2500 μg was administered in the reservoir. As a result, the concentration of amrinone (Amcoral, Meiji Pharmaceutical, Tokyo, Japan) in the perfusate rose to 10 μg·ml^{-1} or 100 μg·ml^{-1}, respectively (total volume of the perfusate was 25 ml). Saline 0.5 ml was administered in the reservoir in the control group.

Ten minutes after the start of perfusion, all hearts were rendered globally ischemic for 8 min by clamping the venous return and reducing pneumatic resistance to zero. Subsequently, the preparations were reperfused for 10 min by regulating venous return and pneumatic resistance (Fig. 2). The recovery time was recorded when the cardiac output and the mean arterial pressure returned to preischemic values (30 ml·min^{-1} and 70 mmHg, respectively). At the end of the experiment, the hearts were frozen by liquid nitrogen and freeze-dried for 6 days. An aliquot supernatant was extracted with perchloric acid and centrifuged at 3000 rpm (r = 16.2 cm). Myocardial high-energy phosphates [ATP, adenosine diphosphate (ADP), and adenosine monophosphate (AMP)] were measured by high-performance liquid chromatography [8]. The lactate level was determined spectrophotometrically by standard techniques [9]. Another piece of freeze-dried sample was placed in 30% KOH and digested at 100°C. Tissue glycogen was extracted, hydrolyzed, and assayed as glucose equivalents [10].

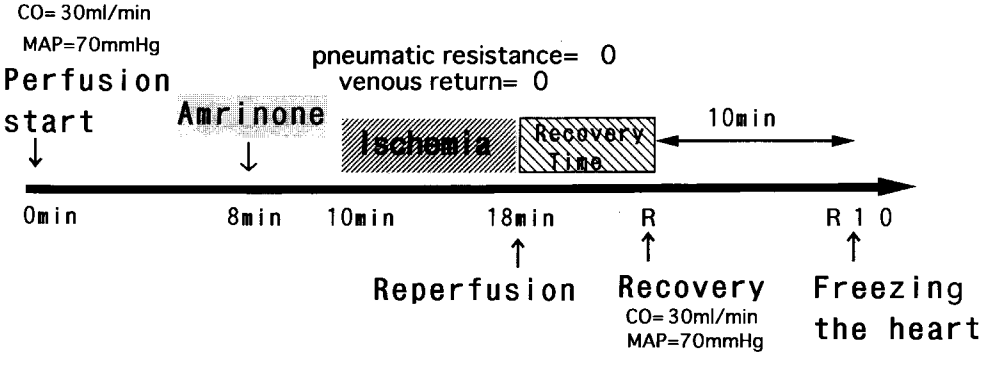

Fig. 2. Experimental protocol. Ten minutes after the start of perfusion, all hearts were rendered globally ischemic for 8 min by clamping the venous return and reducing the pneumatic resistance to zero. Subsequently, the preparations were reperfused for 10 min by regulating the venous return and the pneumatic resistance. *CO*, cardiac output; MAP, mean arterial pressure

The values were expressed as micromoles per gram of dry weight.

Hemodynamic data within groups were analyzed by two-way analysis of variance with repeated measures. Recovery time was analyzed by the Kruskal-Wallis test. The other data were analyzed by one-way analysis of variance followed by the Dunnett test for multiple comparisons. A probability of $P < 0.05$ was regarded as statistically significant. The data are given as mean ± SD.

Results

The ventricular rate and atrial rate in all groups decreased significantly during ischemia. The ventricular rate decreased more than the atrial rate during ischemia due to atrioventricular (AV) conduction block in all groups. However, there were no significant differences in ventricular rate and atrial rate among the groups, respectively (Fig. 3). Cardiac output at 10 min after recovery (R10) in the 10 μg·ml^{-1} and 100 μg·ml^{-1} groups

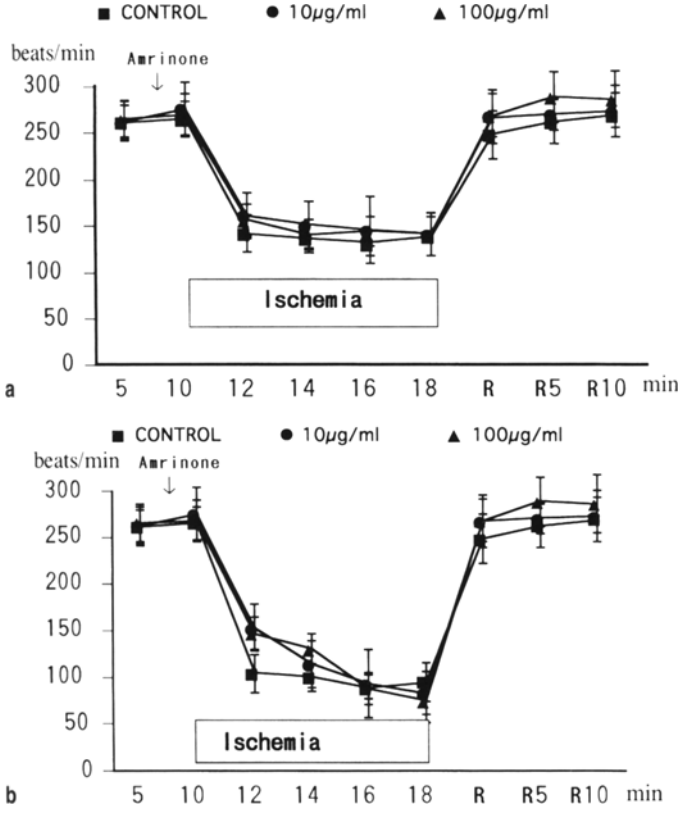

Fig. 3. Changes in atrial (**a**) and ventricular (**b**) rate after administration of amrinone and from the time of recovery from ischemia. Arial and ventricular rate in all groups decreased significantly during ischema. However, there were no significant differences in heart rate among the groups. Data are presented as means ± SD

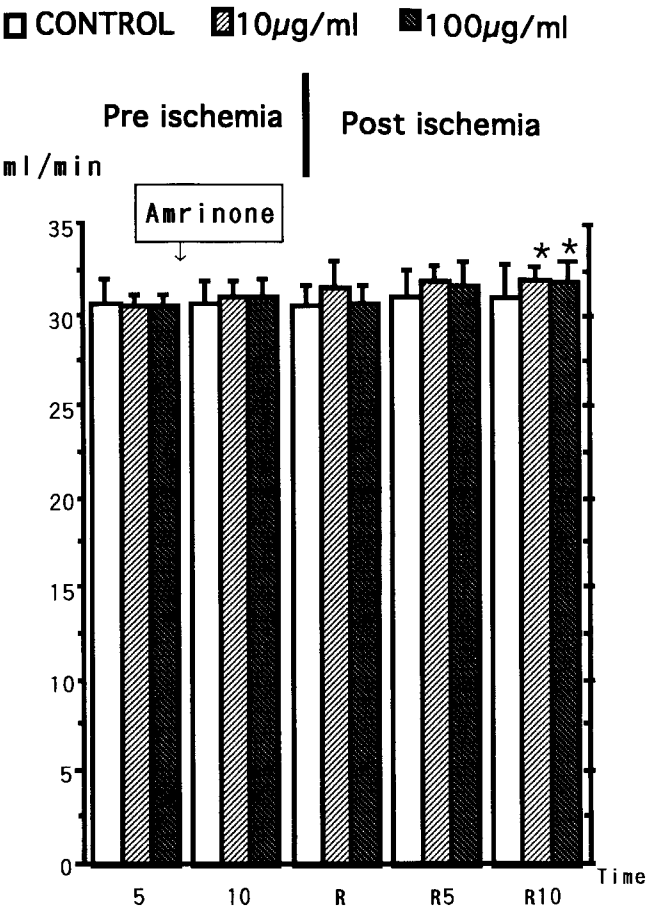

Fig. 4. Changes in cardiac output after administration of amrinone and from the time of recovery from ischemia. Data are presented as means ± SD. *$P < 0.05$ *vs* the values at 5 min

increased slightly when compared with those at 5 min after the start of the perfusion. However, there were no significant differences in cardiac output among the groups (Fig. 4). Left ventricular (LV) dP/dt_{max} was not significantly different among the groups (Fig. 5). There were also no significant differences in recovery time and right atrial pressure among the groups (Figs. 6,7). The myocardial ATP level in the 100 μg·ml^{-1} group was significantly higher than that in the control group. ADP and AMP levels in the 100 μg·ml^{-1} group were significantly lower than those in the control group (Fig. 8). Therefore, the energy charge (EC) in the 100 μg·ml^{-1} group was significantly higher than that in the control group (0.82 ± 0.04 *vs* 0.79 ± 0.02; $P < 0.05$). However, myocardial lactate, pyruvate, and glycogen levels were not significantly different among the groups (Fig. 9).

Discussion

In the present study, amrinone improved postischemic myocardial metabolism. It is reported that amrinone did

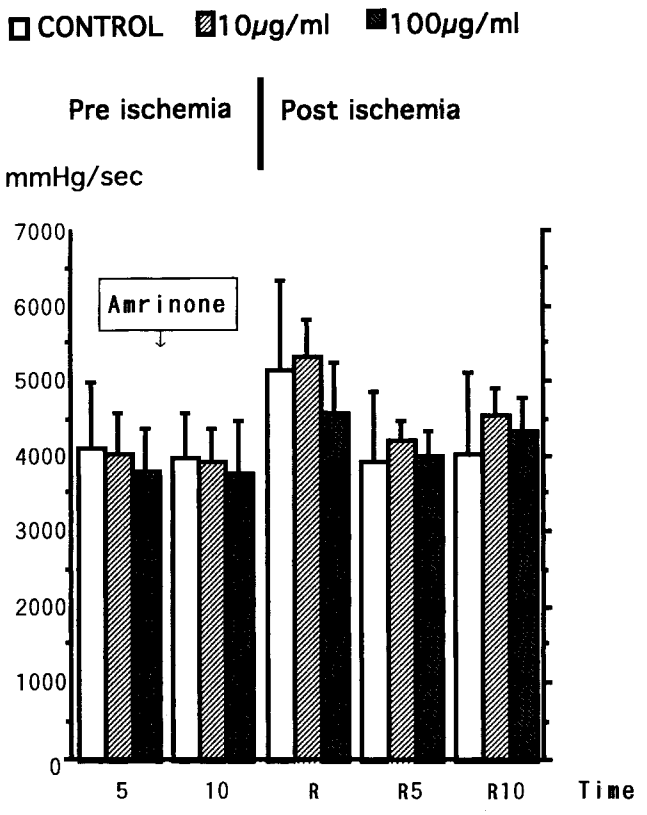

Fig. 5. Changes in left ventricular (LV) dP/dt_{max} after administration of amrinone and from the time of recovery from ischemia. Data are presented as means ± SD

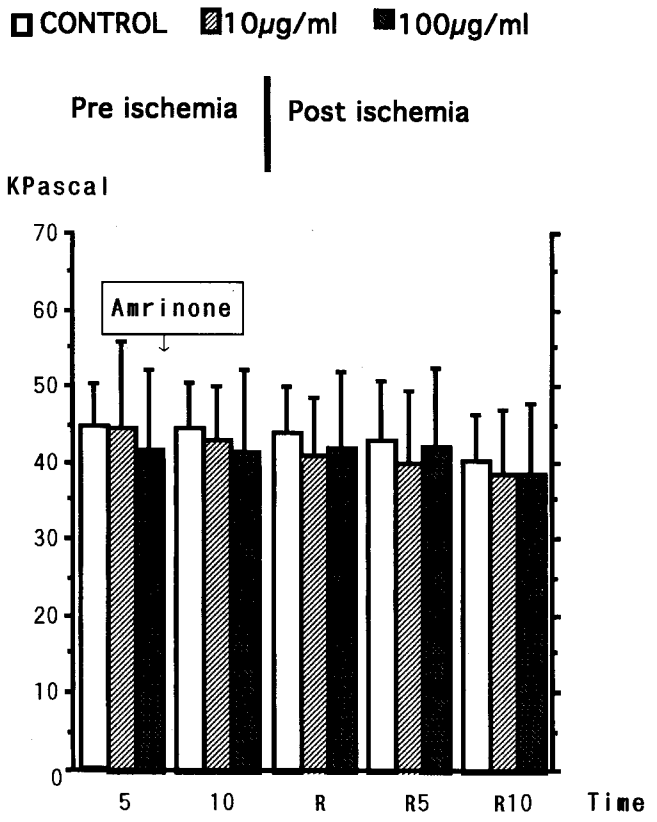

Fig. 6. Changes in right atrial pressure after administration of amrinone and from the time of recovery from ischemia. There were no significant differences in right atrial pressure among the groups. Data are presented as means ± SD

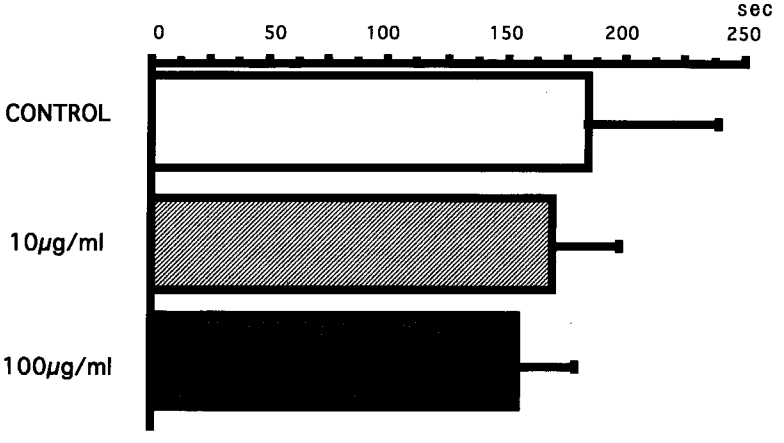

Fig. 7. Recovery time after ischemia. There were no significant differences in recovery time among the groups. Data are presented as means ± SD

not increase anatomic infarct size induced by coronary occlusion and reperfusion in anesthetized, open-chest dogs [11] and isolated rabbit hearts [12]. Another study showed that amrinone did not have a deleterious effect on myocardial metabolism [13]. For what reason did amrinone improve postischemic myocardial metabolism in this study?

In general, inotropic agents tend to increase myocardial oxygen consumption through an augmentation of myocardial contractility. In the setting of acute myocardial ischemia any possible beneficial effect of a positive inotropic agent on myocardial oxygen consumption will depend on the balance between augmentation in contractility and reduction in ventricular wall tension [14]. Jentzer et al. [15] reported that the net improvement in myocardial oxygen consumption during amrinone-treated left ventricular failure was caused by a reduction in ventricular wall tension, offsetting the increase in

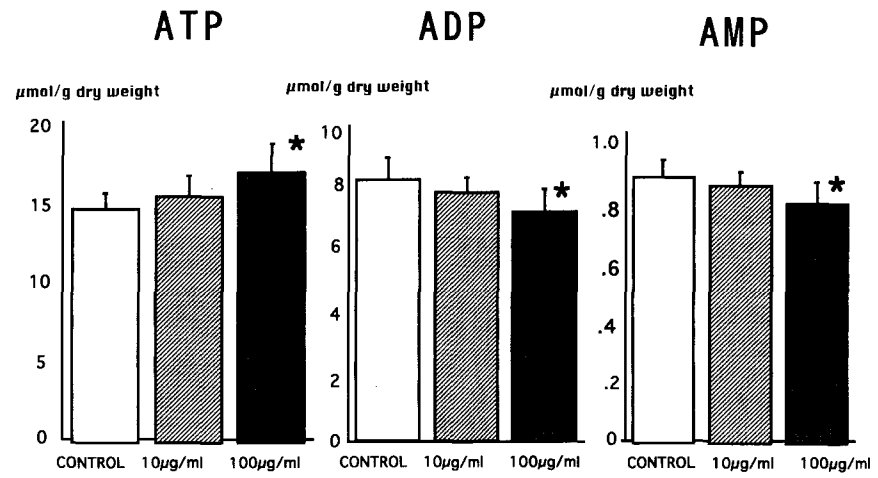

Fig. 8. Myocardial concentrations of ATP, adenosine diphosphate (*ADP*), and adenosine monophosphate (*AMP*). Data are presented as means ± SD. $*P < 0.05$ *vs* control

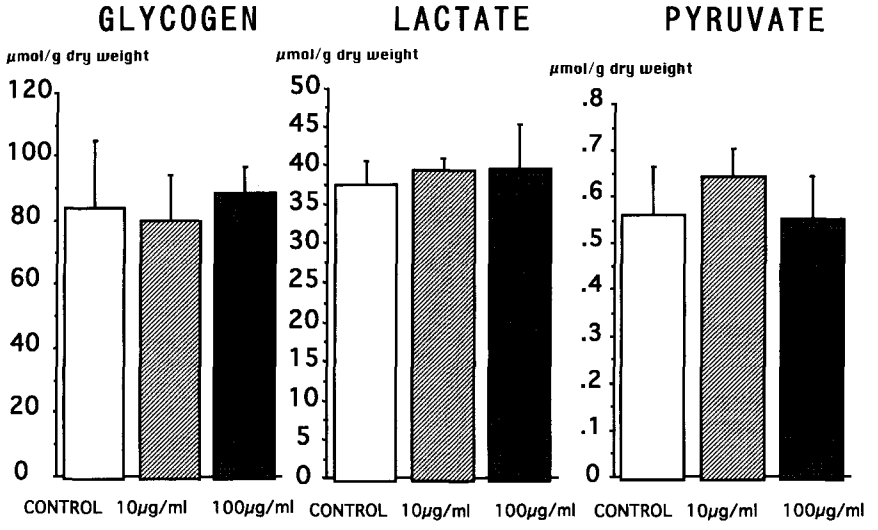

Fig. 9. Myocardial concentrations of glycogen, lactate, and pyruvate. Myocardial lactate, pyruvate, and glycogen levels were not significantly different among the groups. Data are presented as means ± SD

myocardial contractility due to amrinone. Ventricular wall tension is dependent on the pressure generated during systole and the left ventricular volume according to the Laplace relation. However, in our heart-lung preparation, preload and afterload were kept constant throughout the experiment. Moreover, right atrial pressure (RAP) and LV dP/dt_{max} were not significantly different among the groups. These results suggest that amrinone did not affect the ventricular wall tension in our model. Thus, the improvement of myocardial metabolism in our study seems not to have resulted from the reductions in wall tension and oxygen consumption.

It is well known that the myocardial ATP level after reperfusion is lower and ADP and AMP levels are higher than those in the normally perfused heart. These were the same with all groups in this study because ATP was about 27, ADP was about 5.5, and AMP was $0.41\,\mu mol \cdot g^{-1}$ in nonischemic hearts [6]. However, the $100\,\mu g \cdot ml^{-1}$ group tended to recover from ischemia more quickly than the control group, though there was no significant difference in recovery time among the groups. The sooner the recovery from ischemia, the more the ATP level was restored. This may have contributed to higher ATP and EC levels in the $100\,\mu g \cdot ml^{-1}$ group than those in the control group.

The most probable reason may be the coronary circulation. Rooney et al. [16] have observed that amrinone did not increase coronary flow to a level greater than the metabolic requirement of the heart and that amrinone was not a direct coronary vasodilator. However, other studies have reported that amrinone caused direct coronary vasodilation and increased myocardial oxygen supply in excess of the increase in myocardial oxygen demand [13,17]. Milrinone, a bipyridine-positive inotropic agent that is closely related to amrinone, increased coronary blood flow without significant changes in systemic hemodynamics in the conscious rat [18]. Although we did not measure coronary flow, amrinone might have increased coronary flow with direct coronary vasodilation which would have caused the im-

provement in the myocardial ATP and EC levels in the $100\,\mu g \cdot ml^{-1}$ group.

This study indicated that amrinone had a cardioprotective effect probably due to improving myocardial perfusion and oxygen supply to the myocardium. However, we cannot determine whether the beneficial effect of amrinone on the myocardial metabolism occurred during ischemia or postischemia or both. Sidi et al. [19] reported that administration of amrinone before coronary occlusion appeared to improve left ventricular performance and increase blood flow to the ischemic myocardium while not worsening regional metabolic effects during ischemia. In our heart-lung preparation, all hearts were rendered globally ischemic for 8 min by clamping the venous return and reducing pneumatic resistance to zero during the ischemic period. In this period all hearts were beating by themselves and we did not clamp outflow; thus, the coronary blood flow might not have been completely blocked during the ischemic period.

Usually amrinone causes a positive inotropic effect when it is administered to maintain a therapeutic plasma concentration of $1.5–2.0\,\mu g \cdot ml^{-1}$ [1–4]. In ischemia-damaged hearts, $100\,\mu g \cdot ml^{-1}$ amrinone had an apparent inotropic action, provoking significant increases in cardiac output (11%) and aortic flow (10%) in the isolated working rat heart [20]. However, in the present study we could not observe significantly positive inotropic effects of amrinone in the $10\,\mu g \cdot ml^{-1}$ group or even in the $100\,\mu g \cdot ml^{-1}$ group when compared with the control group. Onuaguluchi and Tanz [21] also reported that amrinone, $50–1000\,\mu g \cdot ml^{-1}$, produced a dose-dependent inotropic action on rabbit papillary muscle. It may be possible to observe the inotropic effects of amrinone if we administer a higher dose of amrinone than the one we used. Rude et al. [22] have shown that amrinone, when infused to produce positive inotropic effects, yielded greater acute myocardial ischemic injury as evidenced by augmentation of the epicardial ST segment elevation and intramyocardial carbon dioxide pressure in the normal canine heart during coronary artery occlusion. However, they administered a much higher dose of amrinone ($1–3\,mg \cdot kg^{-1}$ intravenous bolus followed by a constant infusion dose of $420\,\mu g \cdot kg^{-1} \cdot min^{-1}$ required to maintain the increased LV dP/dt) compared with the therapeutic dose.

The activity and distribution of phosphodiesterase differs from species to species, especially in the rat. Milrinone, which is closely related to amrinone, exerts significant regional vasodilating effects in the conscious rat model, being most enhanced in the coronary artery at a dosage that does not alter the central hemodynamics [18]. On the other hand, in the isolated, perfused working guinea pig heart, amrinone was a positive inotropic drug with vasodilating properties only at high doses [23]. Therefore, the results from animal studies have an only limited applicability to human beings.

In conclusion, $100\,\mu g \cdot ml^{-1}$ amrinone increased postischemic myocardial ATP and EC levels in the rat heart-lung preparation, which means that amrinone improved myocardial metabolism. Although the effects of amrinone on coronary artery and myocardial contractility differ from species to species, the above dose of amrinone may dilate the coronary artery at a level without any positive inotropic effect. Amrinone may be a useful drug for patients who have undergone open heart surgery using cardiopulmonary bypass, and patients with heart failure associated with acute myocardial infarction.

References

1. Bailey JM, Levy JH, Rogers G, Szlam F, Hug Jr CC (1991) Pharmacokinetics of amrinone during cardiac surgery. Anesthesiology 75:961–968
2. Royster RL, Butterworth JF, Prielipp RC, Zaloga GP, Lawless SG, Spray BJ, Kon ND, Wallenhaupt SL, Cordell AR (1993) Combined inotropic effects of amrinone and epinephrine after cardiopulmonary bypass in humans. Anesth Analg 77:662–672
3. Lathi KG (1992) Amrinone in patients undergoing cardiac surgery. Anesthesiology 77:215
4. Butterworth JF, Royster RL, Robertie PG, Zaloga GP, Prielipp RC, Dudas LM (1990) Hemodynamic effects of amrinone in patients recovering from aortocoronary bypass surgery. Anesth Analg 70:S45 (abstract)
5. Kashimoto S, Tsuji Y, Kumazawa T (1987) Effects of halothane and enflurane on myocardium during postischemic reperfusion in the rat. Acta Anesth Scand 31:44–47
6. Kashimoto S, Nakamura T, Nonaka A, Kume M, Oguchi T, Kumazawa T (1994) Effects of artificial blood (FC-43 emulsion) on myocardial energy metabolism in the rat heart-lung preparation. Br J Anaesth 73:380–383
7. Nonaka A, Kashimoto T, Nakamura T, Kumazawa T (1994) Effects of intravenous anaesthetics on function and metabolism in the isolated rat heart-lung preparation. Eur J Anaesth 11:213–219
8. Wynants J, Belle HV (1985) Single-run high-performance liquid chromatography of nucleotides, nucleosides, and major purine bases and its application to different tissue extracts. Analyt Biochem 144:258–266
9. Bergmeyer HU (1975) Neue Werte für die molaren Extinktions-Koeffizienten von NADPH zum Gebrauch im Routine-Laboratorium. Z Klin Chem Klin Biochem 13:507–508
10. Werner W, Rey H-G, Wielinger H (1970) Über die Eigenschaften eines neuen Chromogens für die Blutzuckerbestimmung nach der GOD/POD Methods. Z Analyt Chem 252:224–228
11. Cambell CA, Mehta PM, Wynne J, Kloner A (1987) The cardiotonic agent amrinone does not increase anatomic infarct size. J Cardiovasc Pharmacol 9:225–229
12. Rump AF, Blazincic B, Klaus W (1993) Effects of amrinone and milrinone on myocardial ischemia extent and infarct size in isolated rabbit hearts. Arzneimitt elforschung 43:1262–1266
13. Fujigaki T, Nakamura H, Fukui S, Miyao M, Haseba S, Gotoh Y (1989) Comparison of the effects of amrinone and dobutamine on hemodynamics and myocardial oxygen balance in dogs with experimental left ventricular failure. J Cardiothorac Anesth 3:433–440
14. Kirk ES, LeJemtel TH, Nelson GR, Sonnenblick EH (1978) Mechanisms of beneficial effects of vasodilators and inotropic

stimulation in the experimental failing ischemic heart. Am J Med 65:189–96
15. Jentzer JH, LeJemtel TH, Sonnenblick EH, Kirk ES (1981) Beneficial effect of amrinone on myocardial oxygen consumption during acute left ventricular failure in dogs. Am J Cardiol 48:75–83
16. Rooney RT, Stowe DF, Marijic J, Bosnjak ZJ, Kampine JP (1991) Amrinone reverses cardiac depression and augments coronary vasodilation with isoflurane in the isolated heart. Anesthesiology 74:559–567
17. Takeda K, Matsui K, Nakazawa M, Nakahara H, Imai H, Imai S (1986) Cardiotonic and coronary vasodilatatory effects of amrinone in the canine heart-lung preparation with a support dog as compared with those of dobutamine. Jpn J Pharmacol 40:381–388
18. Drexler H, Hoing S, Faude F, Wollschlager H, Just H (1987) Central and regional vascular hemodynamics following intravenous milrinone in the conscious rat: comparison with dobutamine. J Cardiovasc Pharmacol 9:563–569
19. Sidi A, Pool JM, Rush W (1993) Early administration of amrinone dose not impair regional metabolism of O_2 or lactate and, by improving myocardial performance, preserves myocardial blood flow in the ischemic canine heart. Anesth Analg 76:1201–1212
20. Zucchi R, Poddighe R, Mariani M, Ronca G (1990) Effect of amrinone in the working rat heart: influence of ischemic damage, adenosine and calcium. Drugs Exp Clin Res 16:187–195
21. Onuaguluchi G, Tanz RD (1981) Cardiac effects amrinone on rabbit papillary muscle and guinea pig Langendorff heart preparations. J Cardiovasc Pharmacol 3:1342–1355
22. Rude RT, Kloner RA, Maroko PR, Khuri S, Karaffa S, DeBoer LWV, Braunwald E (1980) Effects of amrinone on experimental acute myocardial ischaemic injury. Cardiovasc Res 14:419–427
23. Zannad F, Juillere Y, Royer RJ (1983) The effects of amrinone on cardiac function, oxygen consumption and lactate production of an isolated, perfused, working guinea-pig heart. Arch Internat Pharmacodyn Ther 263:264–271