ADENOSINE AND DOWN-REGULATION OF MYOCARDIAL OXYGEN DEMAND

DISSERTATION

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This investigation studied the physiological means by which myocardium can survive and function properly when oxygen supply is limited and can not initially match oxygen demand. The effects of isoproterenol (ISO) stimulations during low coronary perfusion pressure or hypoxemia on myocardial oxygen demand, work, and oxygen utilization efficiency were investigated in 39 anesthetized, open-chest dogs. The anterior descending coronary artery (LAD) was cannulated and perfused with normoxic arterial blood or with moderately hypoxic blood. Coronary perfusion pressure was decreased from 100 mmHg to 60 mmHg. During low coronary perfusion pressure, ISO-stimulated myocardium increased oxygen utilization efficiency and decreased oxygen demand when compared to normal perfusion pressure. During hypoxemia (O_2 content = 9.8 ± 0.2 ml O_2/dl), ISO-stimulated myocardium decreased myocardial work and decreased oxygen demand when compared to normoxemia. Exogenous adenosine also increased oxygen utilization efficiency and decreased oxygen demand in ISO-stimulated myocardium during normal perfusion pressure. Adenosine deaminase (ADA) decreased and erythro-9-(2-hydroxy-

3-nonyl)-adenine (EHNA) increased oxygen utilization efficiency of ISO-stimulated myocardium during low coronary perfusion pressure and during hypoxemia. Thus, ISO-stimulated myocardium can down-regulate its oxygen demand when oxygen supply is limited. Endogenous adenosine released from ISO-stimulated myocardium increases oxygen utilization efficiency and, thus, contributes to down-regulation of myocardial oxygen demand.

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CHAPTER I

INTRODUCTION

Coronary artery disease remains the most serious form of heart disease in the industrialized world. The most common clinical manifestation of coronary artery disease is myocardial ischemia which results from an imbalance between myocardial oxygen supply and demand. Under normal physiologic conditions, the myocardial demand for oxygen is readily balanced by the supply of oxygen. In fact, there is a significant reserve of oxygen supply (8). However, under certain pathologic states, the imbalance of oxygen supply and myocardial demand can be induced by 1) a decrease in oxygen supply caused, for example, by atherosclerotic obstructive coronary artery disease or hypoxia, and/or 2) an increase in oxygen demand caused, for example, by sympathetic stimulation.

In 1963, Berne (12) proposed that whenever the myocardial supply-to-demand ratio for oxygen is upset, more adenosine is formed, which then causes an adaptive increase in coronary blood flow and oxygen delivery. Since then, adenosine has been extensively investigated and proven to possess a wide range of physiological activities in the myocardium. Those include vasodilation (13), slowing of

heart rate (30) and atrioventricular conduction (9), inhibition of ß-adrenergic receptor-mediated responses (60), reduction of superoxide formation in activated neutrophils (60), attenuation of postischemic damage to endothelial cells (9), inhibition of platelet aggregation (9), and optimization of cardiac efficiency (31, 32). The production of adenosine by myocardium with imbalanced supply-to-demand ratio and its varied effects on the myocardium and coronary circulation have resulted in adenosine being considered as a "cardio-protective agent" (31). For instance, exogenous adenosine (2, 4, 9, 23, 37, 38, 39, 57) and mioflazine (85, 86), an adenosine transport inhibitor, have been shown to improve functional and/or metabolic recovery from myocardial ischemia. In addition, Liu et al. (47) observed that adenosine receptor blockade decreases the beneficial effects of preconditioning, i.e., a brief period of myocardial ischemia followed by reperfusion causes minimal injury in itself but makes the heart resistant to necrosis when exposed to a subsequent period of ischemia. They proposed that adenosine is responsible for this pre-conditioning protection from long-term ischemia (56). The mechanisms responsible for adenosine's beneficial effects on the ischemic myocardium are controversial. The proposed mechanisms include restoration of high-energy phosphates, improvement of myocardial perfusion, inhibition of platelet aggregation, neutrophil adherence, free radial radical formation, and endothelial

swelling (9).

If adenosine is indeed released from the myocardium due to a decrease of myocardial oxygen supply-to-demand ratio, and if we consider the imbalance of myocardial oxygen supply-to-demand ratio as an error signal of the feedback system, adenosine is released due to this error signal. Consequently, the error signal should be reduced by some actions of adenosine. There are two ways to increase the oxygen supply-to-demand ratio: 1) to increase oxygen supply, and/or 2) to decrease oxygen demand. Adenosine has been recognized as a vasodilator which can increase oxygen supply for a long time (25). However, the role of adenosine in decreasing myocardial oxygen demand remains unknown.

Oxygen Demand

The heart relies almost exclusively on aerobic metabolism for energy production under normoxic conditions. Fatty acids, the major myocardial substrates, are metabolized via ß-oxidation and the Krebs cycle. Consequently, ATP is produced by oxidative phosphorylation, which is dependent on the presence of oxygen in the myocardial mitochondria. Since almost all energy required for myocyte survival and myocardial performance is oxygen-dependent, oxygen demand can be defined as the quantity of oxygen needed by the myocardium to achieve a certain degree of cardiac work. Under normal physiologic conditions, the demand is easily met by the

supply of oxygen (8). In fact, the literature describes numerous studies in which myocardial oxygen consumption was used as an index of oxygen demand under the non-ischemic conditions (15). If oxygen supply cannot match oxygen demand, myocardial ischemia ensues.

A. Determinants of Myocardial Oxygen Demand.

It has been known for a long time that the metabolism of an arrested, quiescent heart is only a small fraction of that of a working heart. Whereas the oxygen demand of the noncontracting heart is approximately 2 ml/min/100 g, the oxygen demand of the working, beating heart ranges from 8 to 15 ml/min/100 g (15). Of this, the quantity of oxygen required for maintaining ionic electrochemical gradients in the resting heart approximates 0.5 percent of the total oxygen demand by the normal working heart (44). The three most important factors determining myocardial oxygen demand of the working heart are:

a. Heart Rate. An augmentation of rate elevates myocardial oxygen demand per minute by increasing the number of times tension is developed per unit of time, by increasing ion pumping, and by increasing contractility (14, 82). Thus, chronotropy has been suggested to be the most important contributor to the total oxygen demand (8). Tanaka et al. (82) recently reported that oxygen consumption per minute for a constant cardiac external work per minute increased

monotonically with increases in heart rate from 100 to 200 beats/min despite the consequent decreased stroke work. In their experiment, venous return and, thus, cardiac output was kept constant with a constant-flow pump, and mean aortic pressure was also kept constant by inflation or deflation of an intra-aortic balloon, thus, keeping cardiac external work per minute constant. This relationship was thought to be due to an increase in an oxygen demand component for the excitation-contraction coupling (82).

b. Contractility. The velocity of myocardial contraction, a reflection of the heart's contractile state, is another important determinant of oxygen demand. This was shown by Sonnenblick et al. (79) with very similar preparations as used in the experiments of Tanaka et al. (82). Sonnenblick et al. (79) found that administration of calcium, paired electrical stimulation (sustained postextra-systolic potentiation), and administration of norepinephrine exerted similar effects on the velocity of contraction and on oxygen consumption. Thus, contractility is influenced by the autonomic nervous system, heart rate, and blood calcium level, and anything that changes contractility is expected to change oxygen consumption.

c. Systolic Wall Tension. Myocardial wall stress is proportional to ventricular systolic pressure, ventricular radius, and inversely proportional to ventricular wall thickness according to the Laplace relation. Myocardial

preload influences ventricular radius, whereas afterload dictates the magnitude of systolic pressure generation (66) and, indirectly, preload if contractility is constant. Left ventricular hypertrophy in response to aortic pressure overload is therefore a natural compensatory mechanism to reduce systolic wall stress.

B. Estimation of the Myocardial Oxygen Demand.

Myocardial oxygen utilization efficiency is the ratio of the work performed by the myocardium to the amount of oxygen consumed by the myocardium. Braunwald (15) found that myocardial oxygen utilization efficiency varies widely depending on hemodynamic conditions, when work is calculated as pressure times stroke volume. Thus, Braunwald (15) pointed out that it is impossible to determine the myocardial oxygen demand from the work performed by the myocardium. This is because one must also know oxygen utilization efficiency to estimate oxygen demand from the work calculated as pressure times stroke volume. In further attempts to estimate myocardial oxygen demand, several new indexes of total cardiac work, such as the mean blood pressure times heart rate (26, 42), and the tension-time index (70), have then been developed to estimate oxygen demand in different physiological conditions, i.e., different preload, afterload, tension, contractility, and heart rate. A high degree of linear relationship between the indexes of

total cardiac work and oxygen consumption was observed (26, 42, 70) . Thus, when work is calculated as the mean blood pressure times heart rate or as the tension-time index, the oxygen utilization efficiency is independent of the myocardial preload, afterload, tension, contractility, and heart rate in most physiological conditions.

While these indexes accurately predict MVO_2 under most hemodynamic conditions, they underestimate MVO_2 during catecholamine administration. This apparently excessive MVO_2 has been called the oxygen wasting effect of catecholamines, and can be explained by either 1) reduction of oxygen utilization efficiency caused by catecholamines (oxygen wasting) or 2) failure of the indexes to correctly estimate cardiac work during catecholamines stimulation. The excellent study of Rooke and Feigl (66) clearly demonstrated that the latter is the case. They independently varied heart rate, systolic blood pressure, and stroke volume in closed-chest, anesthetized dogs, and reevaluated the ability of various indexes to estimate the oxygen consumption with or without infusion of inotropic agents, including catecholamines. They found high correlation coefficients between the investigated indexes and measured MVO_2 in absence of inotropic stimulation (correlation coefficients ≥ 0.746). With catecholamine stimulation, apparent oxygen wasting was observed with the tension-time, mean pressure-rate, triple

product, and estimated wall tension indexes, but not with new pressure-work or systolic pressure-rate indexes (see below) .

They defined a new index for cardiac work, named the pressure-work index as follows:

MVO_2 = K1 (SBP x HR)

+ K2 x [(0.8 SBP + 0.2 DBP) x HR x SV / BW]

+ 1.43 ml O_2/min/100 g

where MVO_2 = left ventricular myocardial oxygen consumption (ml O_2/min/100 g), SBP = systolic blood pressure in mmHg, DBP = diastolic blood pressure in mmHg, HR = heart rate in beats/min, SV = stroke volume in ml, BW = body weight in kg, K1 = 4.08 x 10^{-4} ml O_2/(mmHg•100 g), and K2 = 3.25 x 10^{-4} (ml O_2/(mmHg•100 g))•(Kg/ml). If cardiac output cannot be measured or estimated, they found the systolic pressure-rate product with the following constants to be the most useful index of left ventricular oxygen consumption:

MVO_2 = K (SBP x HR) + 1.43 ml O_2/min/100 g

where MVO_2 in O_2/min/100 g and K = 7.20 x 10^{-4} ml O_2/(mmHg•100 g).

The correlation coefficients between the pressure-work index and oxygen consumption were 0.985 and 0.944 without and with inotropic stimulations, respectively. Since Rooke and Feigl (66) found that oxygen utilization efficiency was constant despite catecholamine stimulations, they concluded

that catecholamine-induced oxygen wasting does not exist.

Oxygen Supply

The oxygen supply to the myocardium is determined by the magnitude of coronary flow and the oxygen-carrying capacity of the arterial blood. The latter can be compromised by anemia, hypoxemia, and carbon monoxide poisoning. Coronary blood flow, the most important determinant of oxygen supply in most clinical situations, is the result of the pressure gradient across the coronary vascular bed divided by the coronary vascular resistance. The major determinants of coronary blood flow are as follows:

a. Aortic Pressure. This pressure provides the driving force to propel blood through the coronary circulation. The heart's ability to generate aortic pressure depends on an adequate coronary blood flow. In addition, the aortic pressure represents the afterload for the left ventricle, and a change in aortic pressure will also result in a change in myocardial oxygen consumption (26).

b. Myocardial Extravascular Compression. The ventricular myocardium generates sufficient extravascular pressure with each beat to nearly stop left coronary inflow during systole, so most left coronary inflow occurs during diastole (25). If the intramyocardial tissue pressure during diastole is high secondary to an elevated intraventricular cavity pressure, flow may be restricted, particularly in the

subendocardial layers of the heart (25).

c. Myocardium Metabolism. Cardiac metabolism is influenced by heart rate, preload, afterload, and contractility as described above. Coronary vascular resistance is inversely related by a metabolically-linked control system to the rate of cardiac metabolism (60).

d. Neural Control. Coronary vessels are innervated by both the parasympathetic and sympathetic divisions of the autonomic nervous system. Parasympathetic activation causes coronary vasodilation, whereas the direct effect of sympathetic activation causes coronary vasoconstriction (25).

All of the four factors are interrelated and act together to determine coronary blood flow. However, cardiac metabolism probably is the most potent determinant under the physiological conditions (25). Possible mediators between coronary blood flow and cardiac metabolism include O_2, K^+, H^+, CO_2 , EDRF, prostaglandin and adenosine (60). Among them, adenosine has been a favored candidate as the "signal transmitter" from myocardial cells to coronary vascular smooth cells.

Adenosine

A. Adenosine Metabolism.

Adenosine is the product of the enzymatic hydrolysis of either of two substrates, S-adenosylhomocysteine (SAH) or

AMP. The hydrolases that catalyze these two reactions are S-adenosylhomocysteine hydrolase, and 5'-nucleotidase, respectively.

SAH is an adenosine-binding protein (35) that accounts for the sizeable intracellular adenosine compartment found in most tissue (84). In the in situ dog heart, for example, the intracellular compartment appears to account for over 90% of the total adenosine pool (59). However, the possible physiological role of SAH-bound adenosine is obscure since the capacity to generate adenosine via SAH breakdown is very limited (48). In addition, there seems to be no relationship between the catalytic activity of SAH hydrolase and the cellular energetic state, i.e., the ATP phosphorylation potential (60). Thus, the SAH pathway probably has no role in the metabolic regulation of blood flow.

The primary mechanism for the production of adenosine in myocardium is the hydrolysis of AMP by a 5'-nucleotidase in the cytosol (76, 83). This enzyme hydrolyzes all nucleotide 5'-monophosphates, but its catalytic efficiency, i.e., maximum velocity of enzyme reaction divided by its Michaelis constant (Vmax/Km), is highest for AMP. AMP is formed by myokinase, which converts 2 moles of ADP into 1 mole of AMP and 1 mole of ATP. The metabolic pathway that generates adenosine from ATP is shown in Fig. 1 (60). As shown in Fig. 1, AMP, the immediate precursor of adenosine, is degraded to adenosine by the reaction of 5'- nucleotidase. AMP can also

be degraded into IMP, and then to INO by the reaction of AMP deaminase. Thus, these two enzymes, AMP deaminase and 5'-nucleotidase, compete for AMP.

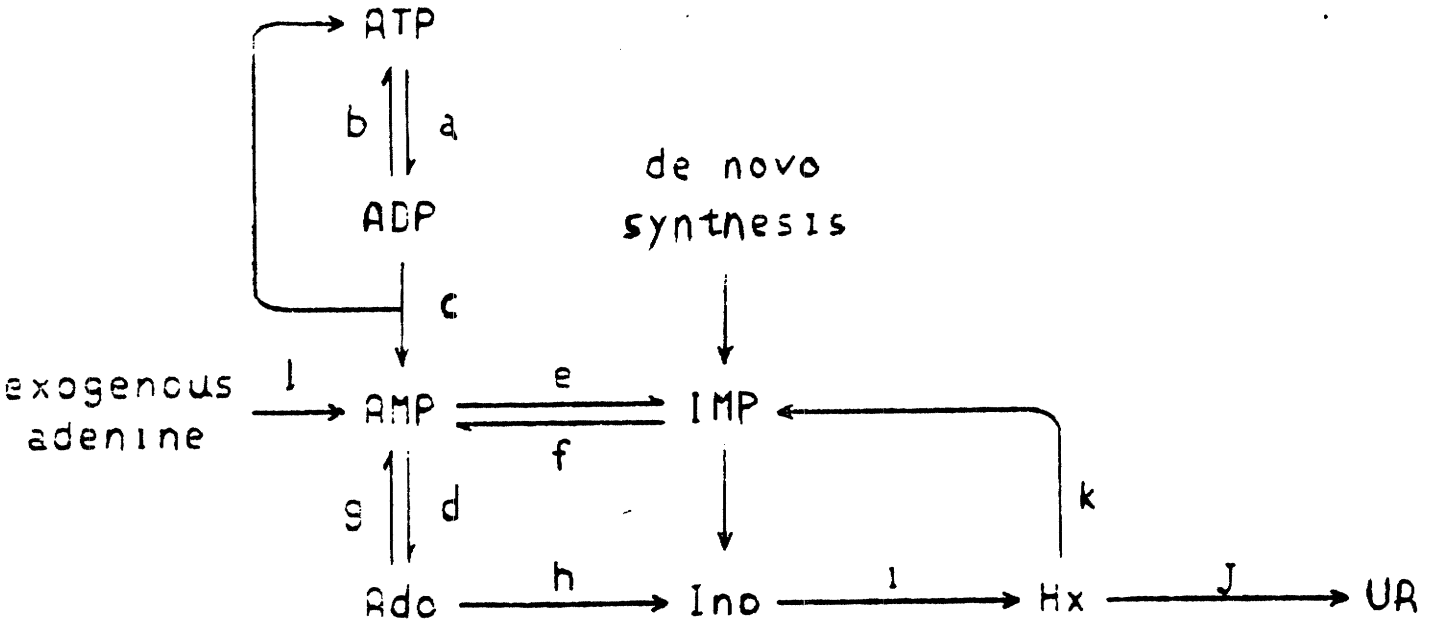

Figure 1. Purine metabolism. Ado, adenosine; Hx, hypoxanthine; Ino, inosine; UA, uric acid, a, ATP-consuming reactions; b, oxidative phosphorylation; c, myokinase; d, 5'-nucleotidase; e, AMP deaminase; f, adenylosuccinate synthase and lyase; g, adenosine kinase; h, adenosine deaminase; i, purine nucleoside phosphorylase; j, xanthine dehydrogenase; k, guanine phosphoribosyl transferase; and l, adenine phosphoribosyl transferase.

The two pathways of AMP catabolism converge on inosine, formed either by the deamination of adenosine or through the dephosphorylation of IMP by the same 5'-nucleotidase that generates adenosine. Which pathway predominates depends on the cell type and the cytosolic ATP phosphorylation potential (60). In oxygenated myocytes in culture (27, 67) as well as in the beating heart exposed to reversible ischemia or hypoxia (36, 39, 81), adenosine is the major metabolite of AMP, whereas IMP is the major metabolite of AMP in the skeletal muscle, blood leukocytes, and liver (60). Furthermore, it has been reported that ATP phosphorylation potential of heart and brain is higher than the ATP phosphorylation potential of skeletal muscle, liver, and red blood cells (17, 88).

B. Adenosine and Energy Metabolism.

In accordance with the linkage between the ATP and AMP shown in Fig. 1, an increase in free ADP concentration in the cytosol ($[ADP]_f$), whether induced by an increase in ATP utilization or by a decrease in the resynthesis of the ATP from ADP, will increase adenosine production. As a result, the release of adenosine plus inosine, formed from degradation of adenosine, should be proportional to cytosolic free AMP ($[AMP]_f$). $[AMP]_f$ can be calculated from the ATP potential, pH, and the equilibrium constant (Keq) of the

myokinase reaction (17). Furthermore, the stoichiometry of the myokinase reaction and the direct relationship between ATP utilization and $[ADP]_f$ predict that there will be a close relationship between ATP utilization and $[AMP]_f$. Since adenosine production is proportional to $[AMP]_f$, this approach predicts that adenosine release is proportional to ATP utilization and, thus, to MVO_2. In fact, Bunger and Soboll (17) showed a proportional relationship among MVO_2, $[AMP]_f$, and adenosine production in the isolated guinea pig heart.

However, Headrick and Willis (33) observed that adenosine release was approximately fourfold greater in glucose-perfused, isolated rat hearts perfused at 12 ml/min/g than in similar hearts perfused at 20 ml/min/g, although MVO_2 was the same in both cases. Since they found adenosine release independent of MVO_2, they concluded that MVO_2 is not a consistent index of adenosine formation. Furthermore, similar findings were reported by Bardenheuer and Shrader (7). Consequently, Headrick and Willis (33) and Bardenheuer and Shrader (7) proposed that the oxygen supply to demand ratio and not MVO_2 is the major determinant of adenosine release, as described originally by Berne (12) in 1963.

Rationale for this Investigation

As mentioned above, the error signal of the feedback

system, i.e. an imbalanced ratio of myocardial oxygen supply and oxygen demand, can be improved by 1) increasing oxygen supply, and/or 2) decreasing oxygen demand. However, when the reserve of oxygen supply is exhausted by a pathological limitation of the oxygen supply, such as atherosclerotic obstructive coronary artery disease or pulmonary disease, oxygen supply cannot be increased further. Consequently, if an error signal of the feedback system is induced by sympathetic stimulation, it can only be reduced by decreasing myocardial oxygen demand. Myocardial oxygen demand can be decreased by either decreasing myocardial work and/or increasing oxygen utilization efficiency.

In fact, Kammermeier et al. (41) recently observed that there was a pronounced negative force-frequency relationship in isolated isometrically working rat hearts during perfusion with hypoxic saline perfusion. In their study, the hearts were perfused with an aqueous buffer, gassed with 5% CO_2 and 95, 45, 30, or 20% O_2 and complementary N_2 to induce four different degrees of hypoxia. Heart rates were changed between 80 and 400/min by electrical stimulation or by the administration of a bradycardia-inducing agent. They demonstrated that when heart rate was increased, contractile force of myocardium decreased in all four different degrees of hypoxia. The calculated energetic state (ATP phosphorylation potential) was decreased as the degree of

hypoxia was increased, and at each degree of hypoxia, the calculated energetic state was constant at the respective degree of hypoxia even if contractile force and beating rate were varied by factors of 4 to 5. This negative force-frequency relationship with maintenance of constant energetic state during hypoxia is an example of myocardial down-regulation of oxygen demand while oxygen supply was limited by hypoxia. However, oxygen utilization efficiency was not calculated, and there was no information about whether those hearts had suffered ischemia. In a related study, Headrick et al. (31) did calculate myocardial oxygen utilization efficiency. After attempting to degrade endogenous adenosine with ADA and to block adenosine receptors with 8 - phenyltheophylline, they reported that endogenous adenosine antagonized the positive inotropic effect of isoproterenol and increased oxygen utilization efficiency in isovolumic, perfused rat hearts stimulated with isoproterenol. Surprisingly, they did not report coronary blood flow, nor did they measure lactate extraction. Therefore, we cannot know the oxygen supply-to-demand ratio of those hearts, or whether those hearts were ischemic during infusions of isoproterenol. Furthermore, both the studies of Kammermeier et al. (41) and Headrick et al. (31) were performed in the isolated heart perfused with non-blood solution. In <u>in vivo</u> conditions, Seitelberger et al. (77) found that there was no antagonistic effect of adenosine on isoproterenol induced

positive inotropy and chronotropy. Therefore, the roles of adenosine in the myocardial oxygen down-regulation in the intact heart preparation is actually unknown.

To examine the myocardial oxygen down-regulation and the role of adenosine in the myocardial oxygen down-regulation, the following hypotheses are proposed.

Hypothesis I: With elevated myocardial work and limited oxygen supply, myocardial oxygen demand can be down-regulated.

If hypothesis I is true, when the myocardium is stimulated by the positive inotropic agent under limited oxygen supply, the elevation of myocardial oxygen demand caused by positive inotropic agent should be less than the normal heart with the same degree of positive inotropic stimulation.

Hypothesis II: With elevated myocardial work and limited oxygen supply, adenosine will decrease oxygen demand of myocardium.

If hypothesis II is true, when myocardial work is elevated and oxygen supply is limited, an increase of myocardial adenosine caused by infusing ADA inhibitor or exogenous ADO should induce a lower MVO_2 (oxygen demand) than in the normal heart. Conversely, a decrease of myocardial adenosine caused by exogenous ADA infusion should induce higher oxygen demand than in the normal heart. However,

since the higher oxygen demand cannot be matched by the limitation of oxygen supply, we will expect a decrease in myocardial work or evidence of myocardial ischemia.

Hypothesis III: With elevated myocardial work and limited oxygen supply, the decrease of oxygen demand caused by adenosine is achieved by increasing myocardial oxygen utilization efficiency and not by decreasing myocardial work.

If hypothesis III is true, an increase of myocardial adenosine caused by infusing ADA inhibitor or exogenous ADO should not decrease myocardial work, and ADA infusion should not increase myocardial work.

CHAPTER II

MATERIALS AND METHODS

Animal Model

The model of this study is regional, controlled coronary perfusion in the *in situ*, working canine heart. This model has several advantages for investigating coronary and myocardial responses to perturbation of oxygen supply/demand parameters. We can control regional coronary arterial blood flow and its oxygen content, the variables which determine oxygen supply. We can also control heart rate, afterload, and contractility, the primary determinants of myocardial oxygen demand. We can change these parameters singularly or in combination to examine interactions between these parameters of oxygen supply and demand, we can also measure local arteriovenous differences in oxygen and other substances, regional contractile function, regional blood flow and its transmural distribution, and regional electrograms. Since only approximately 25% of canine left ventricular free wall is perfused by the LAD (89), we can severely perturb oxygen supply/demand relationships in its perfusion territory without significantly affecting left ventricular function and arterial blood pressure.

Experimental Design

In this study, we used adenosine deaminase (ADA) to decrease myocardial adenosine, erythro-9-(2-hydroxy-3-nonyl) adenine (EHNA), an ADA inhibitor, to increase myocardial adenosine, and exogenous adenosine to mimic endogenous adenosine. We infused isoproterenol (ISO) into the LAD artery to elevate oxygen demand of LAD-perfused myocardium, and we used flow reduction and hypoxemic blood perfusion to depress the reserve of oxygen supply to the LAD-perfused myocardium.

Pharmacological Agents

a. Adenosine deaminase (ADA). ADA has been used to destruct endogenous adenosine for a long time (69). Using labeled ADA, Saito et al. (69) reported that after infusing ADA for 5-10 min at a dose of 5 units/min/kg, the adenosine deaminase in canine myocardium interstitium reaches 1-13 units/ml. The average interstitial ADA concentration, 3 units/ml, the unit defined at 25°C, is equivalent to catalytic activity of 6 units/ml at 37°C. One unit per milliliter of ADA at 10^{-3} M ADO deaminates the nucleoside at a rate of 1020 X 10^{-9} mole/sec/ml. At an ADO concentration of 10^{-7} M, the rate would be 4.8 X 10^{-9} mole/min/ml (68). Given an adenosine production rate of 3-6 X 10^{-9} mole/min/g myocardium in ischemic myocardium (58), this enzyme concentration of 6 units/ml is sufficient to maintain interstitial adenosine

concentration of ischemic myocardium at the levels of 0.1-0.2 μM thought to be obtained under basal conditions. Thus, intracoronary infusion of ADA for 10 min at a dose of 5 units/min/kg should prevent any rise of interstitial adenosine during ISO infusion. In this investigation, ADA (type VIII, Sigma) was diluted in 20 ml of 0.2 mM sodium phosphate buffer in 120 mM sodium chloride to yield a concentration of 100 U/ml. The solution was neutralized to pH 7.40 by adding a few drops of 0.1 N sodium hydroxide, and the neutralized solution was passed through a 0.45-μM filter before use.

b. Erythro-9-(2-hydroxy-3-nonyl)-adenine·HCL (EHNA).

EHNA is an adenosine deaminase inhibitor, and, thus, blocks the conversion of adenosine to inosine. Achterberg et al. (2) reported that EHNA at 50 μmole/l inhibited the deamination of 5 μmole/l adenosine by more than 90%. In the open-chest canine model, infusing EHNA at a dose of 4.74 nmole/min into the LAD artery inhibited endogenous adenosine catabolism (46). In addition, EHNA improved recovery of myocardium after ischemia (46, 92). Thus, intracoronary infusion of EHNA at the rate of 5 nmole/min should decrease adenosine catabolism and increase endogenous adenosine during ISO or under conditions of those experiments. In this investigation, EHNA (Sigma, St. Louis, MO) was dissolved in normal saline to yield a concentration of 1.24×10^{-5} M. The EHNA solution was passed through a 0.45-μM filter before use.

c. Isoproterenol (ISO). ISO is a well-known ß-receptor agonist. ISO stimulates ß-adrenergic receptors on the myocyte, and increases myocardial contractility and myocardial work. Intracoronary infusion of ISO at a dose of 0.5 µg/min increased myocardial contractility, myocardial work, and oxygen consumption to about 200% of control value under normoxic condition in this study. Isoproterenol (1 mg/5 ml), (Elkins-Sinn, Inc., Cherry Hill, NJ), was diluted by normal saline to yield a concentration of 5 µg/ml.

In addition, the following pharmacological drugs were used in this investigation: surital (thiamylal sodium, 25 mg/ml), anesthesia (Park-Davis, Morris, NY); alpha-chloralose (130 mg/ml), anesthesia, (Nutritional Biochemical, Cheveland, OH); heparin (5000 U/ml), anticoagulant (Elkins-Sinn Inc., Cherry Hill, NJ); sodium bicarbonate (75 mg/ml), systemic alkalizer (Fisher Chemical Company, Fair Lawn, NJ). Alpha-chloralose and surital were dissolved in distilled water. Sodium bicarbonate was dissolved in normal saline. Adenosine (Sigma, St. Louis, MO) was dissolved in normal saline to yield a final concentration of 500 µg/ml.

Experimental Preparation

Experiments were performed on mongrel dogs of either sex weighing 15 to 35 kg. The dogs were anesthetized with thiamylal sodium (20 mg/kg i.v.) followed by alpha-chloralose (80 mg/kg i.v., plus supplements), and were ventilated via

tracheotomy with room air by a Harvard Apparatus 606 respirator. Positive end-expiratory pressure was held at 2 cm H_2O to prevent atelectasis. Vinyl catheters were inserted into the thoracic aorta via the left carotid artery to measure mean aortic blood pressure (MAP) and into the left femoral vein to administer supplementary alpha-chloralose and fluids. Blood from a donor dog was infused via the left femoral vein as required to maintain MAP around 100 mmHg. The right femoral artery and vein were catheterized to supply blood for coronary artery perfusion (see below). Arterial blood was frequently sampled and analyzed for PO_2, PCO_2, and pH; ventilation was adjusted to keep these parameters within physiological limits. $NaHCO_3$ was injected as needed to maintain normal arterial pH in the presence of normal PCO_2.

The heart was exposed through a left thoracotomy in the fifth intercostal space. Left intraventricular pressure (LVP) was measured with a Konigsberg pressure transducer inserted through the left atrial appendage and advanced across the mitral valve. Heart rate and left ventricular dP/dt were obtained from the LVP signal with a cardiotachometer and a differentiator, respectively. Piezoelectric crystals for measuring segment lengths of left anterior descending (LAD)-perfused myocardium were placed approximately 1 cm apart in pairs in the mid-myocardial layer, near the second diagonal branch of the LAD. The

crystals were oriented perpendicular to the axis of the heart (base to apex), since the myocardial fibers in the mid-myocardial layer are also perpendicular to the axis (80). Signals from the piezoelectric crystals were processed by an Ultrasonic Dimension System (Schuessler & Associates, model 401) and monitored with a Tektronix 2215A oscilloscope. Segment lengths measured at the beginning of the positive deflection of the dP/dt record were considered to reflect end-diastolic length (EDL), and those measured at the beginning of the negative deflection of the dP/dt record were considered to reflect end-systolic length (ESL). Percent shortening of myocardial segments (S%) was computed as [(EDL - ESL) / EDL] x 100.

The LAD was isolated distal to its first major diagonal branch. The anterior interventricular vein was also isolated at this location. After heparinization (500 U/kg i.v.), the anterior interventricular vein was cannulated with PE-50 tubing. Coronary venous blood drained freely into a beaker and was reinfused intermittently via the left femoral vein. The LAD was cannulated with a stainless steel cannula (3.0 mm OD, 2.2 mm ID) connected to a perfusion system, as used by Murakami et. al. (55). A schematic diagram of the perfusion system used in these experiments is shown in Fig. 2. The LAD was perfused with either normoxic or hypoxic blood from pressurized reservoirs. The normoxic blood reservoir was

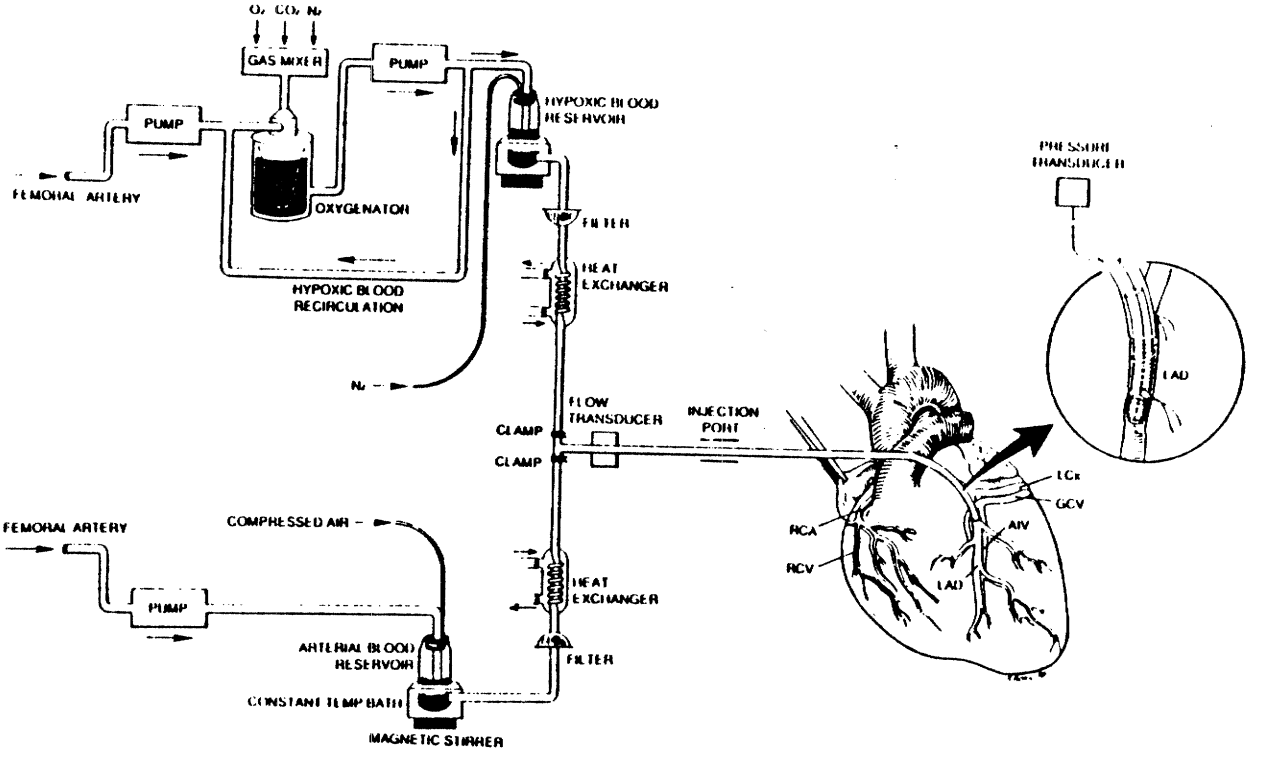

Figure 2. Schematic diagram of the system to perfuse the LAD with normoxic or hypoxemic blood. LAD, left anterior descending coronary artery; LCx, left circumflex coronary artery; GCV, great cardiac vein; AIV, anterior interventricular vein; RCA, right coronary artery; RCV, right coronary vein.

supplied with blood pumped from the right femoral artery. The hypoxic blood reservoir was supplied with blood pumped from the right femoral vein and passed through a Spiraflo infant oxygenator ventilated with O_2, CO_2, and N_2 to produce blood of reduced oxygen content with normal PCO_2.

LAD perfusion pressure (PP) was measured through a PE-50 fluid-filled catheter advanced to the orifice of the LAD cannula. PP and MAP were measured with Statham P23XL pressure transducers (Gould Inc.). LAD blood flow (CBF) was measured with an electromagnetic flowmeter (Carolina Medical Electronics, FM 501) and an in-line flow transducer (Carolina Medical Electronics, EP610). CBF, pressures, dP/dt, segment length, and heart rate were recorded by a SensorMedics R611 eight-channel polygraph.

Coronary vascular resistance (CVR) was calculated as coronary perfusion pressure minus zero flow pressure divided by CBF, as described by Bellamy (10). Zero flow pressure was assumed to equal 20 mmHg in the present study, since the zero flow pressure during reactive hyperemia and full vasodilation are about 20 mmHg. However, one should be aware that coronary vascular capacitance, vascular tone, and extravascular compression can all influence zero flow pressure (10). In fact, Eng et al. (24) have demonstrated that coronary capacitive effects and resistance changes during diastole severely limit the interpretation of

relationship between perfusion pressure and coronary blood flow.

Coronary arterial and venous blood samples were collected anaerobically. PO_2, PCO_2, and pH of these samples were measured with a Corning 175 Automatic pH/Blood Gas System, oxygen content was measured with an Instrumentation Laboratory 282 CO-Oximeter, and plasma lactate concentration (mg/dL) was measured with a Yellow Springs Instrument 2300 STAT L-lactate analyzer. Oxygen consumption of the LAD-perfused myocardium (MVO_2) was computed from CBF times the arteriovenous difference in oxygen content. Lactate extraction (La%) was calculated as $([Lactate]_{arterial} - [Lactate]_{venous})/[Lactate]_{arterial} \times 100$.

At the termination of the experiments, India ink was injected into the coronary perfusion line to delineate the LAD perfusion territory. The dyed tissue was carefully excised and weighed, so that CBF and MVO_2 could be normalized per gram tissue mass.

Evaluation of Myocardial Work, Oxygen Demand, and Oxygen Utilization Efficiency

The three most important factors determining myocardial work are heart rate, contractility, and systolic wall tension (see Introduction). In this experiment model (see above), heart rate, arterial pressure, and left ventricular systolic

pressure were relatively constant throughout the protocols, except for Group IIc (see below). Since the major determinants of the pressure-work index, heart rate and systolic pressure, used by Rooke and Feigle (66) for estimating cardiac work of the whole heart, are relatively constant throughout the protocols, this pressure-work index is not sensitive enough to estimate the regional myocardial work. Unfortunately, there is no other satisfactory myocardial work index that has been described for the regional myocardium in the literature. However, given relatively constant heart rate and systolic pressure, only contractility remains as a major determinant for estimating the changes of myocardial work in our animal model. Thus, it is reasonable to use dP/dt, a global contractility index, or S%, a local contractility index, combining with the product of heart rate and left ventricular developed pressure (LVP) to estimate the changes of myocardial work in this investigation.

Work1 = HR X LVP X dP/dt

Work2 = HR X LVP X S%

Figures 3 and 4 show how these indexes of myocardial work responded to four different doses of ISO. As shown in these two figures, there are satisfactory dose-response relationships between calculated myocardial work indexes and ISO doses. The ISO dose used in all following protocols in this investigation was 0.025 μg/min/kg.

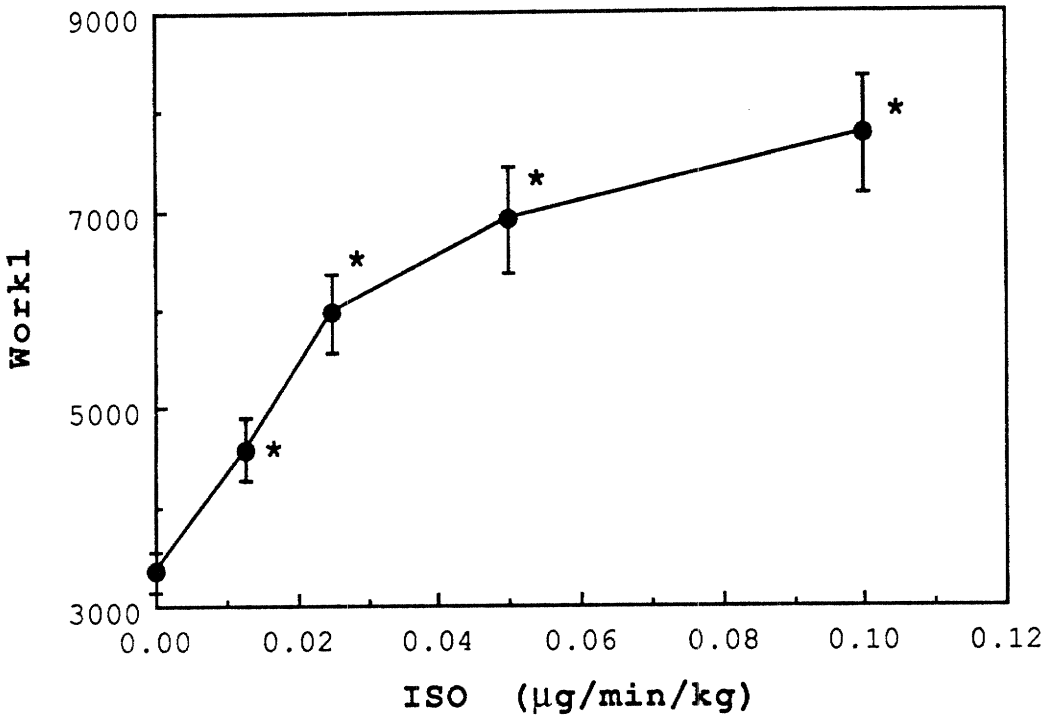

Figure 3. Effect of different doses of intracoronary isoproterenol (ISO) infusions on estimated myocardial work. Myocardial work was estimated as Work1 = HR X LVP X dP/dt. n = 6. Unit of Work1: $10^4 \cdot mmHg^2/sec/min$. *: different from next lower value at $P < 0.05$.

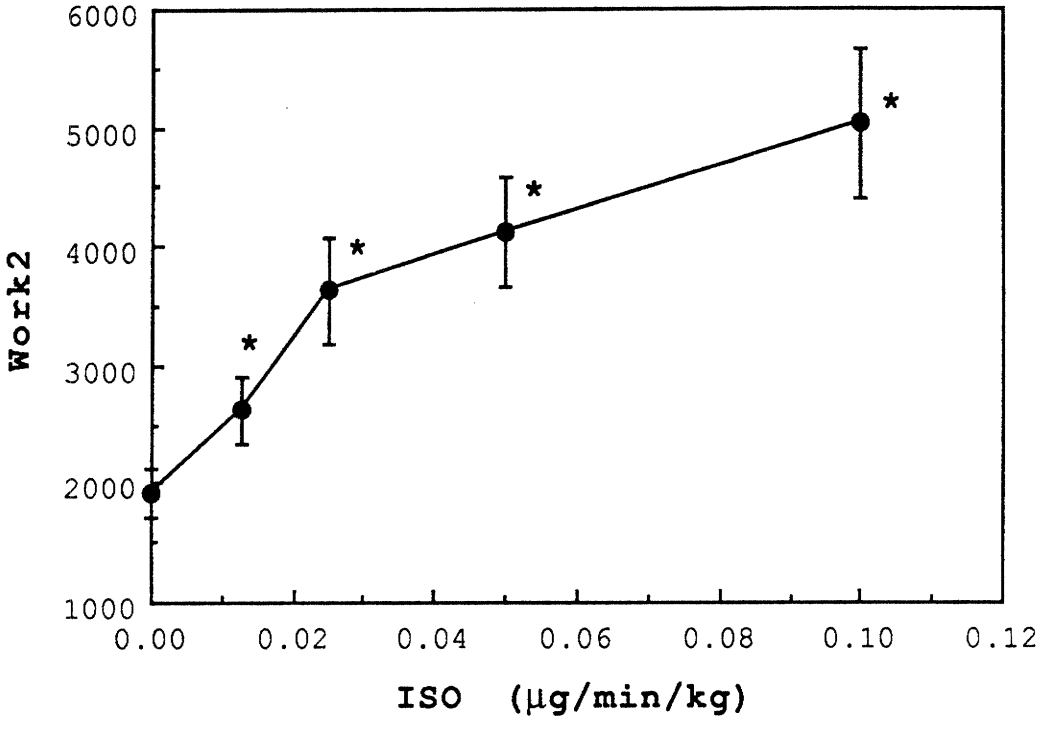

Figure 4. Effect of different doses of intracoronary isoproterenol (ISO) infusions on estimated myocardial work. Myocardial work was estimated as Work2 = HR X LVP X S%. n = 6. Unit of Work2: 10^2·mmHg/min. *: different from next lower value at $P < 0.05$.

Lactate extraction has been widely used as an index of severity of ischemia (19, 63). Since we deliberately chose the degree of perfusion pressure and hypoxemia to avoid myocardium ischemia, it is not surprising that myocardial lactate extraction was maintained relatively constant throughout the protocols in this investigation. In addition, there was no significant decrease in either global or local contractile function due to the low perfusion pressure or hypoxemia. Since there was no evidence of myocardial ischemia, we used MVO_2 as an index of myocardial oxygen demand.

By definition (15), oxygen utilization efficiency is the ratio of myocardial work to myocardial oxygen consumption. Since we calculated two work indexes, Work1 and Work2, we also calculated two oxygen utilization efficiency indexes, O_2EFFIC1 and O_2EFFIC2. As shown in Figures 5 and 6, both O_2EFFIC1 and O_2EFFIC2 remained relatively constant as the dose of ISO was varied from 0.0125 to 0.1 μg/min/Kg, except at a very high dose. At a very high dose (0.1 μg/min/kg), O_2EFFIC1 decreased significantly ($P < 0.05$) during ISO infusion, but O_2EFFIC2 remained constant.

Since we infused ISO into the LAD four times in some groups of the present investigation, it was important to demonstrate the repeatability of the responses to repeated ISO infusions. Table 1 presents systemic hemodynamic values,

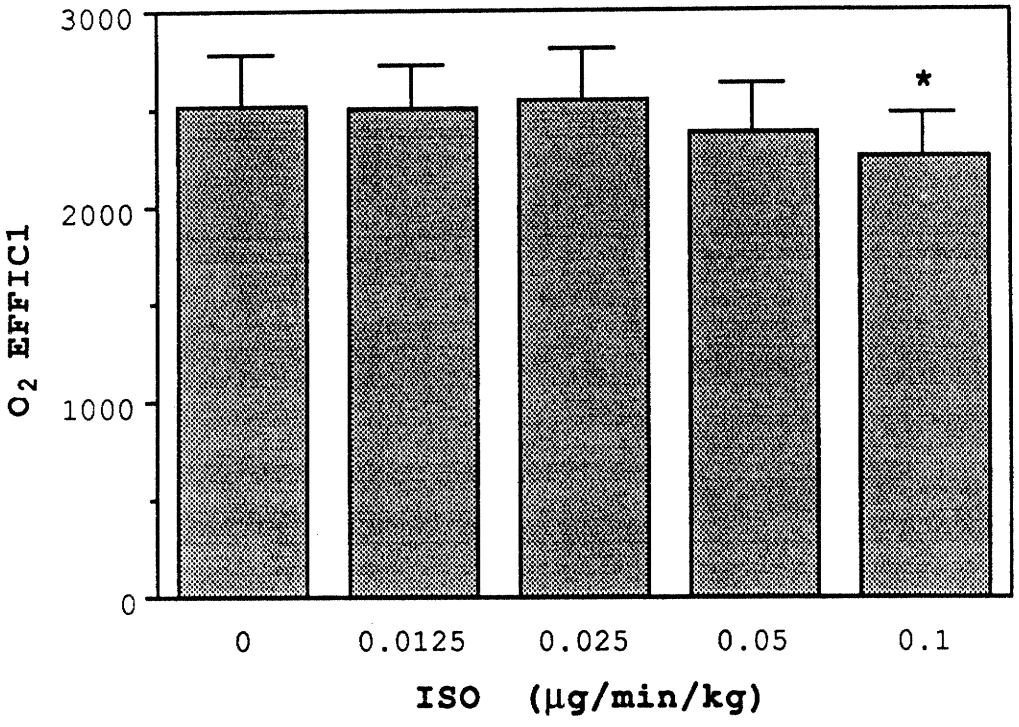

Figure 5. Effect of different doses of intracoronary isoproterenol (ISO) infusions on estimated myocardial oxygen utilization efficiency. Myocardial oxygen utilization efficiency was estimated as O_2EFFIC1 = Work1/MVO_2. n = 6. Unit of O_2EFFIC1: $10^6 \cdot mmHg^2/sec/ml/g$. *: different from other values at $P < 0.05$.

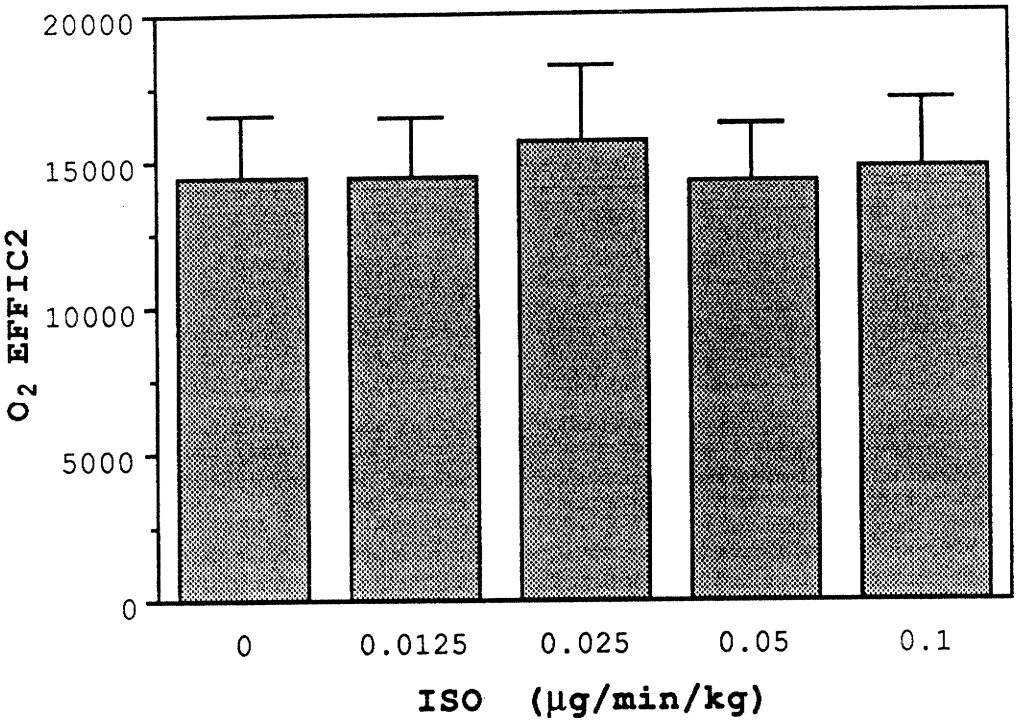

Figure 6. Effect of different doses of intracoronary isoproterenol (ISO) infusions on estimated myocardial oxygen utilization efficiency. Myocardial oxygen utilization efficiency was estimated as $O_2EFFIC2 = Work2/MVO_2$. n = 6. Unit of $O_2EFFIC2$: $10^3 \cdot mmHg/ml/g$.

coronary O_2 extraction, coronary venous O_2 tension, coronary lactate extraction, and myocardial function responses to four repeated ISO infusions of 0.025 μg/min/kg. Data were collected after 1.5 min of each ISO infusion, and designated ISO 1 through 4. A 6.5 min recovery time was allowed after stopping each ISO infusion. Data of Control 2 through 4 were collected 5 min after stopping each ISO infusion. As shown in Table 1, all variables had similar values at Control 1 through 4, and at ISO 1 through 4. Lactate extraction (La%) tended to decrease, but still remained 14.7% for ISO 4. Figures 7 and 8, and Figures 9 and 10 demonstrate the repeatability of ISO-induced elevation in myocardial work, and constant O_2 utilization efficiency, respectively. As shown in Figures 7 and 8, the ISO-induced elevations in Work1 and Work2 were similar for ISO infusions 1 through 4. O_2 utilization efficiency, as estimated by O_2EFFIC1 and O_2EFFIC2, was not significantly influenced by ISO infusions throughout the protocol, as shown in Figures 9 and 10.

Experimental Protocols

Two protocols were performed in this study: I) normal perfusion vs. low perfusion. and II) normoxemia vs. hypoxemia. Control hemodynamic variables, left ventricular dP/dt, and S% were recorded, and coronary arterial and venous blood samples were collected after post-surgical

TABLE I. Repeatability of the responses to repeated ISO infusions.

Conditions	AoP	HR	PP	LVP	dP/dt	CaO_2	CvO_2	PvO_2	CPR	La%	O_2%
Control 1	121 ± 6	149 ± 8	100 ± 0	118 ± 5	1990 ± 80	19.7 ±1.0	6.2 ±0.5	26.0 ±1.1	76.5 ±4.6	35.1 ±4.0	69.0 ±1.2
ISO 1	121 ± 4	155 ± 7	100 ± 1	120 ± 3	3420* ±260	19.7 ±1.0	6.1 ±0.4	25.8 ±1.3	46.1 ±2.2	30.8 ±4.0	69.3 ±0.6
Control 2	122 ± 4	149 ± 7	100 ± 0	117 ± 4	1950 ± 70	20.2 ±1.0	6.5 ±0.5	26.4 ±1.3	75.5 ±4.5	29.1 ±3.7	67.9 ±1.3
ISO 2	121 ± 4	157 ± 7	100 ± 0	119 ± 3	3440* ±260	20.2 ±0.9	6.0 ±0.4	25.7 ±1.2	45.6 ±2.0	26.0 ±2.5	70.2 ±1.1
Control 3	120 ± 4	147 ± 7	101 ± 0	115 ± 4	1940 ± 60	20.7 ±0.9	6.9 ±0.5	28.4 ±2.1	75.7 ±4.5	25.8 ±3.6	66.7 ±1.2
ISO 3	120 ± 4	151 ± 6	100 ± 1	118 ± 3	3380* ±270	20.3 ±1.0	6.1 ±0.4	25.7 ±1.3	45.9 ±1.5	20.5 ±2.3	70.3 ±1.3
Control 4	118 ± 3	140 ± 7	100 ± 0	115 ± 4	1900 ± 80	21.2 ±0.8	7.0 ±0.5	28.5 ±2.2	75.9 ±3.3	21.4 ±1.9	66.9 ±1.4
ISO 4	118 ± 3	150 ± 8	100 ± 1	116 ± 3	3420* ±270	20.4 ±0.9	5.9 ±0.4	25.4 ±1.4	45.3 ±1.4	14.7 ±3.3	70.9 ±1.7

Values are means ± standard errors of the mean (SEM). n = 6. AoP, mean aortic pressure; HR, heart rate; PP, coronary perfusion pressure; LVP, left ventricular pressure development; dP/dt, maximum rate of LVP; CaO_2, coronary arterial oxygen content; CvO_2, coronary venous oxygen content; PvO_2, coronary venous oxygen tension; CVR, coronary vascular resistance; La%, coronary lactate extraction; O_2%, coronary oxygen extraction. *: different from other values at $P < 0.05$.

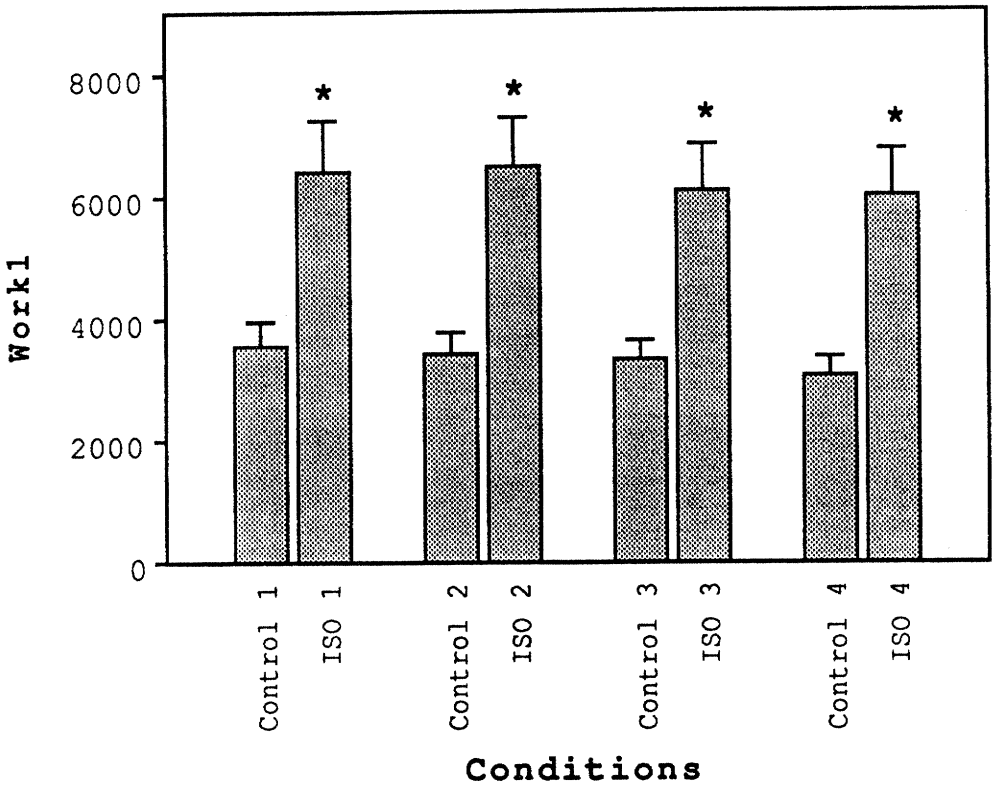

Figure 7. Effect of repeated intracoronary isoproterenol (ISO) infusions on estimated myocardial work. Myocardial work was estimated as Work1 = HR X LVP X dP/dt. n = 6. Unit of Work1: $10^4 \cdot mmHg^2/sec/min$. *: different from control values at $P < 0.05$.

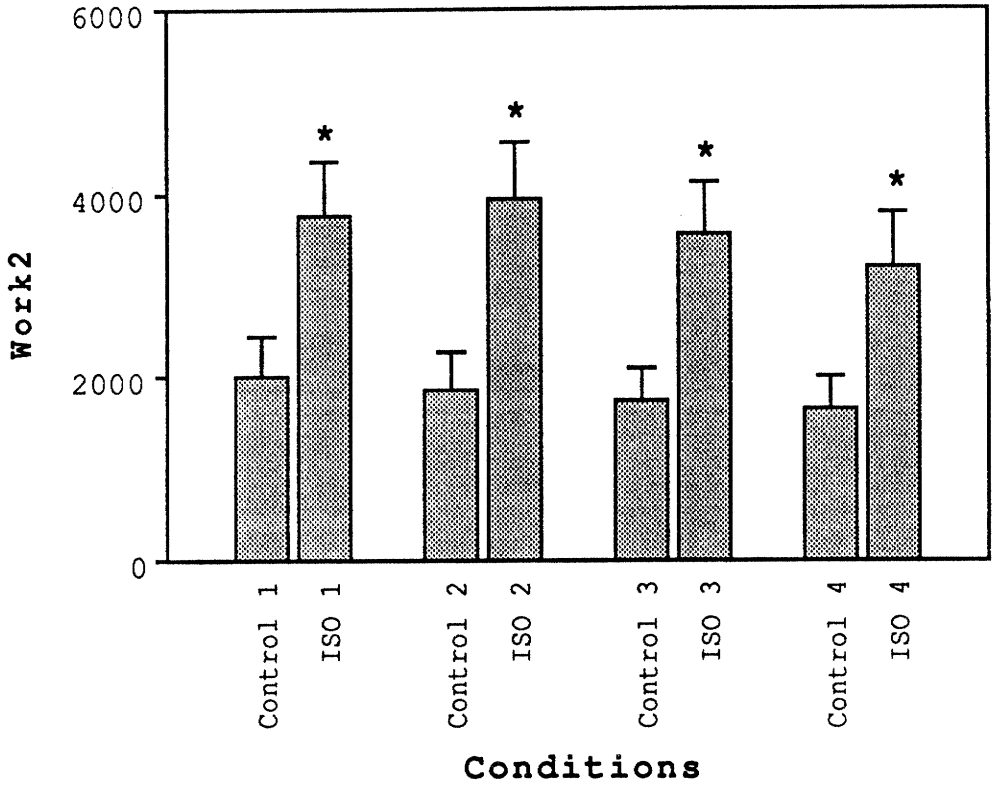

Figure 8. Effect of repeated intracoronary isoproterenol (ISO) infusions on estimated myocardial work. Myocardial work was estimated as Work2 = HR X LVP X S%. n = 6. Unit of Work2: 10^2·mmHg/min. *: different from control values at $P < 0.05$.

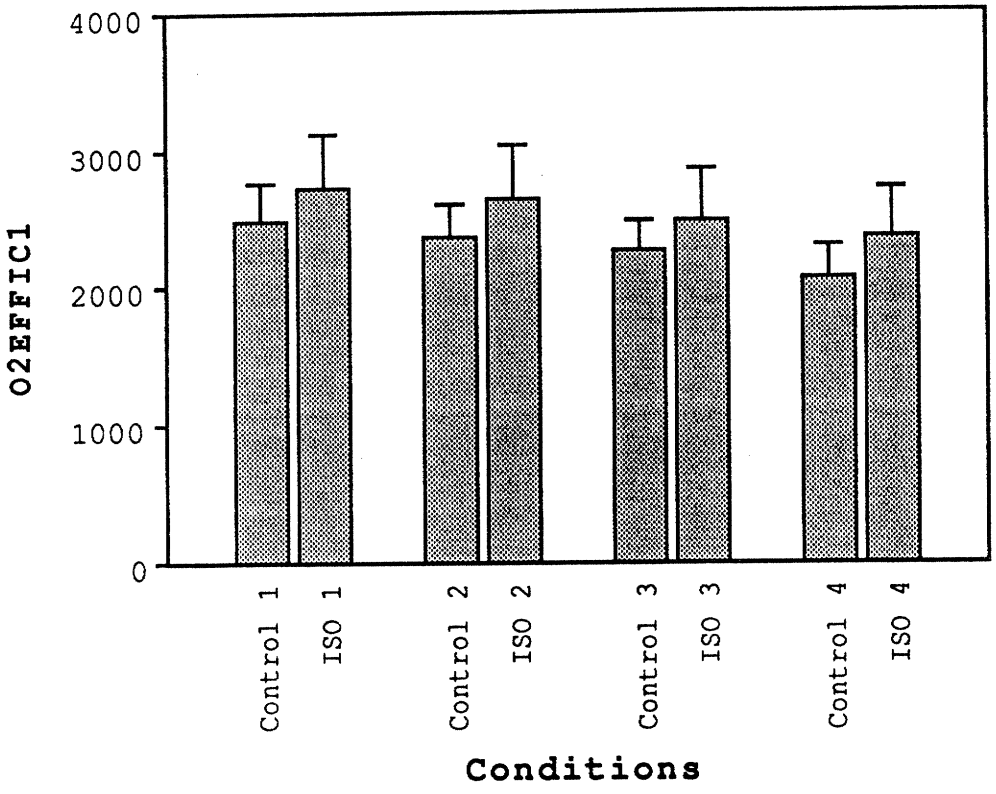

Figure 9. Effect of repeated intracoronary isoproterenol (ISO) infusions on estimated myocardial oxygen utilization efficiency. Myocardial oxygen utilization efficiency was estimated as $O_2EFFIC1 = Work1/MVO_2$. n = 6. Unit of $O_2EFFIC1$: $10^6 \cdot mmHg^2/sec/ml/g$.

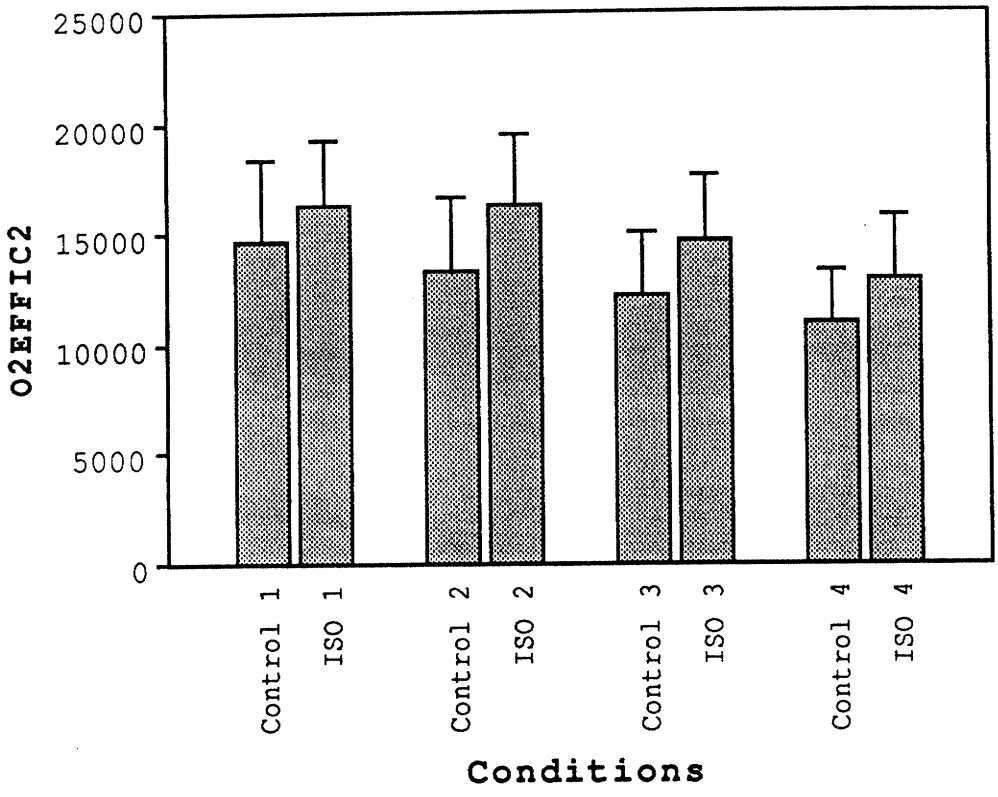

Figure 10. Effect of repeated intracoronary isoproterenol (ISO) infusions on estimated myocardial oxygen utilization efficiency. Myocardial oxygen utilization efficiency was estimated as $O_2EFFIC2 = Work2/MVO_2$. n = 6. Unit of $O_2EFFIC2$: $10^3 \cdot mmHg/ml/g$.

stabilization, the heart was then subjected to one of the protocols described below.

A. Group I: Normal Perfusion pressure (HP) vs. Low Perfusion pressure (LP).

In this protocol the LAD was perfused with normoxemic blood at constant pressure: 100 mmHg for HP and 60 mmHg for LP.

GROUP Ia: ADO & ISO (n = 7). After stabilization, hemodynamic variables, dP/dt, and segment lengths were recorded and coronary arterial and venous blood samples were collected. Data collected at this time were designated Control 1. Isoproterenol solution (5 µg/ml saline) was infused into LAD at a rate of 0.005 ml/min/kg (0.025 µg/min/kg). After 1.5 min, hemodynamic data, dP/dt, and segment length were recorded, and blood samples were collected. These data were designated ISO. The ISO infusion was then stopped. After CBF returned to control and all other recorded hemodynamic variables were stable (about 5 min), hemodynamic data, dP/dt, and segment length were recorded and coronary arterial and venous blood samples were collected. These data were designated Control 2. Adenosine (400 µg/ml saline) was then infused into LAD at a rate of 0.1 ml/min (40 µg/min). After stabilization (about 1 min), hemodynamic data and segment length was recorded, and blood samples were collected. These data were designated ADO.

Isoproterenol solution (5 μg/ml saline) was then infused into LAD at a rate of 0.005 ml/min/kg (0.025 μg/min/kg). After 1.5 min, hemodynamic data, dP/dt, and segment length were recorded, and blood samples were collected. These data were designated ADO+ISO. The ADO and ISO infusions were then stopped. After CBF returned to control and all other recorded hemodynamic variables were stable (about 5 min), hemodynamic data, dP/dt, and segment length were recorded and coronary arterial and venous blood samples were collected. These data were designated Control 3.

Group Ia: LP & ISO (n = 7). After stabilization, hemodynamic variables, dP/dt, and segment lengths were recorded and coronary arterial and venous blood samples were collected. Data collected at this time were designated Control 1. Isoproterenol solution (5 μg/ml saline) was infused into LAD at a rate of 0.005 ml/min/kg (0.025 μg/min/kg). After 1.5 min, hemodynamic data, dP/dt, and segment length were recorded, and blood samples were collected. These data were designated ISO. The ISO infusion was then stopped. After CBF returned to control and all other recorded hemodynamic variables were stable (about 5 min), hemodynamic data, dP/dt, and segment length were recorded and coronary arterial and venous blood samples were collected. These data were designated Control 2. PP was then reduced from 100 mmHg to 60 mmHg. After 3 min, hemodynamic data, dP/dt, and segment length were recorded, and blood

samples were collected. These data were designated LP 1. ISO was then infused into LAD at the same dose as above. After 1.5 min, hemodynamic data, dP/dt, and segment length were recorded, and blood samples were collected. These data were designated LP+ISO. The ISO infusion was then stopped. After 5 min, hemodynamic data, dP/dt, and segment length were recorded, and blood samples were collected. These data were designated LP 2.

Group Ib: ISO+ADA (n = 7). After stabilization, hemodynamic variables, dP/dt, and segment lengths were recorded and coronary arterial and venous blood samples were collected. Data collected at this time were designated Control 1. Isoproterenol solution (5 μg/ml saline) was infused into LAD at a rate of 0.005 ml/min/kg (0.025 μg/min/kg). After 1.5 min, hemodynamic data, dP/dt, and segment length were recorded, and blood samples were collected. These data were designated ISO. The ISO infusion was then stopped. After CBF returned to control and all other recorded hemodynamic variables were stable (about 5 min), hemodynamic data, dP/dt, and segment length were recorded and coronary arterial and venous blood samples were collected. These data were designated Control 2. PP was then reduced to 60 mmHg. After 1.5 min, the same variables were recorded. These data were designated LP 1. Isoproterenol (5 μg/ml saline) was infused into LAD at a rate of 0.005 ml/min/kg (0.025 μg/min/kg). After 1.5 min, the same

variables were recorded and blood samples were collected. These data were designated LP+ISO. The isoproterenol infusion was then stopped. After 5 min, these same variables were recorded. These data were designated LP 2. PP were then increased to 100 mmHg. After 3 min, the same variables were recorded, and blood samples were collected. These data were designated Control 3. 2 μg adenosine (0.1 ml) were then injected into the LAD. After CBF returned to control (1 min), ADA was then infused into LAD at a rate of 5 units/min/kg. 2 μg adenosine (0.1 ml) were again injected into the LAD at 14 min ADA infusion. Data were recorded and blood samples were collected at 15 min ADA infusion. These data were designated ADA 1. Isoproterenol was then infused at the same dose as above. After 1.5 min, data were recorded and blood samples were collected. These data were designated ADA+ISO. ISO infusion was then stopped. After 5 to 7 min, data were recorded and blood samples were collected. These data were designated ADA 2. PP was then reduced to 60 mmHg. After 3 min, data were recorded and blood samples were collected. These data were designated as ADA/LP 1. Isoproterenol was then infused into LAD at the same dose as above. After 1.5 min, data were collected and blood samples were collected. These data were designated ADALP+ISO. ISO infusion was then stopped. After 5 min, data were recorded and blood samples were collected. These data were designated ADALP 2.

Group Ic: EHNA & ISO (n = 6). The protocol of group Ib was used in this group except that we infused EHNA (1.24×10^{-5} M) at a rate of 0.019 ml/min/kg instead of ADA. Data recorded in this group were designated as Control 1, LP 1 Control 2, LP 2, LP+ISO, LP 3, Control 3, EHNA 1, EHNA+ISO, EHNA 2, EHNALP 1, EHNALP+ISO, and EHNALP 2.

B. Group II: Normoxemia vs. Hypoxemia (HYPX).

The LAD was perfused at 100 mmHg with either normoxic or hypoxic blood in this protocol (Figure 2).

Group IIa: HYPX & ISO (n = 6). The protocol of Group Ia: LP & ISO was used in this group, except that we used hypoxic blood perfusion (CaO_2 = 7-10 ml/dl) instead of low pressure perfusion. Data gained in this group were designated as Control 1, ISO, Control 2, HYPX 1, HYPX+ISO, and Control 3.

Group IIb: ADA/HYPX & ISO (n = 7). The protocol of Group IIa was used in this group, except that we infused ADA solution at a rate of 5 units/min/kg for 15 min before ISO infusion. Data gained in this group were designated as ADA 1, ADA+ISO, ADA 2, ADA/HYPX 1, ADA/HYPX+ISO, and ADA 3.

Group IIc: EHNA/HYPX & ISO (n = 6). The protocol of Group IIa was used in this protocol, except that we infused EHNA solution (1.24×10^{-5} M) at a rate of 0.019 ml/min/kg for 15 min before the protocol. Data gained in this group were

designated as EHNA 1, EHNA+ISO, EHNA 2, EHNA/HYPX 1, EHNA/HYPX+ISO, and EHNA 3.

Statistical Analysis

All data were expressed as means ± standard error (SE). Within each group, multiple comparisons of means were performed with an analysis of variance for repeated measures in combination with least significant difference pairwise comparisons. Between the groups, multiple comparisons of means were performed with an analysis of variance for complete randomization in combination with least significant difference pairwise comparisons. Statistical significance was accepted at $P < 0.05$. Changes in CVR and O_2% were not analyzed statistically; however, effects of the LP, hypoxia, and ADO on these variables are clearly evident from mean values ± SE.

CHAPTER III

RESULTS

Normoxemia, PP = 100 mmHg

1) ADO & ISO

Table 2 presents systemic hemodynamic values, coronary oxygen extraction, coronary venous oxygen tension, coronary lactate extraction, and myocardial function responses to ADO infusion and to ISO infusions in the absence and presence of ADO. AoP, HR, PP, LVP, and La% were essentially constant throughout the protocol. All variables were similar at Controls 1, 2, and 3. ADO alone did not change dP/dt, but decreased CVR from 76.2 ± 2.5 to 19.5 ± 0.4 mmHg·min/ml·g, and O_2% from 70.1 ± 0.9% to 19.3 ± 2.0%. ISO alone increased dP/dt from 2210 ± 120 mmHg/s to 3450 ± 200 mmHg/s ($P < 0.05$). O_2% was 69.7 ± 1.0% before and 72.2 ± 0.9% after ISO, and P_vO_2 was 25.6 ± 1.9 mmHg before and 24.8 ± 2.1 mmHg after ISO. When ISO was infused in the presence of ADO, ISO decreased CVR further from 19.5 ± 0.4 mmHg·min/ml·g to 15.5 ± 0.4 mmHg·min/ml·g. As shown in the table, the ISO-induced increase in dP/dt was not significantly influenced by the presence of ADO (3450 ± 200 without ADO vs. 3400 ± 190 with ADO).

TABLE II

The responses to ADO infusion (ADO) and to ISO infusions in the absence (ISO) and presence (ADO+ISO) of ADO.

Conditions	AoP	HR	PP	LVP	dP/dt	CaO_2	CvO_2	PvO_2	CVR	La%	O_2%
Control 1	122 ± 3	141 ± 6	100 ± 1	114 ± 2	2210 ±120	19.9 ±0.5	6.1 ±0.3	25.6 ±1.9	76.2 ± 2.4	39.5 ±2.6	69.7 ±1.0
ISO	123 ± 3	148 ± 4	100 ± 1	121 ± 4	3450* ±200	20.1 ±0.6	5.6 ±0.2	24.8 ±2.1	46.0 ± 2.0	37.5 ±2.9	72.2 ±0.9
Control 2	123 ± 4	141 ± 6	100 ± 1	114 ± 2	2140 ±110	20.4 ±0.7	6.1 ±0.3	26.7 ±1.8	76.2 ± 2.5	42.1 ±5.0	70.1 ±0.9
ADO	117 ± 2	142 ± 6	101 ± 1	111 ± 2	2160 ±110	20.2 ±0.6	16.2 ±0.3	53.5 ±3.7	19.5 ± 0.4	36.2 ±4.4	19.3 ±2.0
ADO+ISO	123 ± 4	143 ± 3	101 ± 1	120 ± 3	3400* ±190	20.1 ±0.5	16.2 ±0.2	53.3 ±3.8	15.5 ± 0.4	43.7 ±4.7	19.1 ±1.8
Control 3	123 ± 4	143 ± 6	100 ± 1	113 ± 2	2090 ± 90	20.0 ±0.5	6.5 ±0.3	27.0 ±2.3	76.2 ± 2.3	35.4 ±3.1	67.9 ±0.8

Values are means ± standard errors of the mean (SEM). n = 7. AoP, mean aortic pressure; HR, heart rate; PP, coronary perfusion pressure; LVP, left ventricular pressure development; dP/dt, maximum rate of LVP; CaO_2, coronary arterial oxygen content; CvO_2, coronary venous oxygen content; PvO_2, coronary venous oxygen tension; CVR, coronary vascular resistance; La%, coronary lactate extraction; O_2%, coronary oxygen extraction. *: different from other values at $P < 0.05$.

Figures 11 through 18 illustrate the CBF, dP/dt, S%, Work1, Work2, MVO_2, O_2EFFIC1, and O_2EFFIC2 responses to ADO infusion and to ISO infusions in absence and presence of ADO, respectively. As shown in Figure 11, ADO increased CBF about 300%, and ISO caused another 100% increase in CBF when infused with ADO. As shown in Figures 12 and 13, the ISO-induced increases in dP/dt and S% were not significantly influenced by the presence of ADO. As shown in Figures 14 and 15, the ISO-induced increases in Work1 and Work2 were not significantly influenced by the presence of ADO. As shown in Figure 16, ISO-induced elevation in MVO_2 was decreased from 25.8 ± 1.7 ml/min/100 g to 20.4 ± 2.6 ml/min/100 g ($P < 0.05$) in the presence of ADO. This reduction in the MVO_2 is apparently due to the ADO infusion, and it is this reduction that resulted in a significantly higher oxygen utilization efficiency during ISO infusion in the presence of ADO than during ISO infusion alone. Compared to ISO alone, ISO infusion in the presence of ADO increased O_2EFFIC1 from 2489 ± 274 to 3141 ± 420 X 10^6·$mmHg^2$/sec/ml/g ($P < 0.05$) and O_2EFFIC2 from 15520 ± 2733 to 19660 ± 3722 X 10^3·mmHg/ml/g ($P < 0.05$), as shown in Figures 17 and 18, respectively. Thus, ISO+ADO increased oxygen utilization efficiency by 23% when compared to ISO alone.

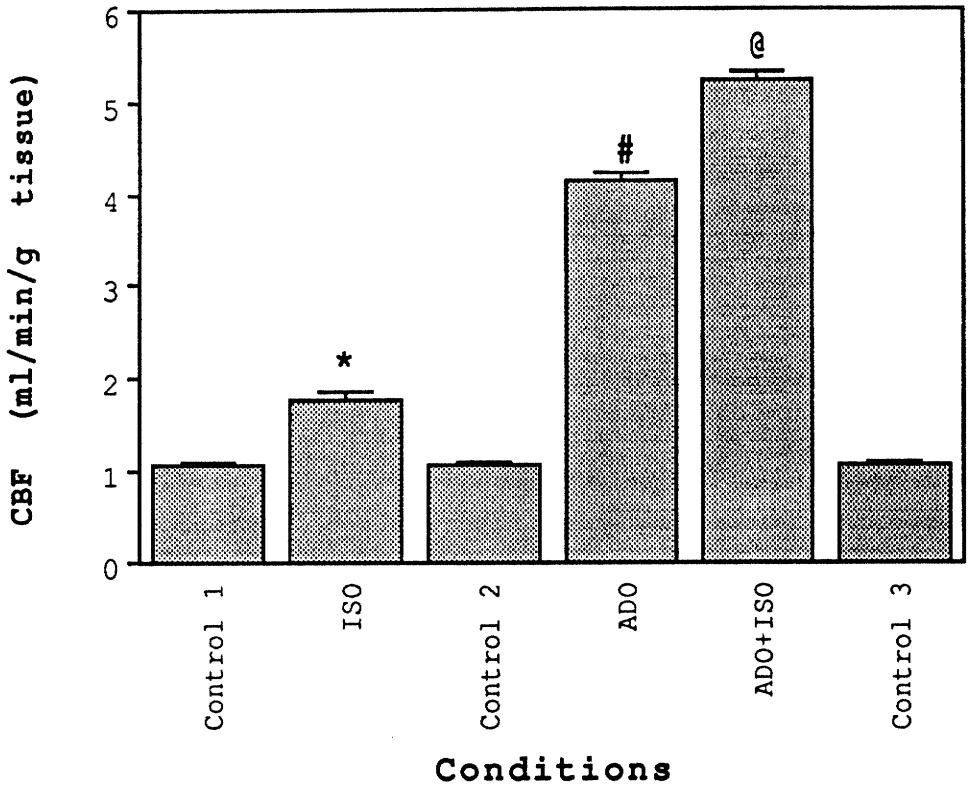

Figure 11. The coronary blood flow (CBF) response to ADO infusion (ADO) and to ISO infusions in absence (ISO) and presence (ADO+ISO) of ADO. n = 7. *, #, @: different from other values at $P < 0.05$.

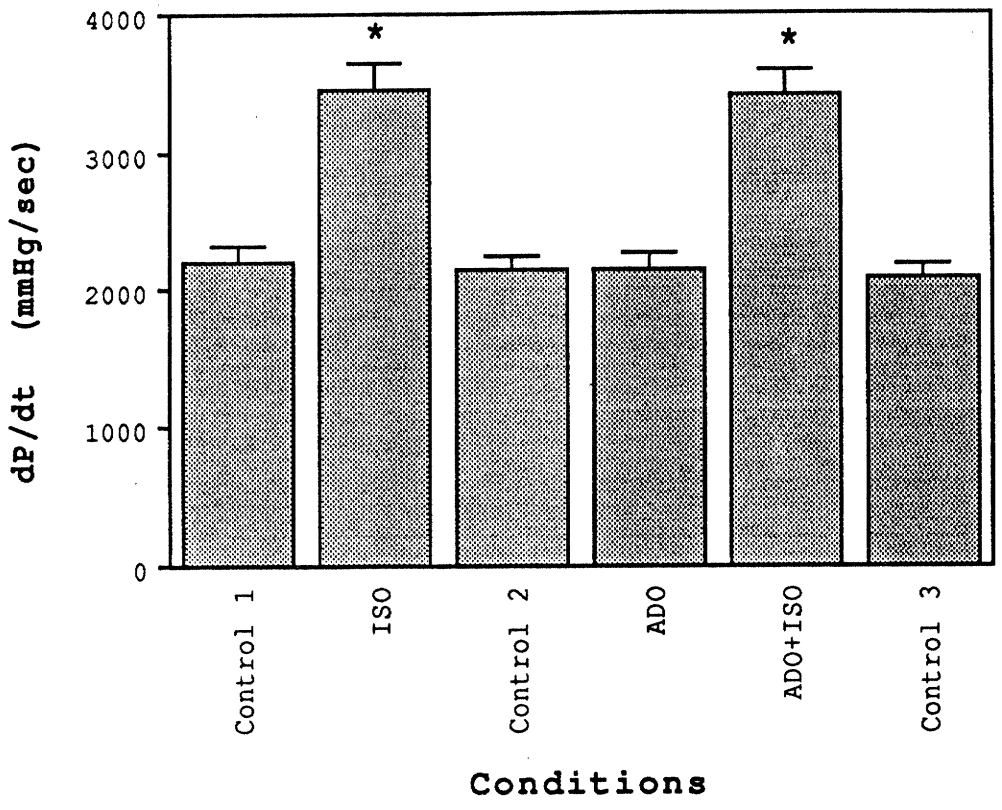

Figure 12. The maximum rate of LVP (dP/dt) response to ADO infusion (ADO) and to ISO infusions in absence (ISO) and presence (ADO+ISO) of ADO. n = 7. *: different from other values at $P < 0.05$.

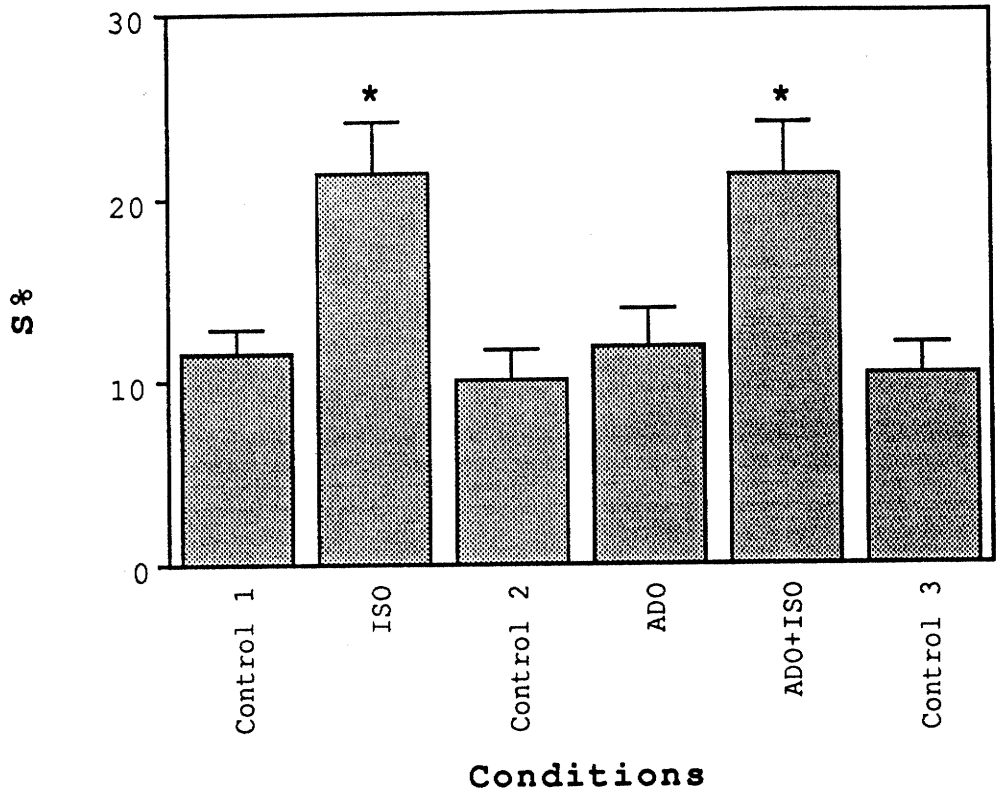

Figure 13. The percent segment shortening (S%) response to ADO infusion (ADO) and to ISO infusions in absence (ISO) and presence (ADO+ISO) of ADO. n = 7. *: different from other values at $P < 0.05$.

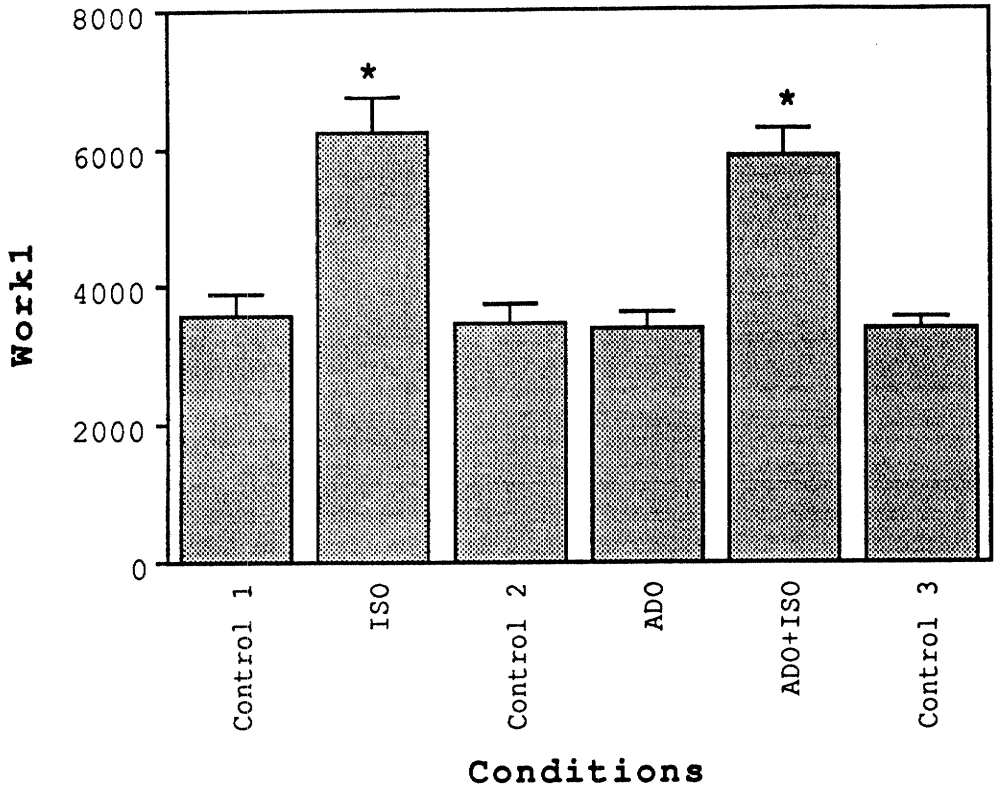

Figure 14. The estimated myocardial work response to ADO infusion (ADO) and to ISO infusions in absence (ISO) and presence (ADO+ISO) of ADO. Myocardial work was estimated as Work1 = HR X LVP X dP/dt. n = 7. Unit of Work1: $10^4 \cdot mmHg^2/sec/min$. *: different from other values at $P < 0.05$.

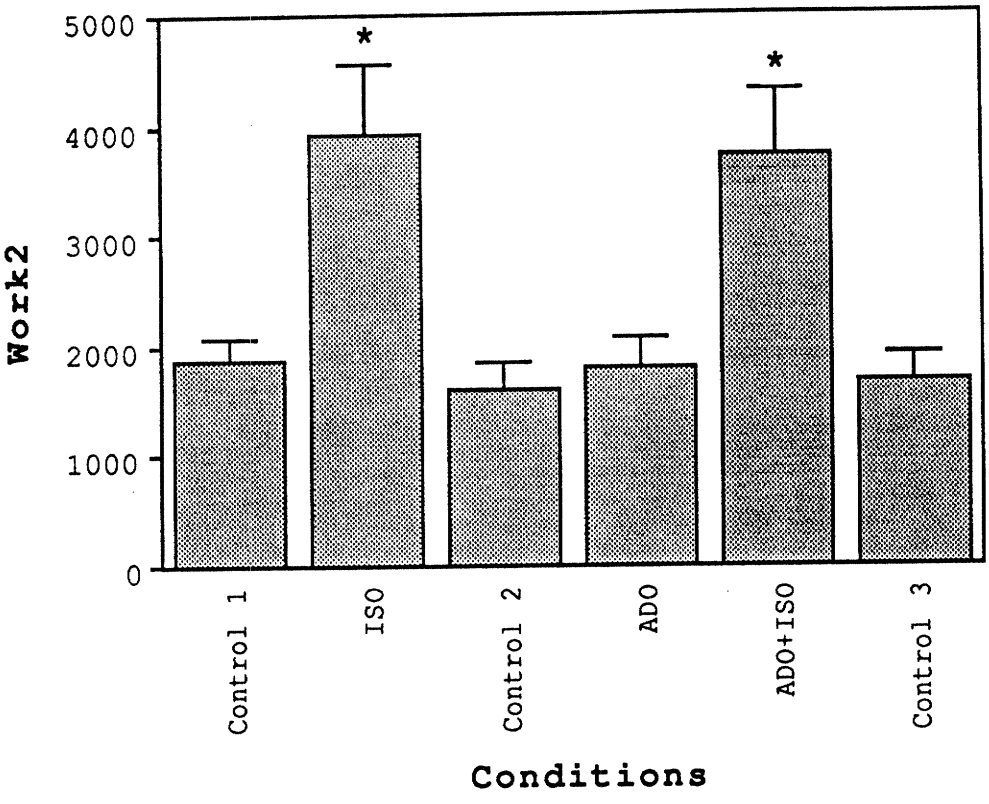

Figure 15. The estimated myocardial work response to ADO infusion (ADO) and to ISO infusions in absence (ISO) and presence (ADO+ISO) of ADO. Myocardial work was estimated as Work2 = HR X LVP X S%. n = 7. Unit of Work2: 10^2·mmHg/min. *: different from other values at $P < 0.05$.

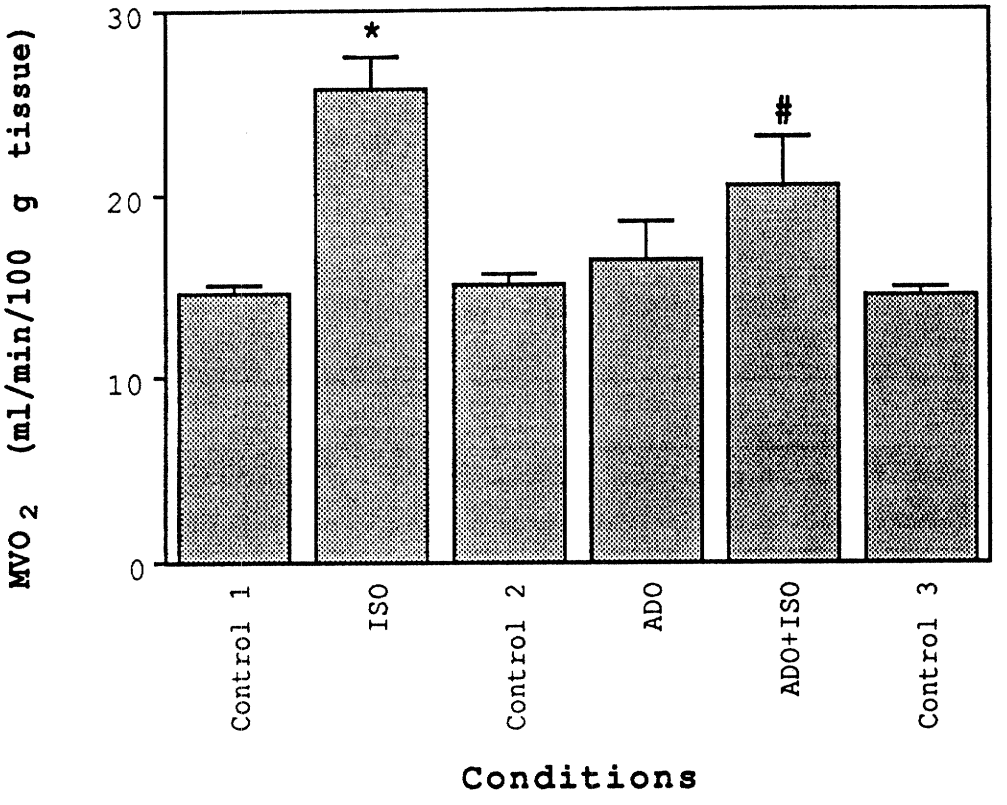

Figure 16. The myocardial oxygen consumption (MVO_2) response to ADO infusion (ADO) and to ISO infusions in absence (ISO) and presence (ADO+ISO) of ADO. n = 7. *, #: different from other values at $P < 0.05$.

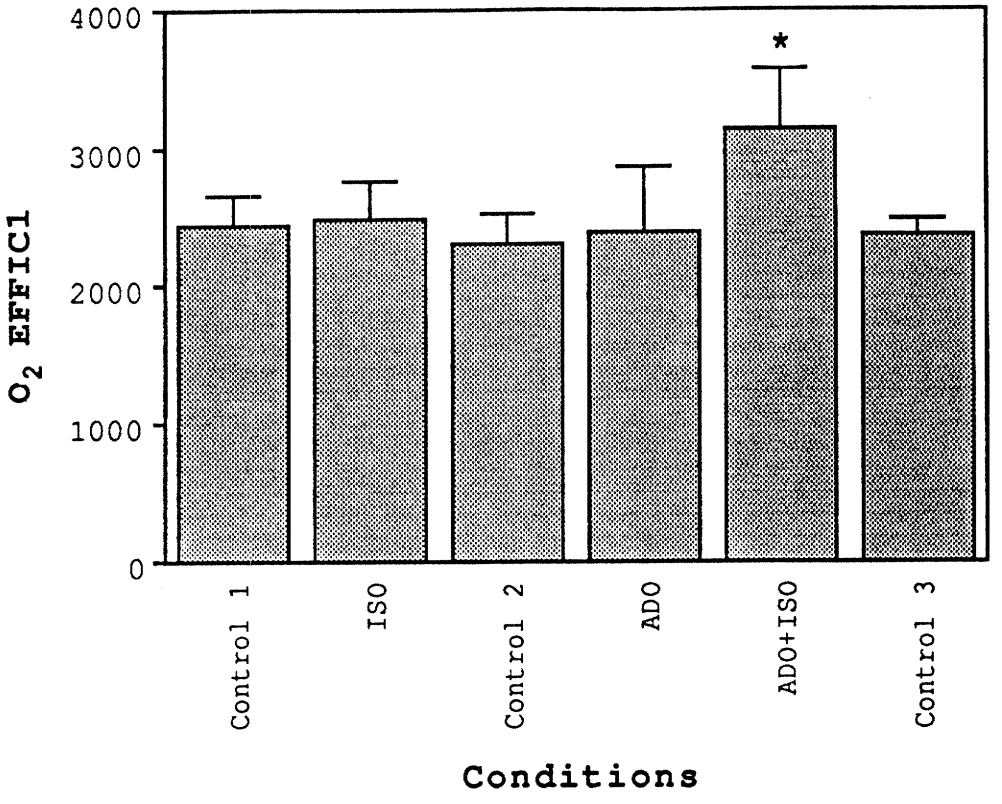

Figure 17. The estimated myocardial oxygen utilization efficiency response to ADO infusion (ADO) and to ISO infusions in absence (ISO) and presence (ADO+ISO) of ADO. Myocardial oxygen utilization efficiency was estimated as $O_2EFFIC1 = Work1/MVO_2$. n = 7. Unit of $O_2EFFIC1$: $10^6 \cdot mmHg^2/sec/ml/g$. *: different from other values at $P < 0.05$.

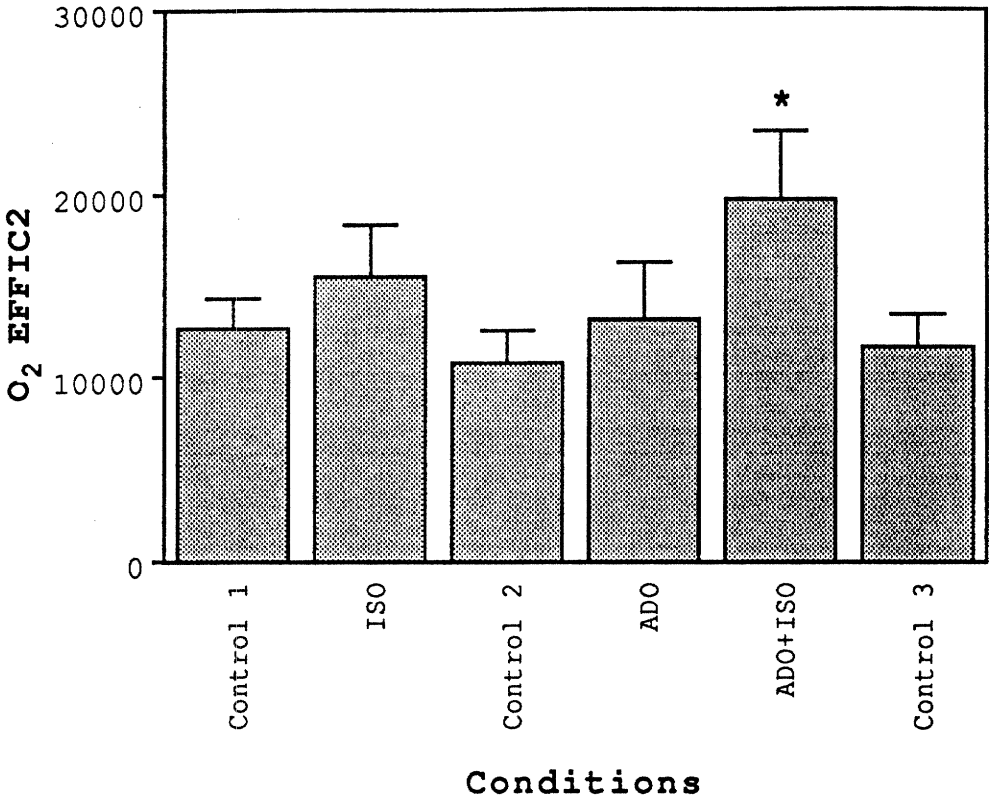

Figure 18. The estimated myocardial oxygen utilization efficiency response to ADO infusion (ADO) and to ISO infusions in absence (ISO) and presence (ADO+ISO) of ADO. Myocardial oxygen utilization efficiency was estimated as O_2EFFIC2 = Work2/MVO_2. n = 7. Unit of O_2EFFIC2: 10^3·mmHg/ml/g. *: different from other values at $P < 0.05$.

2) ADA & ISO

Table 3 presents systemic hemodynamic values, coronary oxygen extraction, coronary venous oxygen tension, coronary lactate extraction, and myocardial function responses to ADA infusion and to ISO infusions in the absence and presence of ADA. AoP, HR, PP, LVP, and La% were essentially constant throughout the protocol. All variables had similar values at Control 1 and 2, and at ADA 1 and 2. CBF response to ADO injections (50 μg, i.c.) was significantly decreased from 96 ± 6% above control before ADA to 35 ± 4% above control after 14 min ADA infusion ($P < 0.05$). ADA alone did not change dP/dt, CVR, P_vO_2, or O_2%. ISO alone increased dP/dt from 2160 ± 110 mmHg/s to 3590 ± 150 mmHg/s ($P < 0.05$). O_2% was 69.5 ± 1.1% before and 72.9 ± 0.9% after ISO, and CVR was 81.2 ± 3.5 mmHg·min/ml·g before and 45.6 ± 1.1 mmHg·min/ml·g after ISO. When ISO was infused in the presence of ADA, ISO decreased CVR from 78.3 ± 2.7 mmHg·min/ml·g to 54.7 ± 1.9 mmHg·min/ml·g. As shown in the table, the ISO-induced increase in dP/dt was decreased by the presence of ADA (3590 ± 150 without ADA vs. 3190 ± 120 with ADA, $P < 0.05$).

TABLE III

The responses to ADA infusion (ADA) and to ISO infusions in the absence (ISO) and presence (ADA+ISO) of ADA.

Conditions	AoP	HR	PP	LVP	dP/dt	CaO_2	CvO_2	PvO_2	CVR	La%	O_2%
Control 1	122 ± 3	142 ± 6	100 ± 1	112 ± 4	2160 ±110	19.9 ±0.5	6.1 ±0.8	25.4 ±2.1	81.2 ± 3.5	31.5 ±3.3	69.5 ±1.1
ISO	123 ± 4	149 ±11	99 ± 1	120 ± 9	3590# ±150	20.5 ±1.3	5.5 ±0.6	25.2 ±1.8	45.6 ± 1.1	33.8 ±3.4	72.9 ±0.9
Control 2	123 ± 4	142 ± 7	101 ± 1	114 ± 6	2270 ±160	20.3 ±0.6	6.2 ±0.7	25.8 ±2.0	76.5 ± 2.3	42.9 ±4.5	69.7 ±0.9
ADA 1	117 ± 2	143 ± 5	100 ± 0	111 ± 6	2310 ±150	20.0 ±0.6	6.2 ±0.7	25.9 ±1.9	78.3 ± 2.7	35.9 ±3.0	69.2 ±0.8
ADA+ISO	123 ± 3	144 ± 9	100 ± 1	118 ± 5	3190* ±120	20.3 ±0.5	5.5 ±0.6	25.0 ±2.1	54.7 ± 1.9	31.4 ±2.5	72.8 ±2.6
ADA 2	123 ± 4	143 ±15	100 ± 1	113 ± 4	2110 ± 80	20.0 ±0.5	6.5 ±0.6	26.2 ±1.9	74.8 ± 1.1	33.8 ±3.0	67.3 ±1.7

Values are means ± standard errors of the mean (SEM). n = 7. AoP, mean aortic pressure; HR, heart rate; PP, coronary perfusion pressure; LVP, left ventricular pressure development; dP/dt, maximum rate of LVP; CaO_2, coronary arterial oxygen content; CvO_2, coronary venous oxygen content; PvO_2, coronary venous oxygen tension; CVR, coronary vascular resistance; La%, coronary lactate extraction; O_2%, coronary oxygen extraction. *, #: different from other values at $P < 0.05$.

Figures 19 through 26 illustrate the CBF, dP/dt, S%, Work1, Work2, MVO_2, O_2EFFIC1, and O_2EFFIC2 responses to ADA infusion and to ISO infusions in absence and presence of ADA, respectively. As shown in Figure 19, ADA did not change CBF significantly, and ISO-induced elevation in CBF was decreased from 1.77 ± 0.06 ml/min/g to 1.48 ± 0.04 ml/min/g ($P < 0.05$) in the presence of ADA. As shown in Figures 20 and 21, the ISO-induced elevations in dP/dt and S% were reduced by 12% and 20%, respectively, in the presence of ADA. As shown in Figures 22 and 23, the ISO-induced elevations in Work1 and Work2 were reduced by 16% and 25%, respectively, in the presence of ADA. As shown in Figure 24, the ISO-induced elevation in MVO_2 was reduced from 26.4 ± 1.3 ml/min/100 g to 22.0 ± 0.7 ml/min/100 g ($P < 0.05$) in the presence of ADA. This 17% reduction in the MVO_2 and similar percentage decreases in Work1 and Work2 resulted in the nearly constant oxygen utilization efficiencies, as shown in Figures 25 and 26. Thus, ADA+ISO decreased myocardial work and MVO_2, and maintained oxygen utilization efficiency when compared to ISO alone.

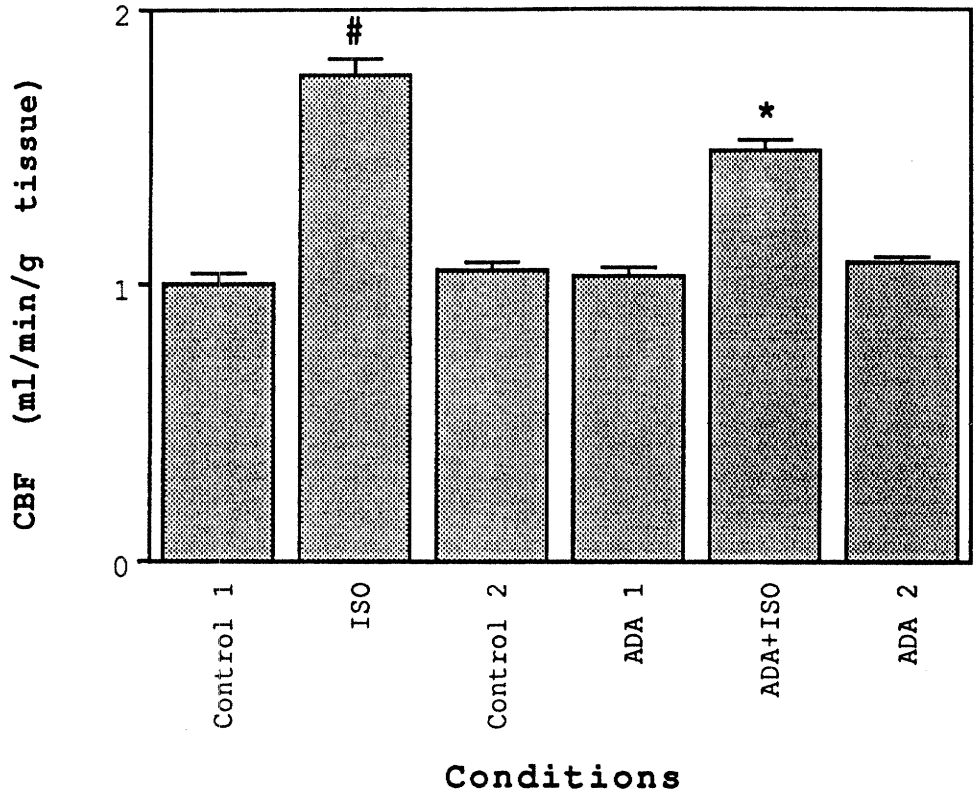

Figure 19. The coronary blood flow (CBF) response to ADA infusion (ADA) and to ISO infusions in absence (ISO) and presence (ADA+ISO) of ADA. n = 7. *, #: different from other values at $P < 0.05$.

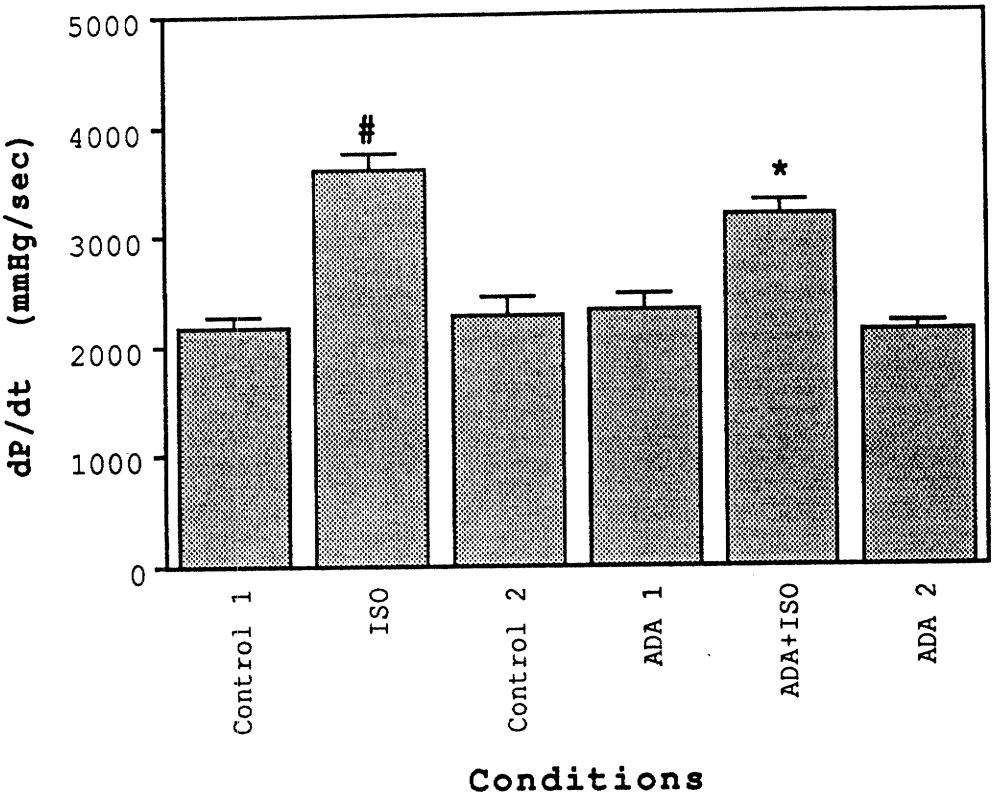

Figure 20. The maximum rate of LVP (dP/dt) response to ADA infusion (ADA) and to ISO infusions in absence (ISO) and presence (ADA+ISO) of ADA. n = 7. *, #: different from other values at $P < 0.05$.

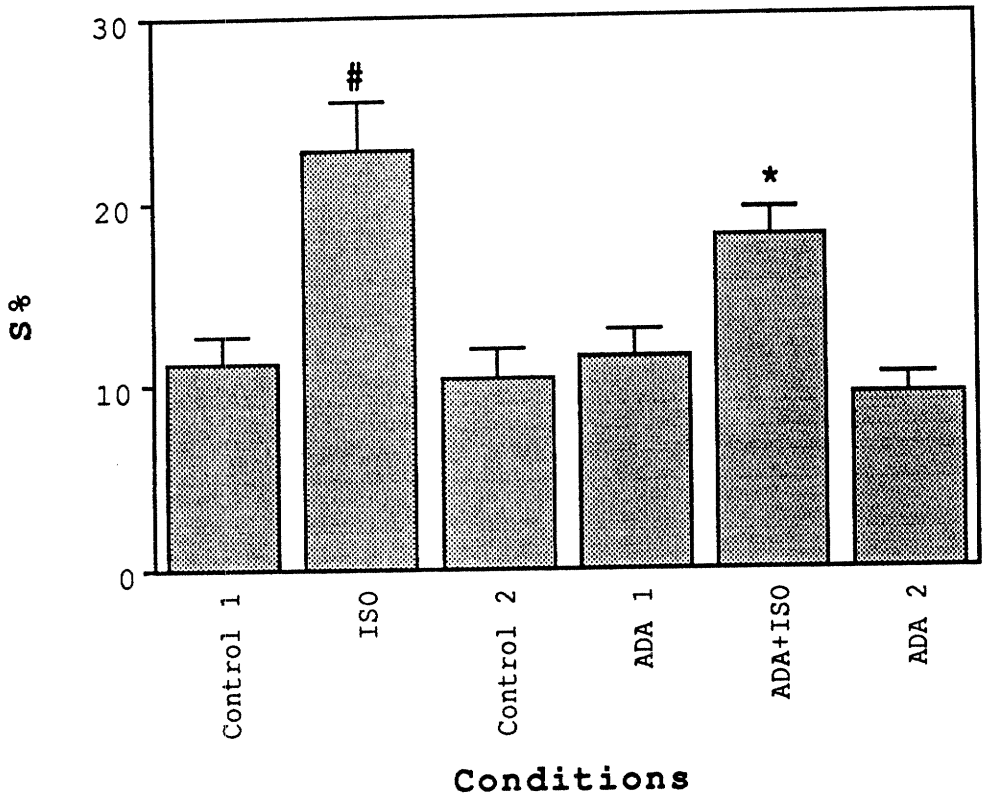

Figure 21. The percent segment shortening (S%) response to ADA infusion (ADA) and to ISO infusions in absence (ISO) and presence (ADA+ISO) of ADA. n = 7. *, #: different from other values at $P < 0.05$.

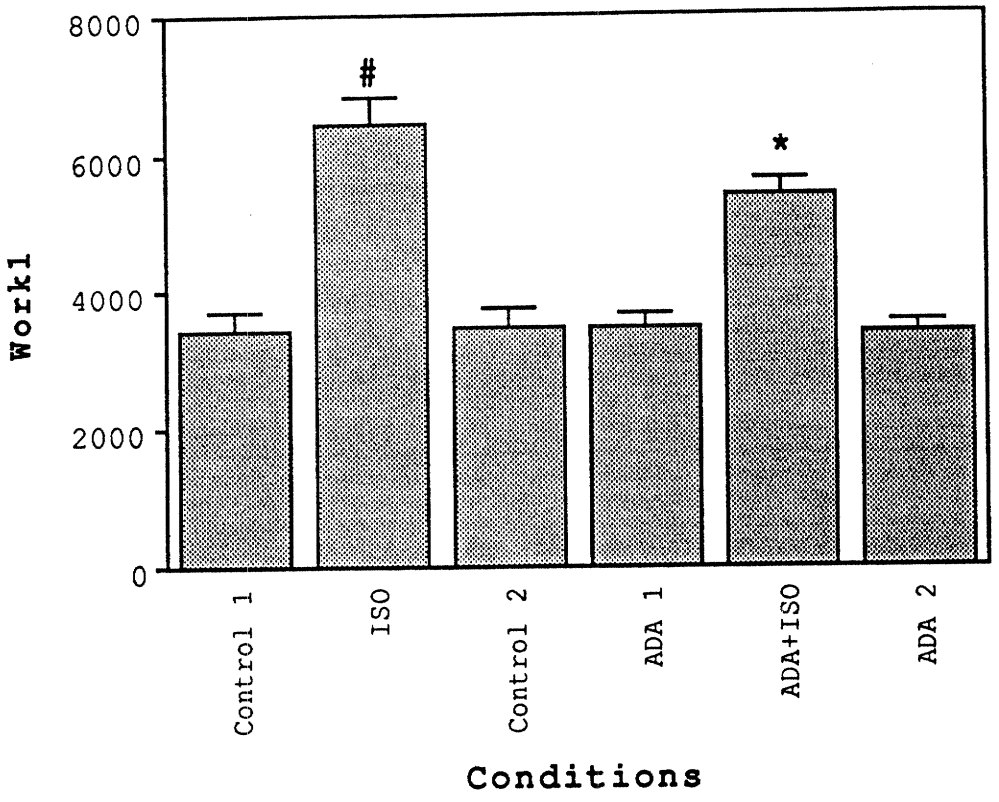

Figure 22. The estimated myocardial work response to ADA infusion (ADA) and to ISO infusions in absence (ISO) and presence (ADA+ISO) of ADA. Myocardial work was estimated as Work1 = HR X LVP X dP/dt. n = 7. Unit of Work1: $10^4 \cdot mmHg^2/sec/min$. *, #: different from other values at $P < 0.05$.

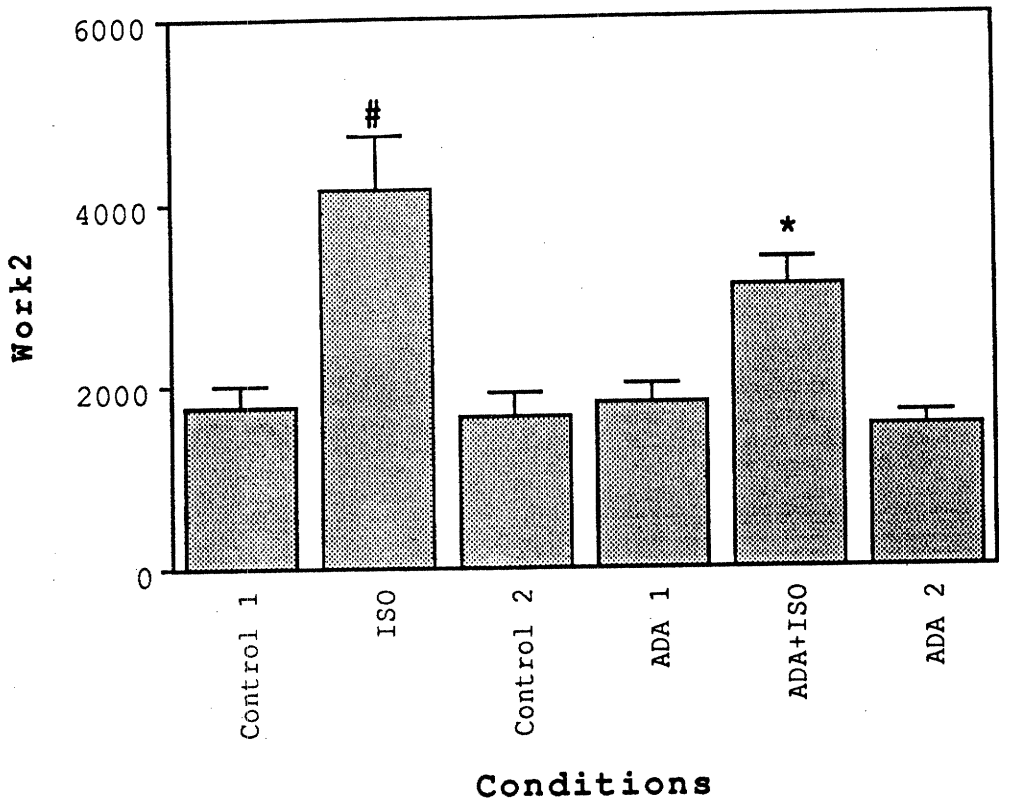

Figure 23. The estimated myocardial work response to ADA infusion (ADA) and to ISO infusions in absence (ISO) and presence (ADA+ISO) of ADA. Myocardial work was estimated as Work2 = HR X LVP X S%. n = 7. Unit of Work2: 10^2·mmHg/min. *, #: different from other values at $P < 0.05$.

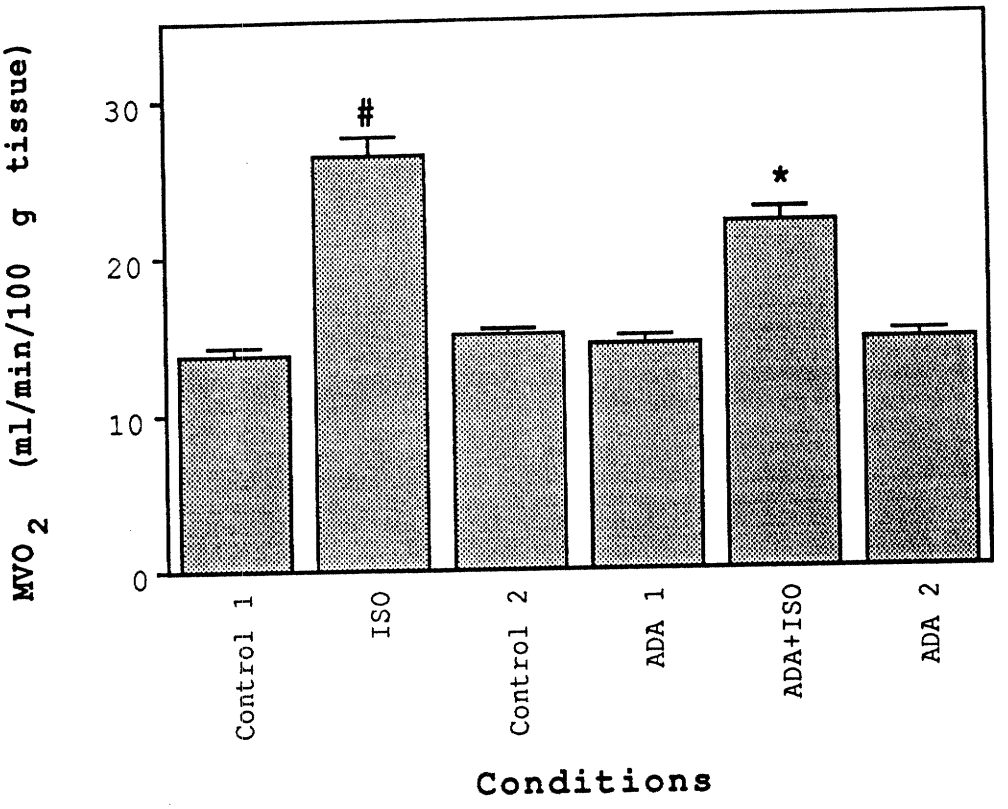

Figure 24. The myocardial oxygen consumption (MVO_2) response to ADA infusion (ADA) and to ISO infusions in absence (ISO) and presence (ADA+ISO) of ADA. n = 7. *, #: different from other values at $P < 0.05$.

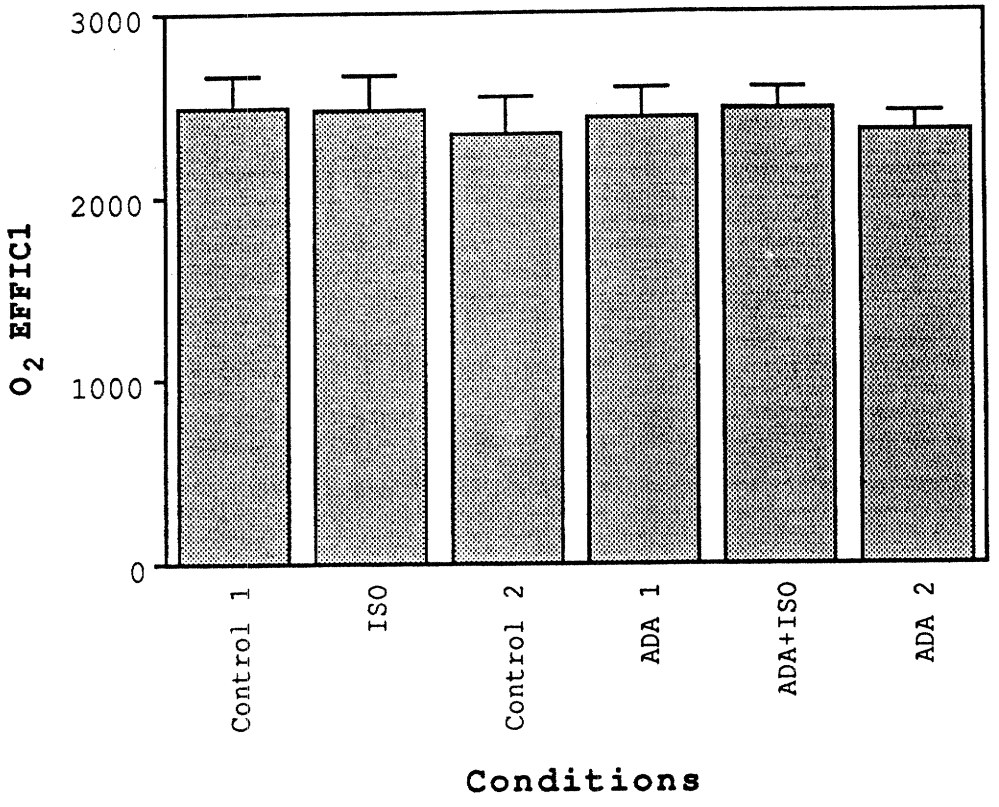

Figure 25. The estimated myocardial oxygen utilization efficiency response to ADA infusion (ADA) and to ISO infusions in absence (ISO) and presence (ADA+ISO) of ADA. Myocardial oxygen utilization efficiency was estimated as $O_2EFFIC1 = Work1/MVO_2$. n = 7. Unit of $O_2EFFIC1$: $10^6 \cdot mmHg^2/sec/ml/g$.

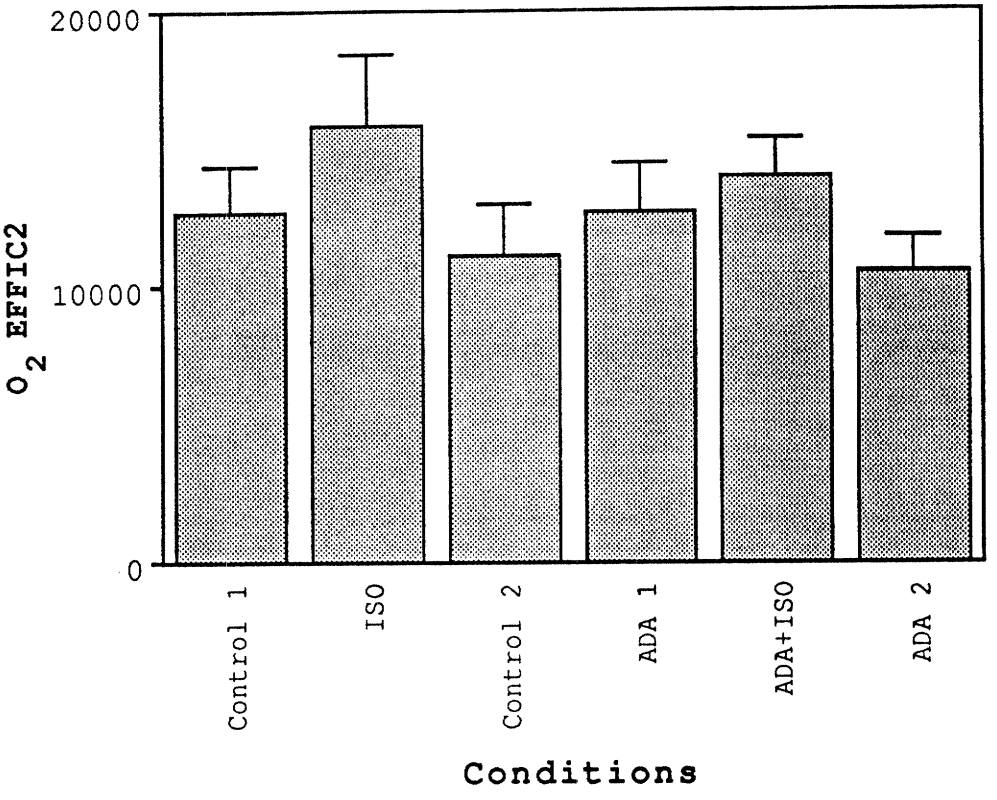

Figure 26. The estimated myocardial oxygen utilization efficiency response to ADA infusion (ADA) and to ISO infusions in absence (ISO) and presence (ADA+ISO) of ADA. Myocardial oxygen utilization efficiency was estimated as O_2EFFIC2 = Work2/MVO_2. n = 7. Unit of O_2EFFIC2: 10^3·mmHg/ml/g.

3) EHNA & ISO

Table 4 presents systemic hemodynamic, coronary oxygen extraction, coronary venous oxygen tension, coronary lactate extraction, and myocardial function responses to EHNA infusion and to ISO infusions in the absence and presence of EHNA. AoP, HR, PP, LVP, and La% were essentially constant throughout the protocol. All variables had similar values at Controls 1 and 2, and at EHNA 1 and 2. CBF response to ADO injections (2 μg, i.c.) was significantly increased from 98 ± 7% above control before EHNA to 152 ± 14% above control after 14 min EHNA infusion ($P < 0.05$). EHNA alone did not change dP/dt, CVR, P_vO_2, or O_2%. ISO alone increased dP/dt from 2220 ± 110 mmHg/s to 3540 ± 190 mmHg/s ($P < 0.05$). O_2% was 70.1 ± 1.6% before and 73.3 ±1.3% after ISO, and CVR was 77.8 ± 3.7 mmHg·min/ml·g before and 41.9 ± 1.6 mmHg·min/ml·g after ISO. When ISO was infused in the presence of EHNA, ISO decreased CVR from 74.1 ± 3.4 mmHg·min/ml·g to 41.6 ± 1.5 mmHg·min/ml·g. As shown in the table, the ISO-induced increase in dP/dt was not influenced by the presence of EHNA (3540 ± 190 without EHNA vs. 3720 ± 150 with EHNA).

TABLE IV

The responses to EHNA infusion (EHNA) and to ISO infusions in the absence (ISO) and presence (EHNA+ISO) of EHNA.

Conditions	AoP	HR	PP	LVP	dP/dt	CaO_2	CvO_2	PvO_2	CVR	La%	O_2%
Control 1	121 ± 5	146 ± 7	101 ± 1	118 ± 4	2220 ±110	20.3 ±0.9	6.1 ±0.4	25.3 ±2.0	77.8 ± 3.7	34.7 ±2.3	70.1 ±1.6
ISO	125 ± 4	155 ±10	99 ± 1	121 ± 4	3540* ±190	20.7 ±0.9	5.5 ±0.3	24.7 ±1.7	41.9 ± 1.6	35.9 ±3.1	73.3* ±1.3
Control 2	119 ± 6	146 ± 7	101 ± 1	116 ± 5	2180 ±130	20.5 ±0.9	6.2 ±0.4	25.7 ±1.9	75.1 ± 2.3	34.3 ±2.5	69.6 ±1.7
EHNA 1	122 ± 5	146 ± 7	101 ± 1	120 ± 5	2210 ±120	20.4 ±1.0	6.0 ±0.4	25.1 ±2.2	74.1 ± 3.4	33.1 ±2.3	70.4 ±1.7
EHNA+ISO	123 ± 4	155 ±10	100 ± 1	130 ± 3	3720* ±150	20.7 ±1.0	6.1 ±0.3	25.2 ±2.3	41.6 ± 1.5	32.4 ±3.4	69.9 ±1.2
EHNA 2	117 ± 6	145 ± 6	101 ± 1	119 ± 6	2140 ± 60	20.5 ±0.8	6.3 ±0.3	26.0 ±1.8	72.3 ± 1.2	33.2 ±2.9	69.2 ±1.5

Values are means ± standard errors of the mean (SEM). n = 6. AoP, mean aortic pressure; HR, heart rate; PP, coronary perfusion pressure; LVP, left ventricular pressure development; dP/dt, maximum rate of LVP; CaO_2, coronary arterial oxygen content; CvO_2, coronary venous oxygen content; PvO_2, coronary venous oxygen tension; CVR, coronary vascular resistance; La%, coronary lactate extraction; O_2%, coronary oxygen extraction. *: different from other values at $P < 0.05$.

Figures 27 through 34 illustrate the CBF, dP/dt, S%, Work1, Work2, MVO_2, O_2EFFIC1, and O_2EFFIC2 responses to EHNA infusion and to ISO infusions in absence and presence of EHNA, respectively. As shown in Figure 27, EHNA did not change CBF significantly, and the ISO-induced elevation in CBF was not influenced by the presence of EHNA. As shown in Figures 28 and 29, the ISO-induced elevations in dP/dt and S% were not influenced by the presence of EHNA. As shown in Figures 30 and 31, the ISO-induced elevations in Work1 and Work2 were not influenced by the presence of EHNA. As shown in Figure 32, the ISO-induced elevation in MVO_2 was not influenced by the presence of EHNA. However, as shown in Figure 33, O_2EFFIC1 was 2796 ± 323 X 10^6·$mmHg^2$/sec/ml/g during EHNA+ISO and 2393 ± 336 X 10^6·$mmHg^2$/sec/ml/g during ISO alone; as shown in Figure 34, O_2EFFIC2 was higher during EHNA+ISO than during ISO alone (15590 ± 1447 X 10^3·mmHg/ml/g during EHNA+ISO vs. 12810 ± 1814 X 10^6·mmHg/ml/g during ISO alone; $P < 0.05$). Thus, EHNA+ISO increased oxygen utilization efficiency slightly, when compared to ISO alone.

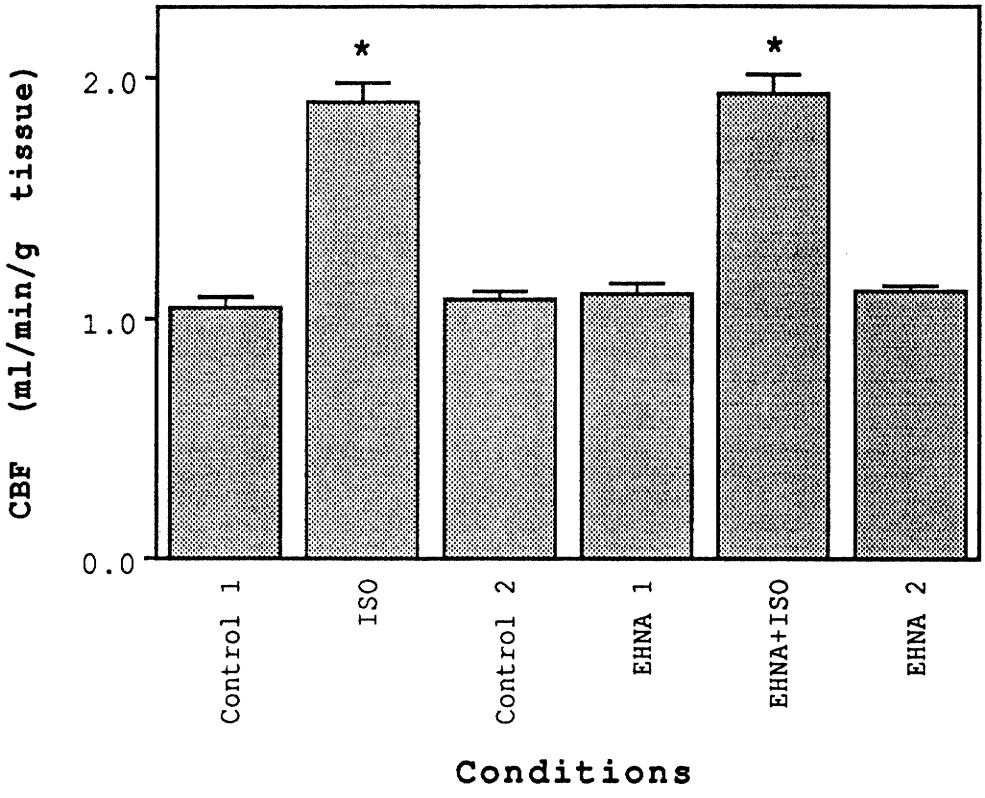

Figure 27. The coronary blood flow (CBF) response to EHNA infusion (EHNA) and to ISO infusions in absence (ISO) and presence (EHNA+ISO) of EHNA. n = 6. *: different from other values at $P < 0.05$.

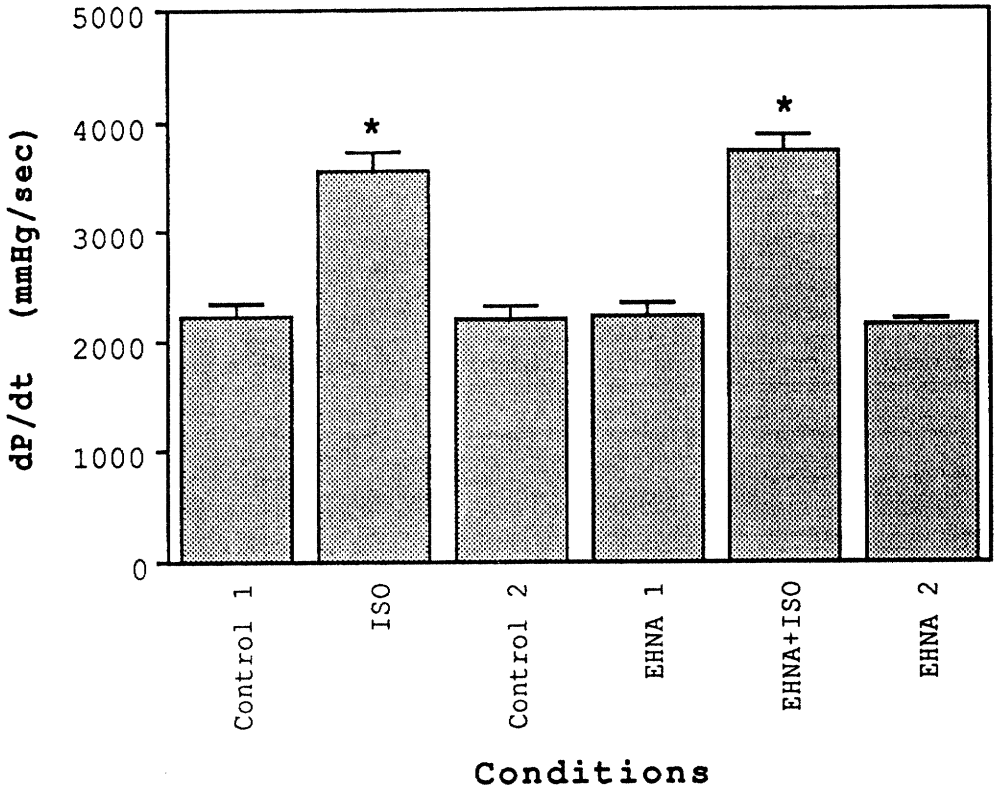

Figure 28. The maximum rate of LVP (dP/dt) response to EHNA infusion (EHNA) and to ISO infusions in absence (ISO) and presence (EHNA+ISO) of EHNA. n = 6. *: different from other values at $P < 0.05$.

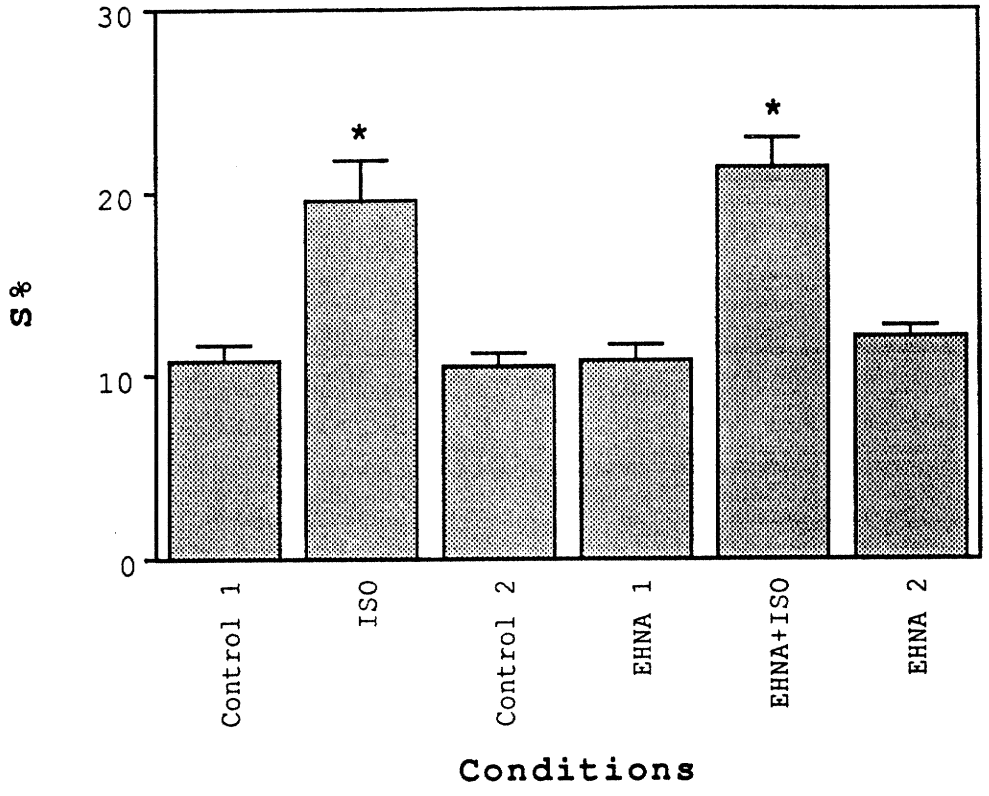

Figure 29. The percent segment shortening (S%) response to EHNA infusion (EHNA) and to ISO infusions in absence (ISO) and presence (EHNA+ISO) of EHNA. n = 6. *: different from other values at $P < 0.05$.

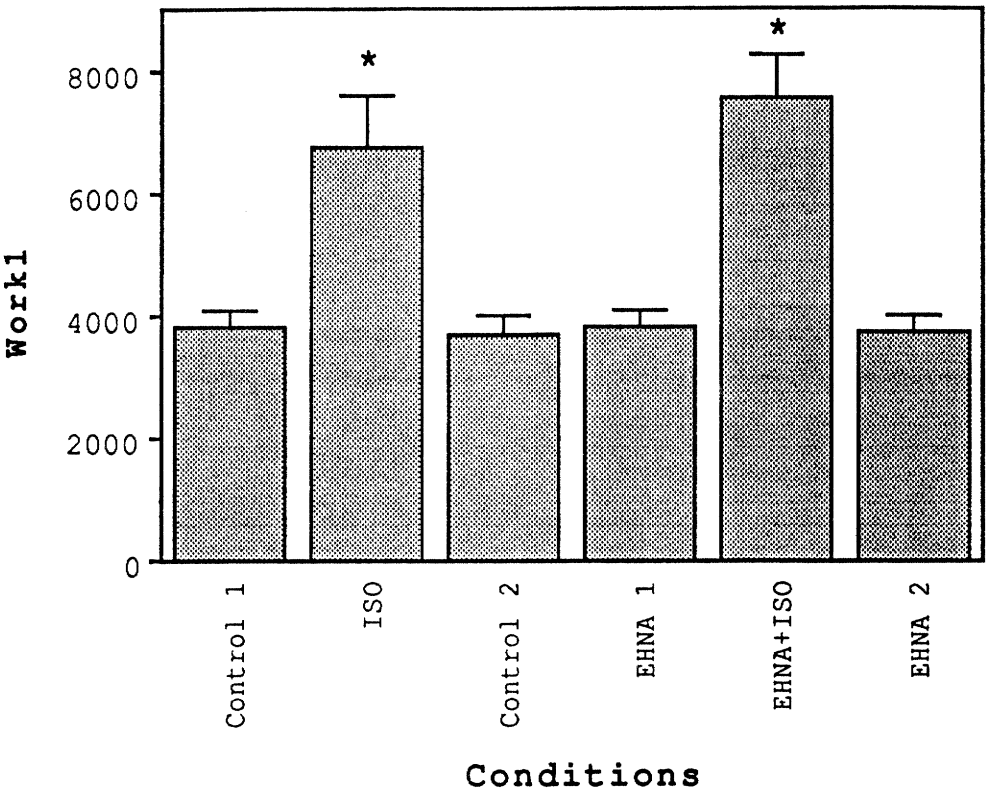

Figure 30. The estimated myocardial work response to EHNA infusion (EHNA) and to ISO infusions in absence (ISO) and presence (EHNA+ISO) of EHNA. Myocardial work was estimated as Work1 = HR X LVP X dP/dt. n = 6. Unit of Work1: $10^4 \cdot mmHg^2/sec/min$. *: different from other values at $P < 0.05$.

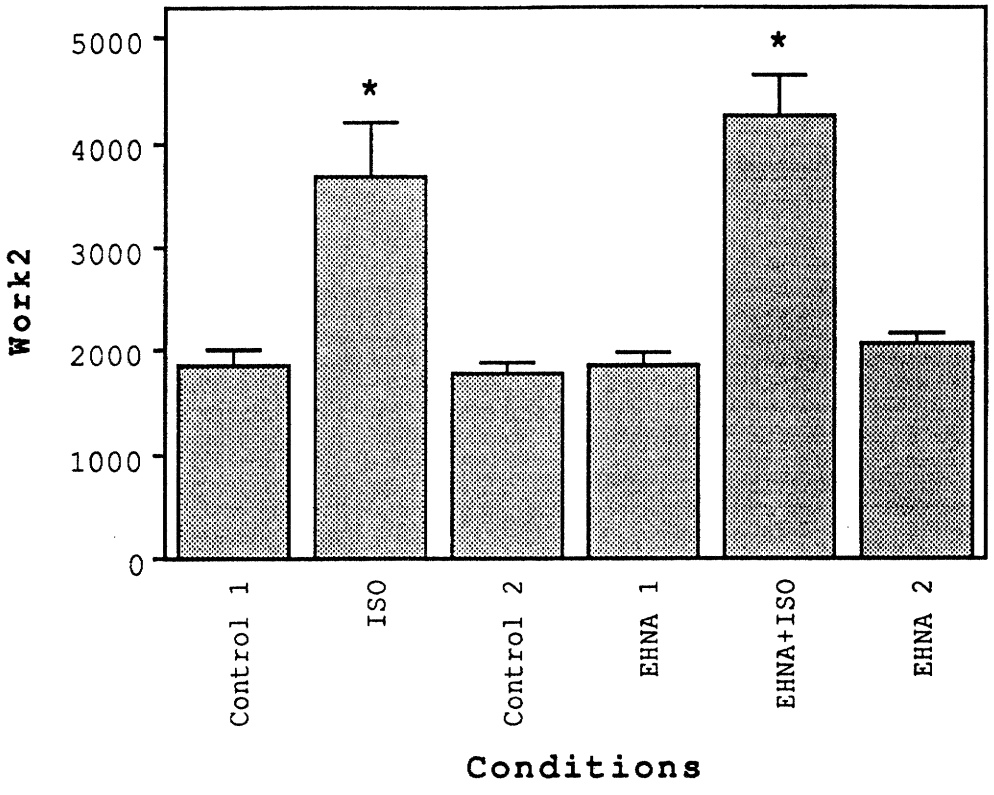

Figure 31. The estimated myocardial work response to EHNA infusion (EHNA) and to ISO infusions in absence (ISO) and presence of EHNA (EHNA+ISO). Myocardial work was estimated as Work2 = HR X LVP X S%. n = 6. Unit of Work2: 10^2·mmHg/min. *: different from other values at $P < 0.05$.

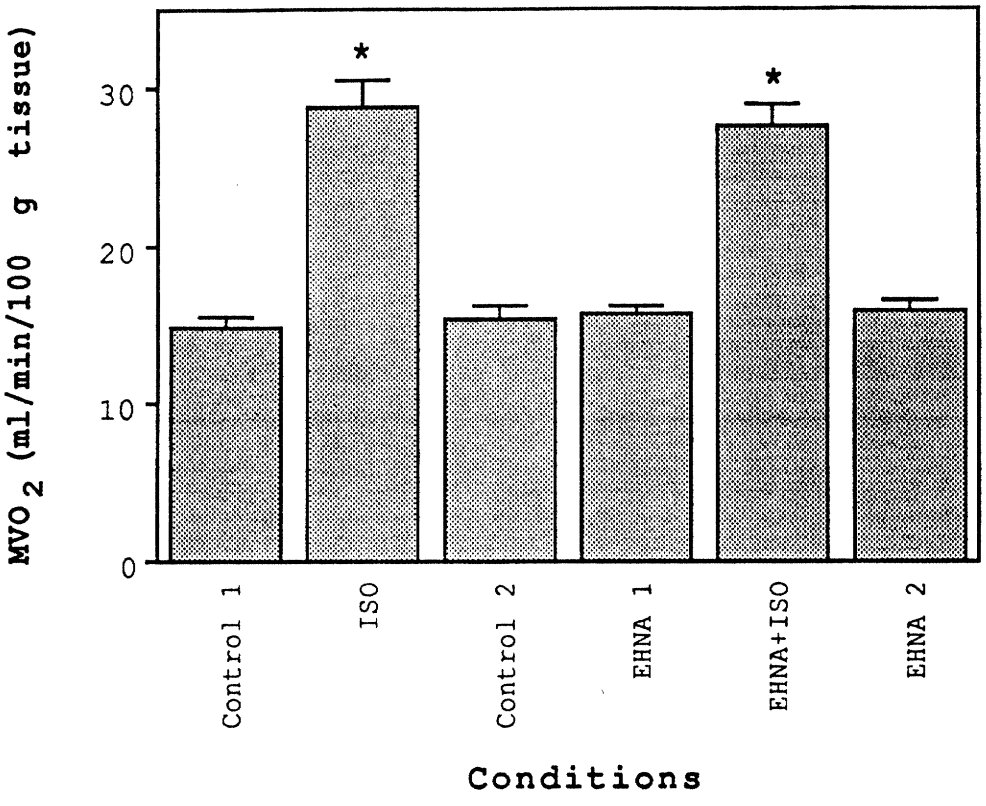

Figure 32. The myocardial oxygen consumption (MVO_2) response to EHNA infusion (EHNA) and to ISO infusions in absence (ISO) and presence (EHNA+ISO) of EHNA. n = 6. *: different from other values at $P < 0.05$.

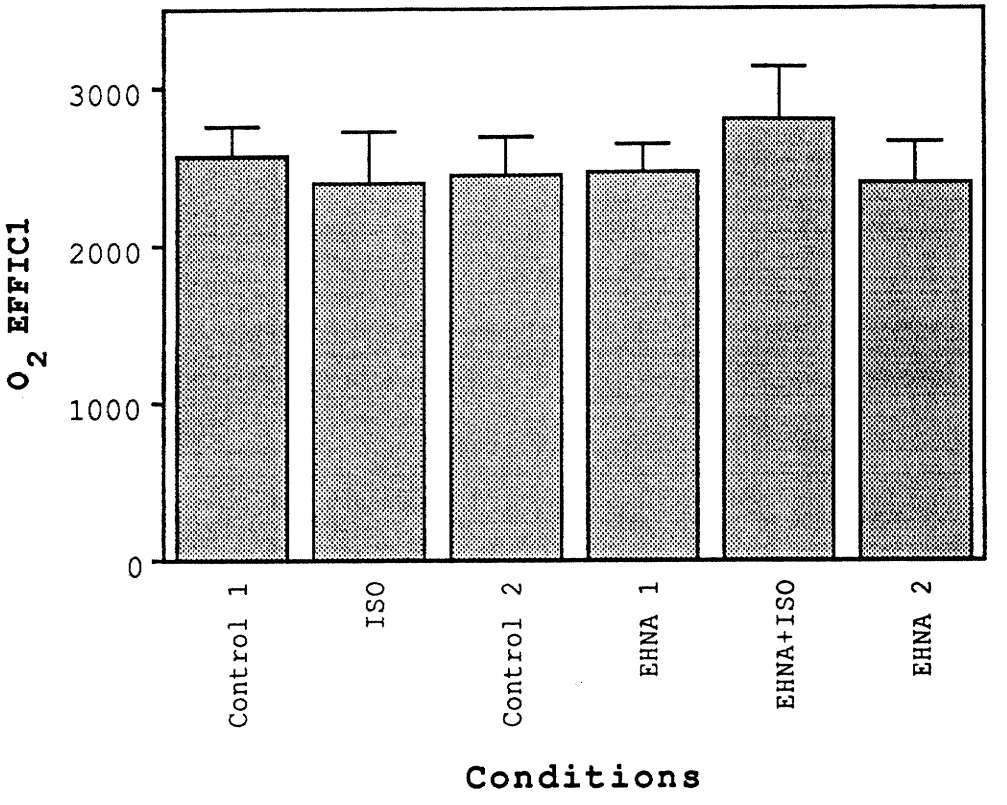

Figure 33. The estimated myocardial oxygen utilization efficiency response to EHNA infusion (EHNA) and to ISO infusions in absence (ISO) and presence (EHNA+ISO) of EHNA. Myocardial oxygen utilization efficiency was estimated as O_2EFFIC1 = Work1/MVO_2. n = 6. Unit of O_2EFFIC1: $10^6 \cdot mmHg^2/sec/ml/g$.

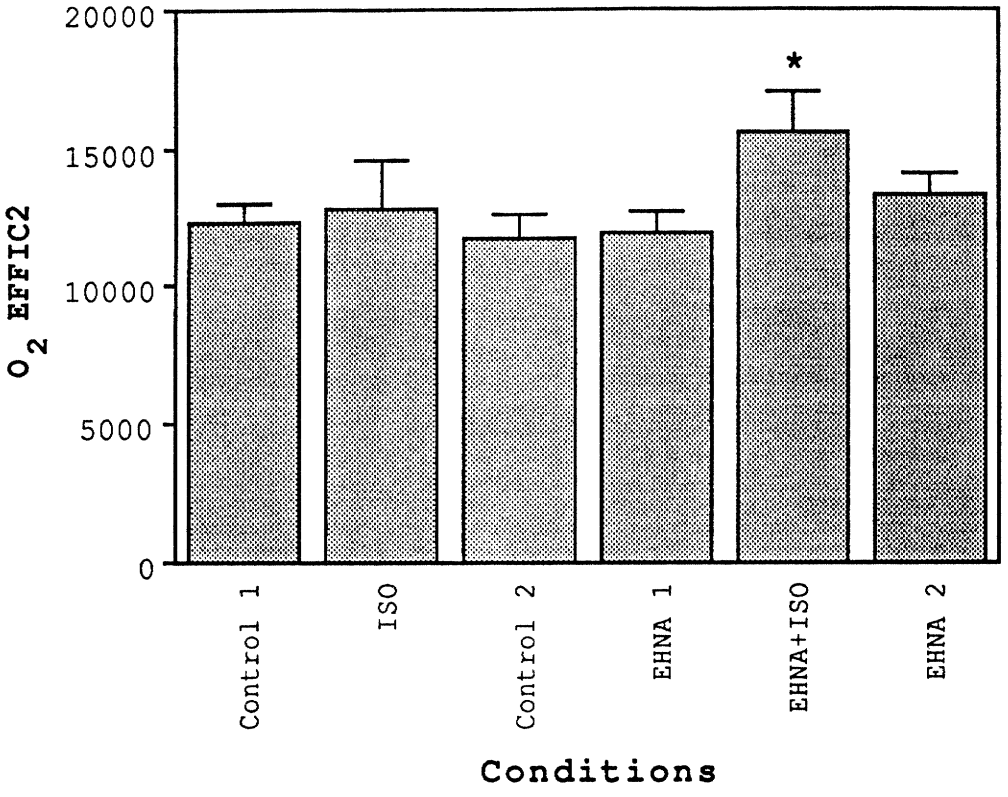

Figure 34. The estimated myocardial oxygen utilization efficiency response to EHNA infusion (EHNA) and to ISO infusions in absence (ISO) and presence (EHNA+ISO) of EHNA. Myocardial oxygen utilization efficiency was estimated as O_2EFFIC2 = Work2/MVO_2. n = 6. Unit of O_2EFFIC2: 10^3·mmHg/ml/g. *: different from other values at $P < 0.05$.

Normoxemia, PP = 60 mmHg

4) LP & ISO

Table 5 presents systemic hemodynamic values, coronary oxygen extraction, coronary venous oxygen tension, coronary lactate extraction, and myocardial function responses to low perfusion pressure and to ISO infusions during normal perfusion pressure, i.e., 100 ± 1 mmHg and low perfusion pressure, i.e., 60 ± 1 mmHg. AoP, HR, LVP, and La% were essentially constant throughout the protocol. All variables had similar values at Control 1 and 2, and at LP 1 and LP 2. LP decreased PP from 100 ± 1 mmHg to 60 ± 1 mmHg, but did not change dP/dt. O_2% was 70.1 ± 1.0% before and 73.3 ± 0.7% after LP, and CVR was 75.6 ± 1.9 mmHg·min/ml·g before and 54.6 ± 1.6 mmHg·min/ml·g after LP. ISO alone increased dP/dt from 2380 ± 150 mmHg/s to 3430 ± 200 mmHg/s ($P < 0.05$). O_2% was 69.6 ± 1.1% before and 72.2 ± 0.9% after ISO, and P_vO_2 was 26.1 ± 1.8 mmHg before and 25.5 ± 2.0 mmHg after ISO. When ISO was infused during LP, ISO decreased CVR from 54.6 ± 1.6 mmHg·min/ml·g to 36.9 ± 1.2 mmHg·min/ml·g. As shown in the table, the ISO-induced increase in dP/dt was not significantly influenced by LP (3430 ± 200 mmHg/sec during ISO vs. 3490 ± 160 mmHg/sec during LP+ISO).

TABLE V

The responses to low perfusion pressure (LP) and to ISO infusions in the absence (ISO) and presence (LP+ISO) of low perfusion pressure.

Conditions	AoP	HR	PP	LVP	dP/dt	CaO_2	CvO_2	PvO_2	CVR	La%	O_2%
Control 1	119 ± 2	141 ± 6	100 ± 1	114 ± 2	2380 ±150	19.9 ±0.5	6.1 ±0.3	26.1 ±1.8	76.8 ± 2.3	37.4 ±2.8	69.6 ±1.1
ISO	120 ± 2	147 ± 3	100 ± 1	121 ± 4	3430* ±200	20.2 ±0.5	5.6 ±0.2	25.5 ±2.0	45.6 ± 1.6	36.6 ±5.9	72.2 ±0.9
Control 2	123 ± 3	141 ± 6	101 ± 1	114 ± 3	2170 ± 90	20.4 ±0.7	6.1 ±0.3	27.1 ±1.6	75.6 ± 1.9	41.4 ±3.7	70.1 ±1.0
LP 1	119 ± 2	141 ± 5	60* ± 0	111 ± 2	2180 ±100	20.3 ±0.5	5.4 ±0.2	25.0 ±1.6	54.6 ± 1.6	30.9 ±4.0	73.3 ±0.7
LP+ISO	126 ± 3	144 ± 2	59* ± 1	121 ± 3	3490* ±160	20.0 ±0.5	5.3 ±0.1	24.6 ±1.7	36.9 ± 1.2	43.6 ±3.7	73.5 ±0.7
LP 2	123 ± 4	143 ± 6	59* ± 1	114 ± 2	2070 ± 90	20.1 ±0.6	5.6 ±0.3	25.2 ±1.8	55.0 ± 1.2	35.6 ±2.8	71.9 ±0.5

Values are means ± standard errors of the mean (SEM). n = 7. AoP, mean aortic pressure; HR, heart rate; PP, coronary perfusion pressure; LVP, left ventricular pressure development; dP/dt, maximum rate of LVP; CaO_2, coronary arterial oxygen content; CvO_2, coronary venous oxygen content; PvO_2, coronary venous oxygen tension; CVR, coronary vascular resistance; La%, coronary lactate extraction; O_2%, coronary oxygen extraction. *: different from other values at $P < 0.05$.

Figures 35 through 42 illustrate the CBF, dP/dt, S%, Work1, Work2, MVO_2, O_2EFFIC1, and O_2EFFIC2 responses to low perfusion pressure and to ISO infusions during normal perfusion pressure, i.e., 100 ± 1 mmHg and low perfusion pressure, i.e., 60 ± 1 mmHg, respectively. As shown in Figure 35, LP decreased CBF from 1.07 ± 0.02 ml/min/g to 0.73 ± 0.02 ml/min/g ($P < 0.05$), and ISO increased CBF from 0.73 ± 0.02 ml/min/g to 1.06 ± 0.03 ml/min/g when infused during LP. As shown in Figures 36 and 37, the ISO-induced increases in dP/dt and S% were not significantly influenced by LP. As shown in Figures 38 and 39, the ISO-induced increases in Work1 and Work2 were not significantly influenced by LP. As shown in Figure 40, LP decreased MVO_2 from 15.2 ± 0.5 ml/min/100 g to 10.8 ± 0.5 ml/min/100 g, and ISO-induced elevation in MVO_2 was decreased from 26.0 ± 1.5 ml/min/100 g to 15.7 ± 2.6 ml/min/100 g ($P < 0.05$) in the presence of LP. This 40% reduction in the MVO_2 is apparently due to the low oxygen delivery, and it is this reduction that resulted in a significantly higher oxygen utilization efficiency during ISO+LP than during ISO infusion alone. Compared to ISO alone, ISO infusion during LP increased O_2EFFIC1 and O_2EFFIC2 significantly, as shown in Figures 41 and 42, respectively. Thus, ISO+LP increased oxygen utilization efficiency by 60% when compared to ISO alone.

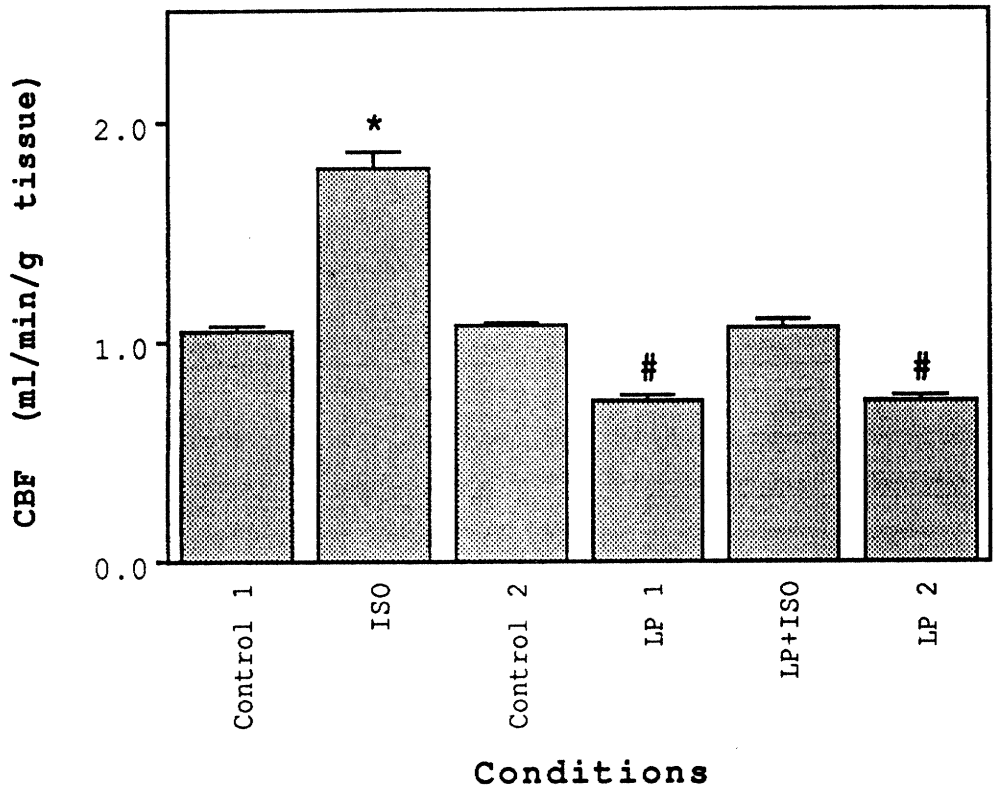

Figure 35. The coronary blood flow (CBF) response to low perfusion pressure (LP) and to ISO infusions in absence (ISO) and presence (LP+ISO) of low perfusion pressure. n = 7. *, #: different from other values at $P < 0.05$.

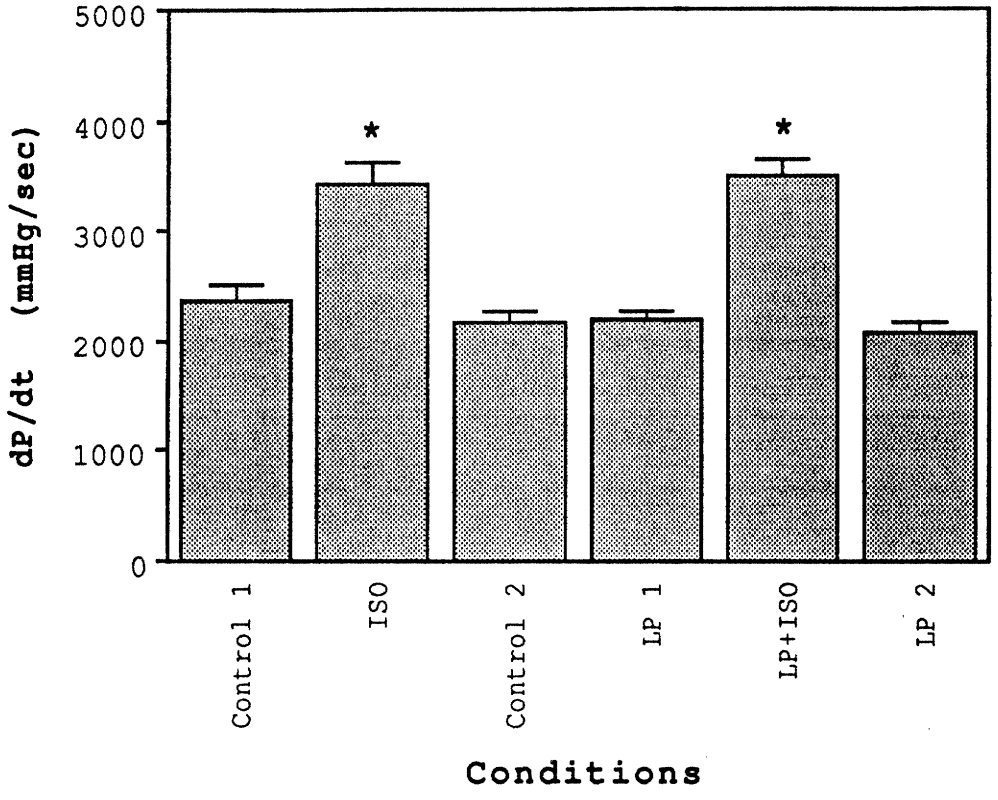

Figure 36. The maximum rate of LVP (dP/dt) response to low perfusion pressure (LP) and to ISO infusions in absence (ISO) and presence (LP+ISO) of low perfusion pressure. n = 7. *: different from other values at $P < 0.05$.

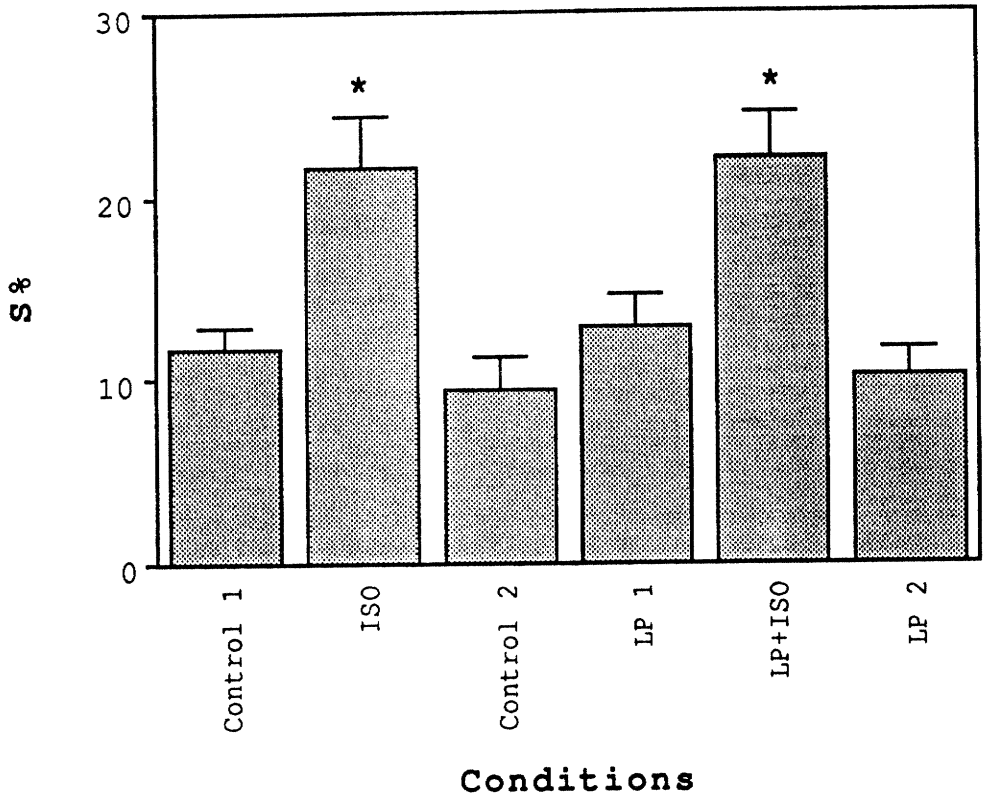

Figure 37. The percent segment shortening (S%) response to low perfusion pressure (LP) and to ISO infusions in absence (ISO) and presence (LP+ISO) of low perfusion pressure. n = 7. *: different from other values at $P < 0.05$.

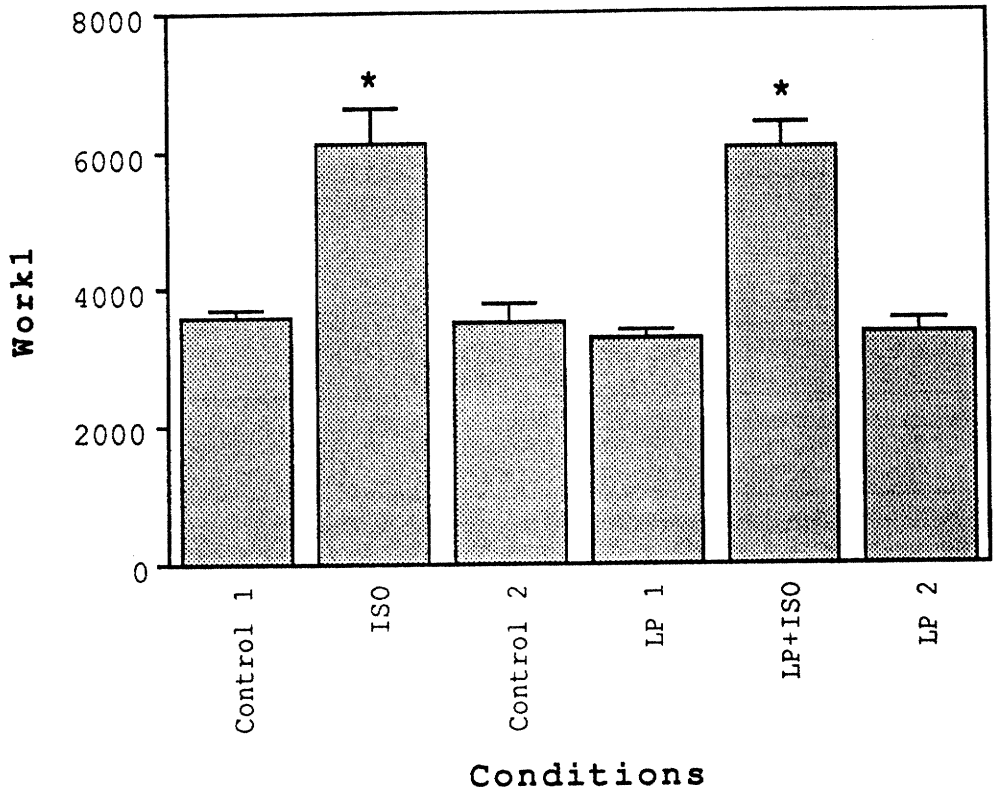

Figure 38. The estimated myocardial work response to low perfusion pressure (LP) and to ISO infusions in absence (ISO) and presence (LP+ISO) of low perfusion pressure. Myocardial work was estimated as Work1 = HR X LVP X dP/dt. n = 7. Unit of Work1: $10^4 \cdot mmHg^2/sec/min$. *: different from other values at $P < 0.05$.

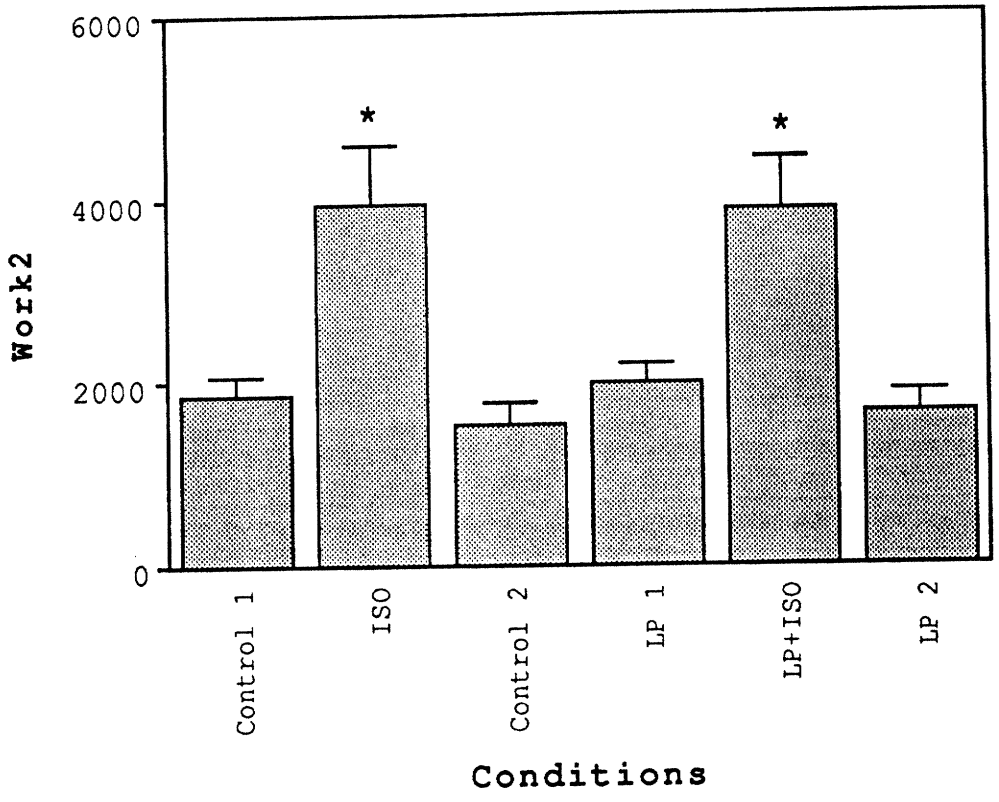

Figure 39. The estimated myocardial work response to low perfusion pressure (LP) and to ISO infusions in absence (ISO) and presence (LP+ISO) of low perfusion pressure. Myocardial work was estimated as Work2 = HR X LVP X S%. n = 7. Unit of Work2: 10^2·mmHg/min. *: different from other values at $P < 0.05$.

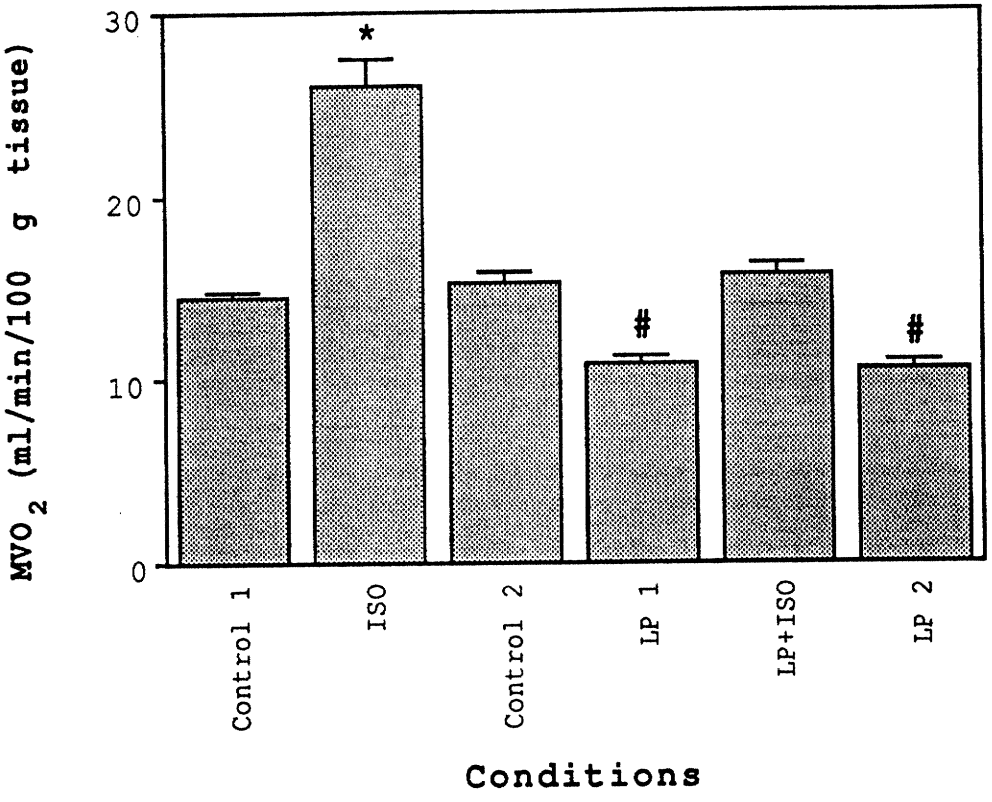

Figure 40. The myocardial oxygen consumption (MVO_2) response to low perfusion pressure (LP) and to ISO infusions in absence (ISO) and presence (LP+ISO) of low perfusion pressure. n = 7. *, #: different from other values at $P < 0.05$.

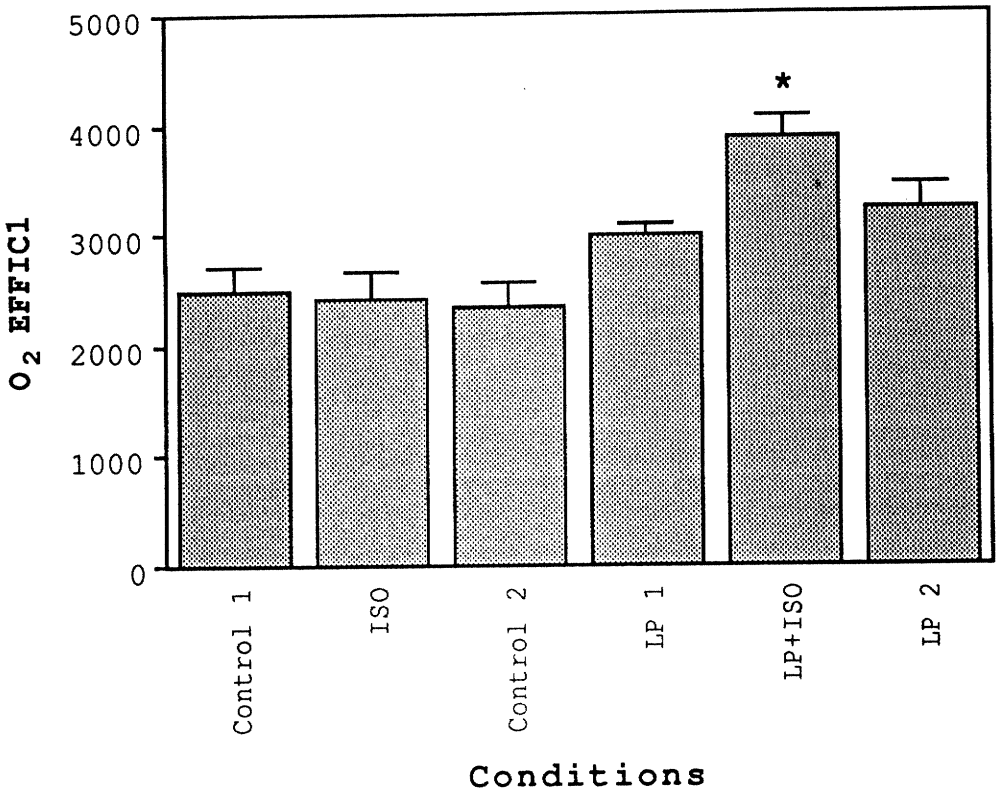

Figure 41. The estimated myocardial oxygen utilization efficiency response to low perfusion pressure (LP) and to ISO infusions in absence (ISO) and presence (LP+ISO) of low perfusion pressure. Myocardial oxygen utilization efficiency was estimated as $O_2EFFIC1 = Work1/MVO_2$. n = 7. Unit of $O_2EFFIC1$: $10^6 \cdot mmHg^2/sec/ml/g$. *: different from other values at $P < 0.05$.

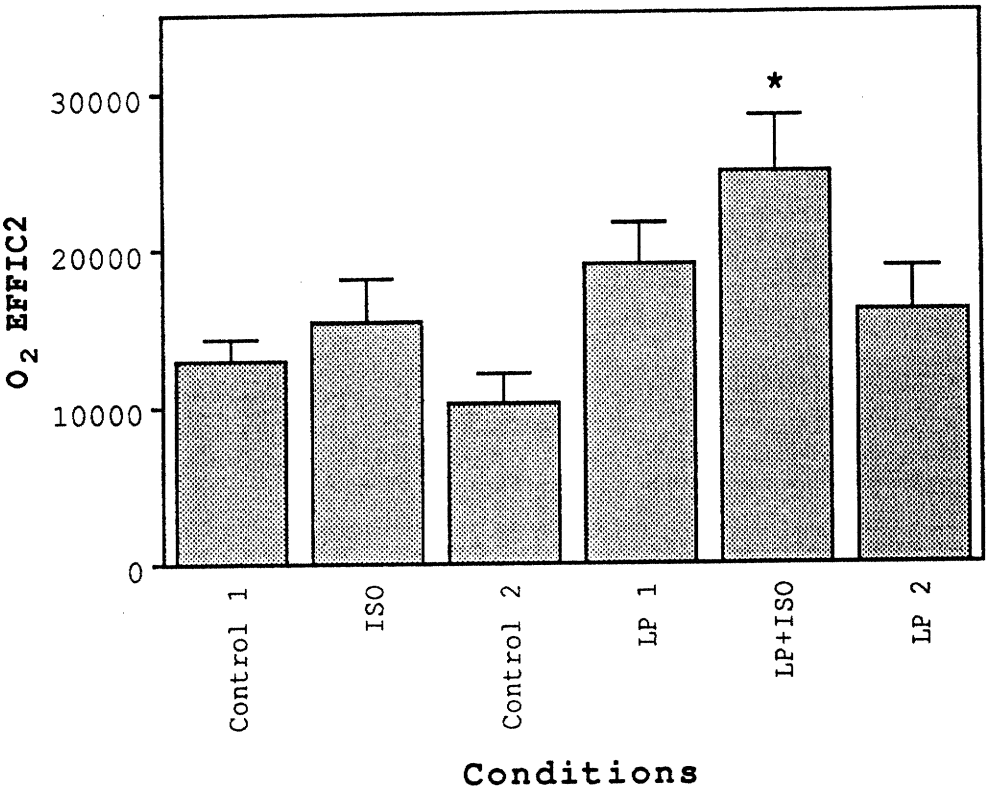

Figure 42. The estimated myocardial oxygen utilization efficiency response to low perfusion pressure (LP) and to ISO infusions in absence (ISO) and presence (LP+ISO) of low perfusion pressure. Myocardial oxygen utilization efficiency was estimated as $O_2EFFIC2 = Work2/MVO_2$. n = 7. Unit of $O_2EFFIC2$: $10^3 \cdot mmHg/ml/g$. *: different from other values at $P < 0.05$.

5) ADA/LP & ISO

Table 6 presents systemic hemodynamic values, coronary oxygen extraction, coronary venous oxygen tension, coronary lactate extraction, and myocardial function responses to ADA infusion and to ISO infusions in the absence and presence of ADA. PP were held at 60 ± 1 mmHg throughout the protocol. AoP, HR, LVP, La%, CaO_2, P_vO_2, and O_2% were essentially constant throughout the protocol except ADA/LP 2. All variables had similar values at LP 1 and 2. ADA alone did not change dP/dt or CVR. ISO alone increased dP/dt from 2140 ± 110 mmHg/s to 3610 ± 140 mmHg/s ($P < 0.05$). O_2% was 73.0 ± 0.9% before and 73.3 ± 0.8% after ISO, and CVR was 55.0 ± 2.8 mmHg·min/ml·g before and 38.9 ± 1.2 mmHg·min/ml·g after ISO. When ISO was infused in the presence of ADA, ISO decreased CVR from 53.7 ± 2.7 mmHg·min/ml·g to 37.8 ± 1.0 mmHg·min/ml·g. As shown in the table, the ISO-induced increase in dP/dt was decreased by the presence of ADA (3610 ± 140 without ADA vs. 3230 ± 180 with ADA, $P < 0.05$).

TABLE VI

The responses to ADA infusion (ADA/LP) and to ISO infusions in the absence (LP+ISO) and presence (ADA/LP+ISO) of ADA when PP was held at 60 mmHg.

Conditions	AoP	HR	PP	LVP	dP/dt	CaO_2	CvO_2	PvO_2	CVR	La%	O_2%
LP 1	126 ± 5	142 ±10	60 ± 1	118 ± 3	2140 ±110	20.0 ±0.5	5.4 ±0.5	24.9 ±1.3	55.0 ± 2.8	36.9 ± 4.8	73.0 ±0.9
LP+ISO	132 ± 4	150 ± 7	59 ± 1	124 ± 8	3610* ±140	20.1 ±0.4	5.4 ±0.4	24.8 ±1.6	38.9 ± 1.2	30.7 ± 6.0	73.3 ±0.8
LP 2	127 ± 4	143 ± 6	60 ± 1	115 ± 8	2130 ± 90	19.8 ±0.5	5.5 ±0.5	25.0 ±1.4	54.5 ± 2.9	31.4 ± 5.3	72.1 ±0.9
ADA/LP 1	127 ± 3	141 ±10	60 ± 0	116 ± 4	2320 ±220	20.0 ±0.7	5.5 ±0.5	25.2 ±1.9	53.7 ± 2.7	30.3 ± 4.8	72.3 ±0.9
ADA/LP+ISO	135 ± 6	144 ± 9	60 ± 1	123 ± 8	3230* ±180	19.9 ±0.8	5.5 ±0.7	24.8 ±2.1	37.8 ± 1.0	30.3 ± 5.3	72.4 ±0.9
ADA/LP 2	126 ± 4	130 ± 6	60 ± 1	105 ± 6	1870# ±100	19.8 ±0.8	9.2 ±2.5	29.4 ±3.0	46.6 ± 2.7	20.5 ±15.5	53.6 ±4.5

Values are means ± standard errors of the mean (SEM). n = 7. AoP, mean aortic pressure; HR, heart rate; PP, coronary perfusion pressure; LVP, left ventricular pressure development; dP/dt, maximum rate of LVP; CaO_2, coronary arterial oxygen content; CvO_2, coronary venous oxygen content; PvO_2, coronary venous oxygen tension; CVR, coronary vascular resistance; La%, coronary lactate extraction; O_2%, coronary oxygen extraction. *, #: different from other values at $P < 0.05$.

Figures 43 through 50 illustrate the CBF, dP/dt, S%, Work1, Work2, MVO_2, O_2EFFIC1, and O_2EFFIC2 responses to ADA infusion and to ISO infusions in absence and presence of ADA when PP was held at 60 ± 1 mmHg, respectively. As shown in Figure 43, ADA alone did not change CBF significantly, and the ISO-induced increase in CBF was not significantly influenced by ADA. As shown in Figure 44, the ISO-induced elevation in dP/dt was reduced from 3610 ± 140 mmHg/sec to 3230 ± 180 mmHg/sec ($P < 0.09$) in the presence of ADA. As shown in Figure 45, the ISO-induced elevation in S% was reduced by 20% in the presence of ADA. As shown in Figures 46 and 47, the ISO-induced elevations in Work1 and Work2 were reduced by 15% and 35%, respectively, in the presence of ADA. As shown in Figure 48, the ISO-induced increase in MVO_2 was not significantly influenced by ADA. These similar increases in the MVO_2 and the significant decreases in Work1 and Work2 resulted in significant decreases in oxygen utilization efficiencies by 15% and 34%, as shown in Figures 49 and 50, respectively. Thus, ADA/LP+ISO decreased myocardial work and oxygen utilization efficiency, and maintained MVO_2 when compared to ISO alone.

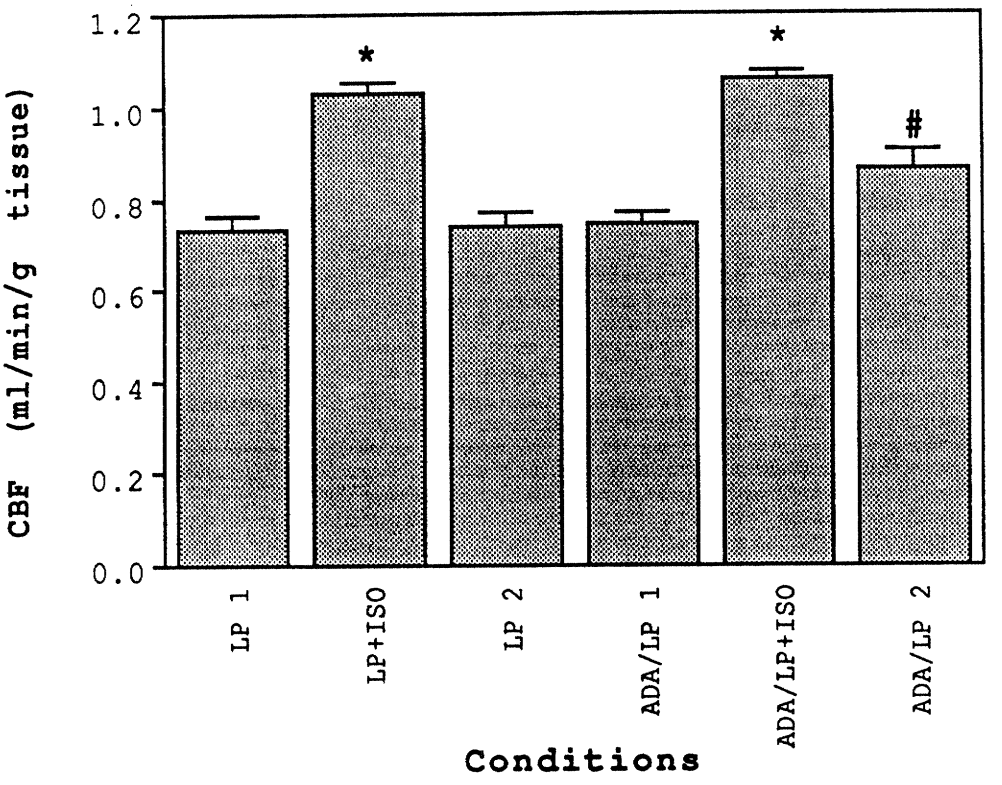

Figure 43. The coronary blood flow (CBF) response to ADA infusion (ADA/LP) and to ISO infusions in absence (LP+ISO) and presence (ADA/LP+ISO) of ADA when PP was held at 60 mmHg. n = 7. *, #: different from other values at $P < 0.05$.

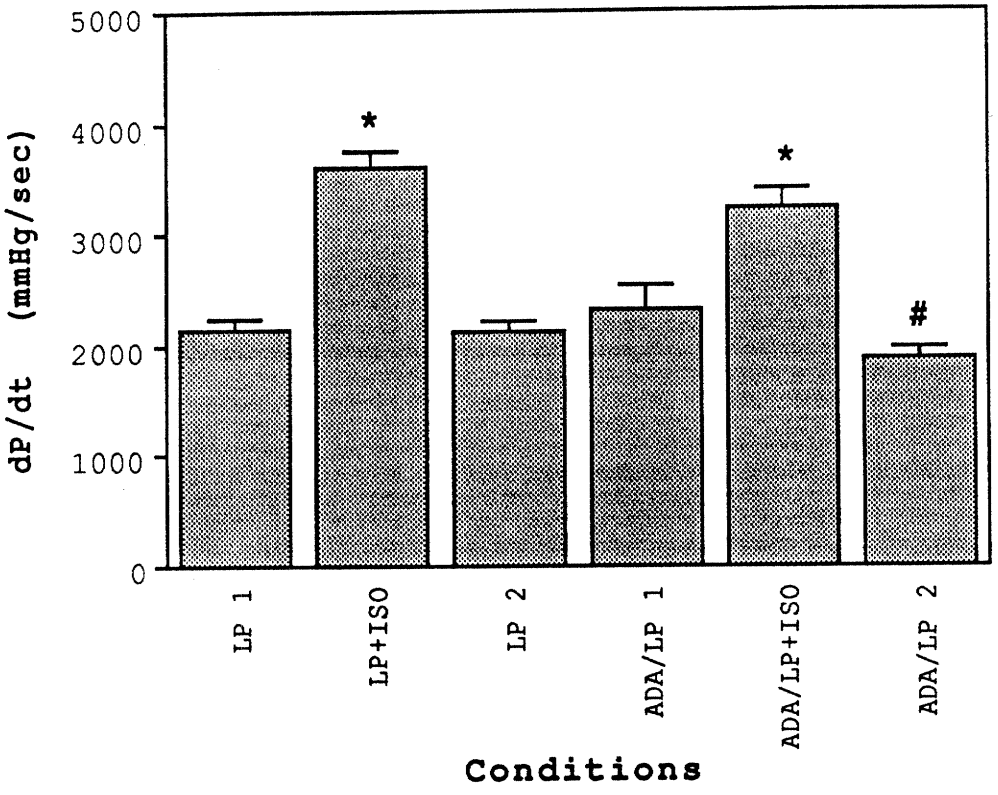

Figure 44. The maximum rate of LVP (dP/dt) response to ADA infusion (ADA/LP) and to ISO infusions in absence (LP+ISO) and presence (ADA/LP+ISO) of ADA when PP was held at 60 mmHg. n = 7. *, #: different from other values at $P < 0.05$.

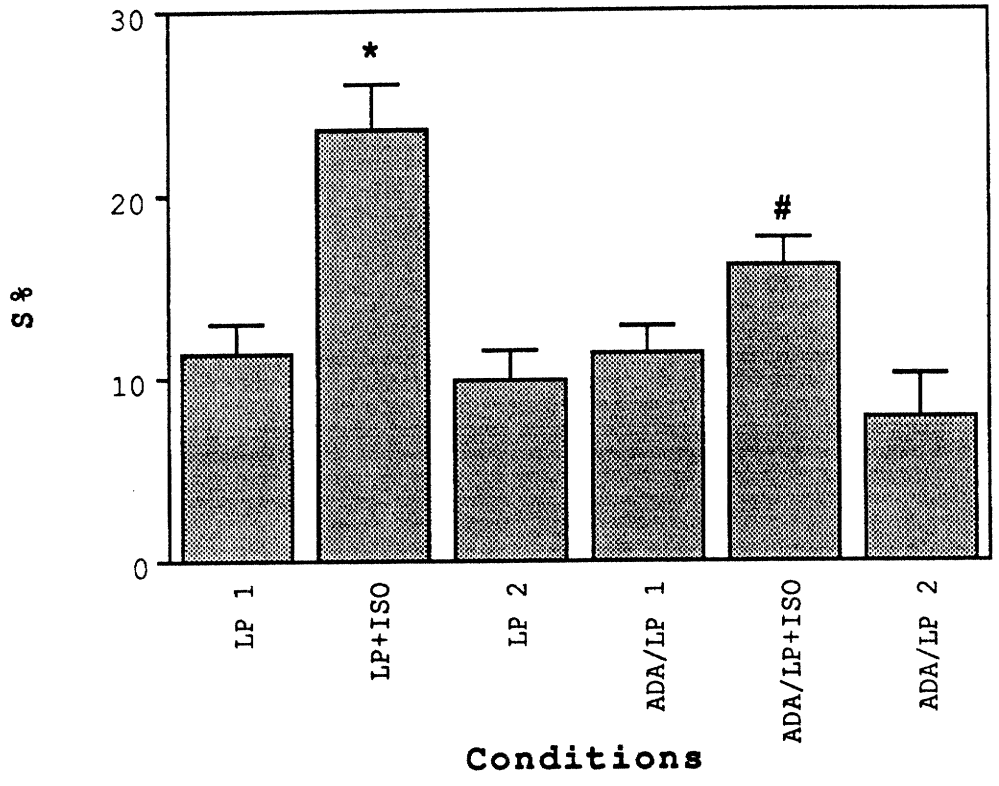

Figure 45. The percent segment shortening (S%) response to ADA infusion (ADA/LP) and to ISO infusions in absence (LP+ISO) and presence (ADA/LP+ISO) of ADA when PP was held at 60 mmHg. n = 7. *, #: different from other values at $P < 0.05$.

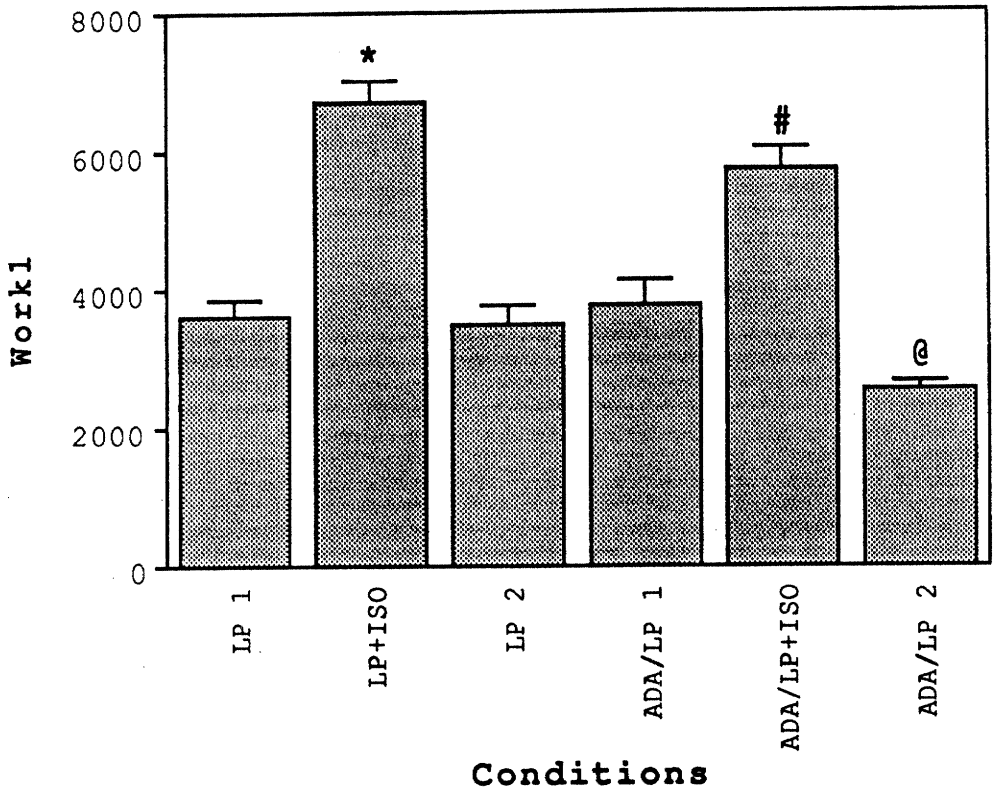

Figure 46. The estimated myocardial work response to ADA infusion (ADA/LP) and to ISO infusions in absence (LP+ISO) and presence (ADA/LP+ISO) of ADA when PP was held at 60 mmHg. Myocardial work was estimated as Work1 = HR X LVP X dP/dt. n = 7. Unit of Work1: $10^4 \cdot mmHg^2/sec/min$. *, #, @: different from other values at $P < 0.05$.

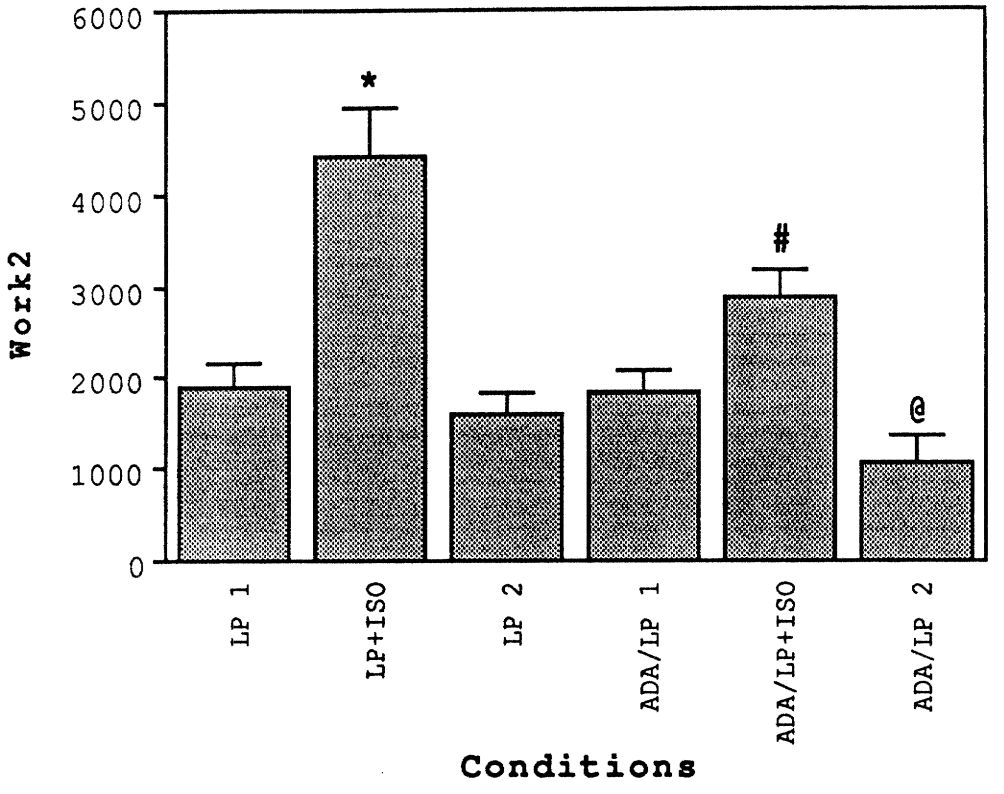

Figure 47. The estimated myocardial work response to ADA infusion (ADA/LP) and to ISO infusions in absence (LP+ISO) and presence (ADA/LP+ISO) of ADA when PP was held at 60 mmHg. Myocardial work was estimated as Work2 = HR X LVP X S%. n = 7. Unit of Work2: 10^2·mmHg/min. *, #, @: different from other values at $P < 0.05$.

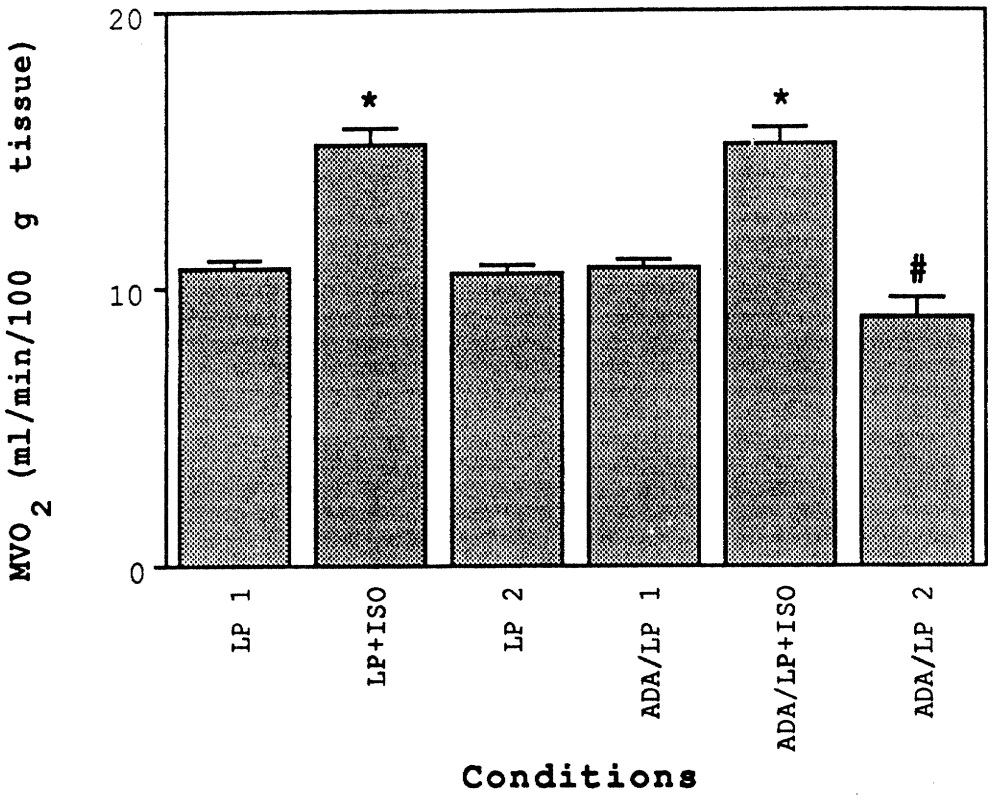

Figure 48. The myocardial oxygen consumption (MVO_2) response to ADA infusion (ADA/LP) and to ISO infusions in absence (LP+ISO) and presence (ADA/LP+ISO) of ADA when PP was held at 60 mmHg. n = 7. *, #: different from other values at $P < 0.05$.

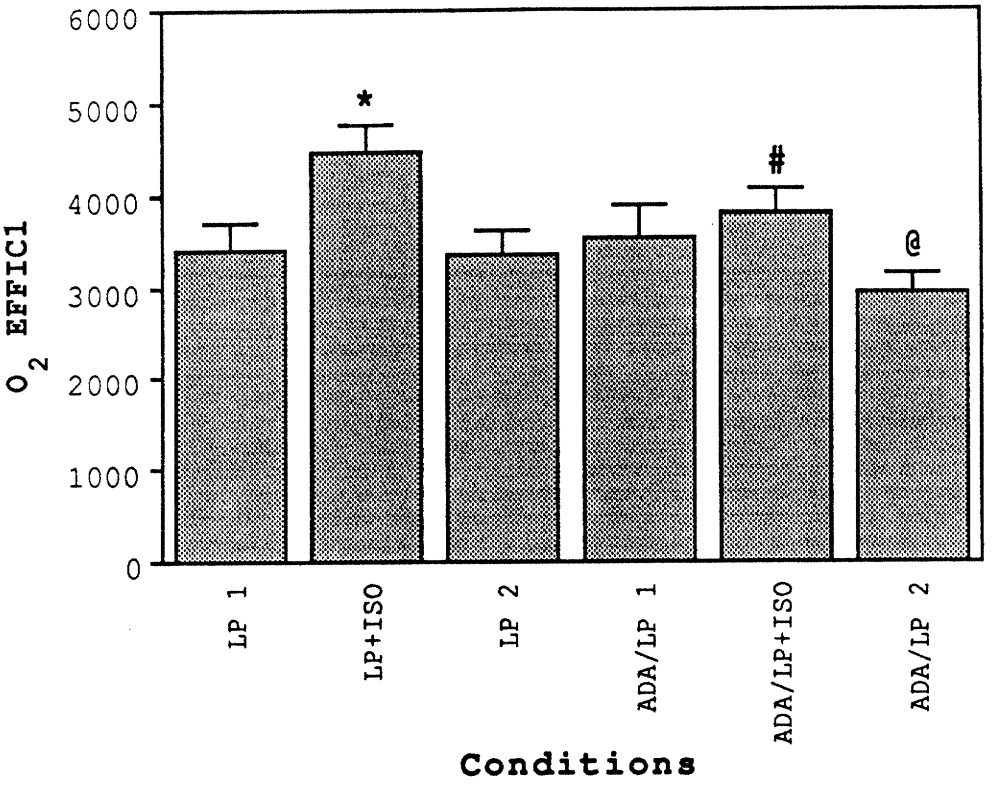

Figure 49. The estimated myocardial oxygen utilization efficiency response to ADA infusion (ADA/LP) and to ISO infusions in absence (LP+ISO) and presence (ADA/LP+ISO) of ADA when PP was held at 60 mmHg. Myocardial oxygen utilization efficiency was estimated as O_2EFFIC1 = Work1/MVO_2. n = 7. Unit of O_2EFFIC1: $10^6 \cdot mmHg^2/sec/ml/g$. *, #, @: different from other values at $P < 0.05$.

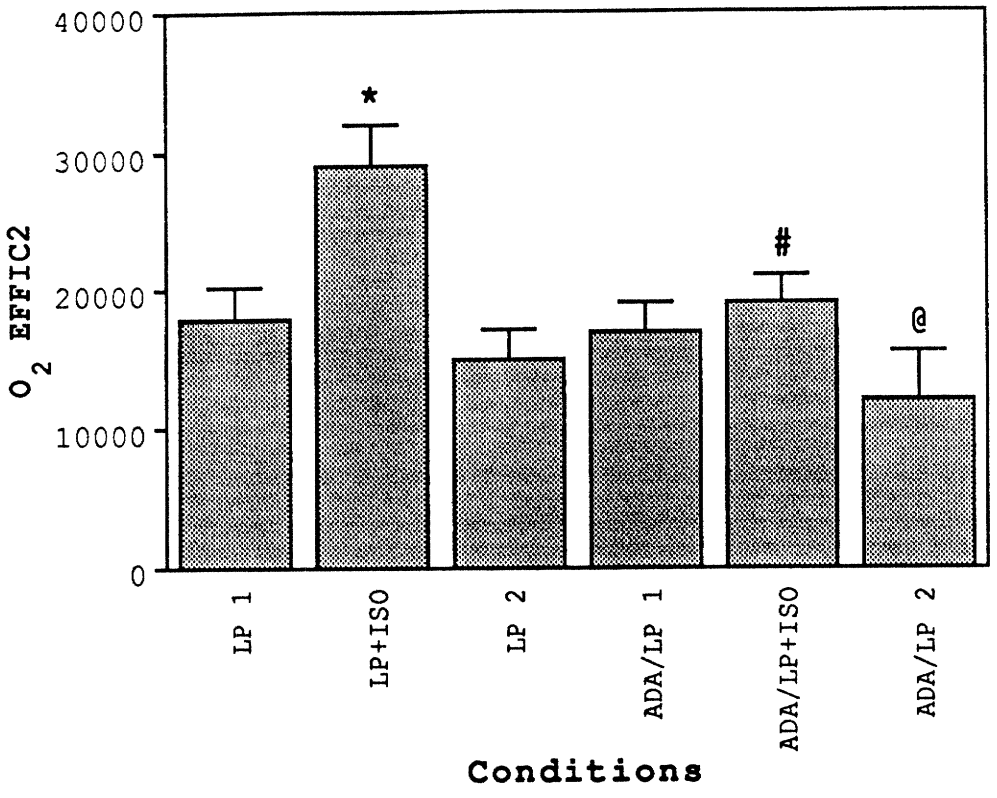

Figure 50. The estimated myocardial oxygen utilization efficiency response to ADA infusion (ADA/LP) and to ISO infusions in absence (LP+ISO) and presence (ADA/LP+ISO) of ADA when PP was held at 60 mmHg. Myocardial oxygen utilization efficiency was estimated as O_2EFFIC2 = Work2/MVO_2. n = 7. Unit of O_2EFFIC2: 10^3·mmHg/ml/g. *, #, @: different from other values at $P < 0.05$.

6) EHNA/LP & ISO

Table 7 presents systemic hemodynamic, coronary oxygen extraction, coronary venous oxygen tension, coronary lactate extraction, and myocardial function responses to EHNA infusion and to ISO infusions in the absence and presence of EHNA. PP were held around 60 mmHg throughout the protocol. AoP, HR, LVP, La%, CaO_2, P_vO_2, and O_2% were essentially constant throughout the protocol. All variables had similar values at LP 1 and 2. EHNA alone did not change dP/dt or CVR. ISO alone increased dP/dt from 2250 ± 120 mmHg/s to 3610 ± 180 mmHg/s ($P < 0.05$). O_2% was 73.1 ± 0.6% before and 73.9 ± 0.5% after ISO, and CVR was 52.6 ± 2.5 mmHg·min/ml·g before and 36.2 ± 2.0 mmHg·min/ml·g after ISO. When ISO was infused in the presence of EHNA, ISO decreased CVR from 53.2 ± 2.2 mmHg·min/ml·g to 34.5 ± 1.6 mmHg·min/ml·g. As shown in the table, the ISO-induced increase in dP/dt was increased by the presence of EHNA (3610 ± 180 without EHNA vs. 4010 ± 200 with EHNA, $P < 0.05$).

TABLE VII

The responses to EHNA infusion (EHNA/LP) and to ISO infusions in the absence (LP+ISO) and presence (EHNA/LP+ISO) of EHNA when PP was held at 60 mmHg.

Conditions	AoP	HR	PP	LVP	dP/dt	CaO_2	CvO_2	PvO_2	CVR	La%	O_2%
LP 1	124 ± 5	146 ± 8	58 ± 1	121 ± 5	2250 ±120	20.4 ±0.9	5.5 ±0.3	24.9 ±2.3	52.6 ± 2.5	29.1 ±1.4	73.1 ±0.6
LP+ISO	126 ± 4	156 ± 9	58 ± 1	125 ± 4	3610* ±180	20.7 ±0.9	5.4 ±0.3	24.6 ±1.7	36.2 ± 2.0	32.7 ±1.7	73.9 ±0.5
LP 2	122 ± 5	146 ± 7	59 ± 1	120 ± 4	2250 ±120	20.8 ±1.0	5.6 ±0.3	25.1 ±2.1	53.1 ± 1.7	27.7 ±2.1	73.2 ±0.8
EHNA/LP 1	122 ± 5	147 ± 7	59 ± 1	122 ± 5	2210 ±120	20.7 ±0.9	5.5 ±0.3	25.0 ±2.2	53.2 ± 2.2	28.2 ±2.7	73.4 ±0.5
EHNA/LP+ISO	127 ± 5	166 ± 8	59 ± 1	133 ± 3	4010# ±200	20.4 ±0.8	5.6 ±0.3	25.0 ±2.0	34.5 ± 1.6	24.9 ±4.2	72.6 ±0.7
EHNA/LP 2	123 ± 4	146 ± 7	59 ± 1	121 ± 4	2190 ± 60	20.6 ±0.8	5.6 ±0.3	25.2 ±2.6	54.5 ± 2.9	27.0 ±3.4	72.9 ±0.7

Values are means ± standard errors of the mean (SEM). n = 6. AoP, mean aortic pressure; HR, heart rate; PP, coronary perfusion pressure; LVP, left ventricular pressure development; dP/dt, maximum rate of LVP; CaO_2, coronary arterial oxygen content; CvO_2, coronary venous oxygen content; PvO_2, coronary venous oxygen tension; CVR, coronary vascular resistance; La%, coronary lactate extraction; O_2%, coronary oxygen extraction. *, #: different from other values at $P < 0.05$.

Figures 51 through 58 illustrate the CBF, dP/dt, S%, Work1, Work2, MVO_2, O_2EFFIC1, and O_2EFFIC2 responses to EHNA infusion and to ISO infusions in absence and presence of EHNA when PP was held around 60 mmHg, respectively. As shown in Figure 51, EHNA alone did not change CBF significantly, and the ISO-induced increase in CBF were not significantly influenced by EHNA. As shown in Figures 52 and 53, the ISO-induced elevations in dP/dt and S% were further increased by 11% and 23%, respectively, in the presence of EHNA. As shown in Figures 54 and 55, The ISO-induced elevations in Work1 and Work2 were further increased by 25% and 38%, respectively, in the presence of EHNA. As shown in Figure 56, the ISO-induced increase in MVO_2 was not significantly influenced by EHNA. This similar increase in the MVO_2 and the significant increases in Work1 and Work2 resulted in significant 23% and 35% increases in oxygen utilization efficiencies, as shown in Figures 57 and 58, respectively. Thus, EHNA/LP+ISO increased myocardial work and oxygen utilization efficiency, and maintained MVO_2 when compared to LP+ISO alone.

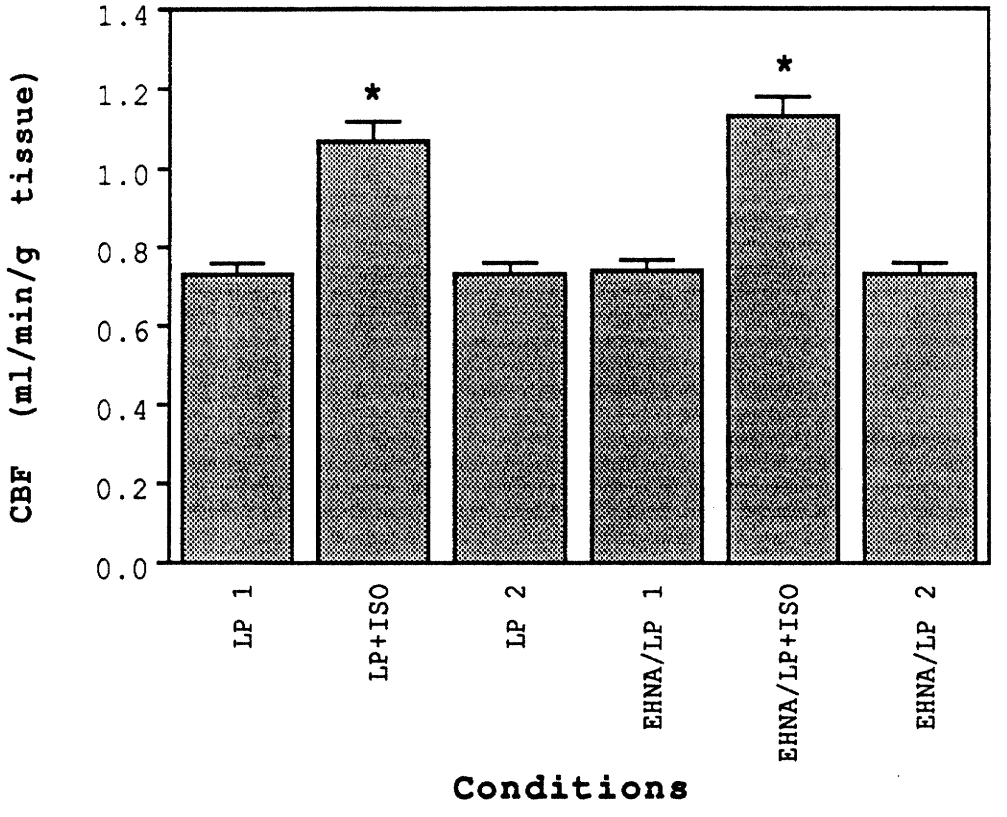

Figure 51. The coronary blood flow (CBF) response to EHNA infusion (EHNA/LP) and to ISO infusions in absence (LP+ISO) and presence (EHNA/LP+ISO) of EHNA when PP was held at 60 mmHg. n = 6. *: different from other values at $P < 0.05$.

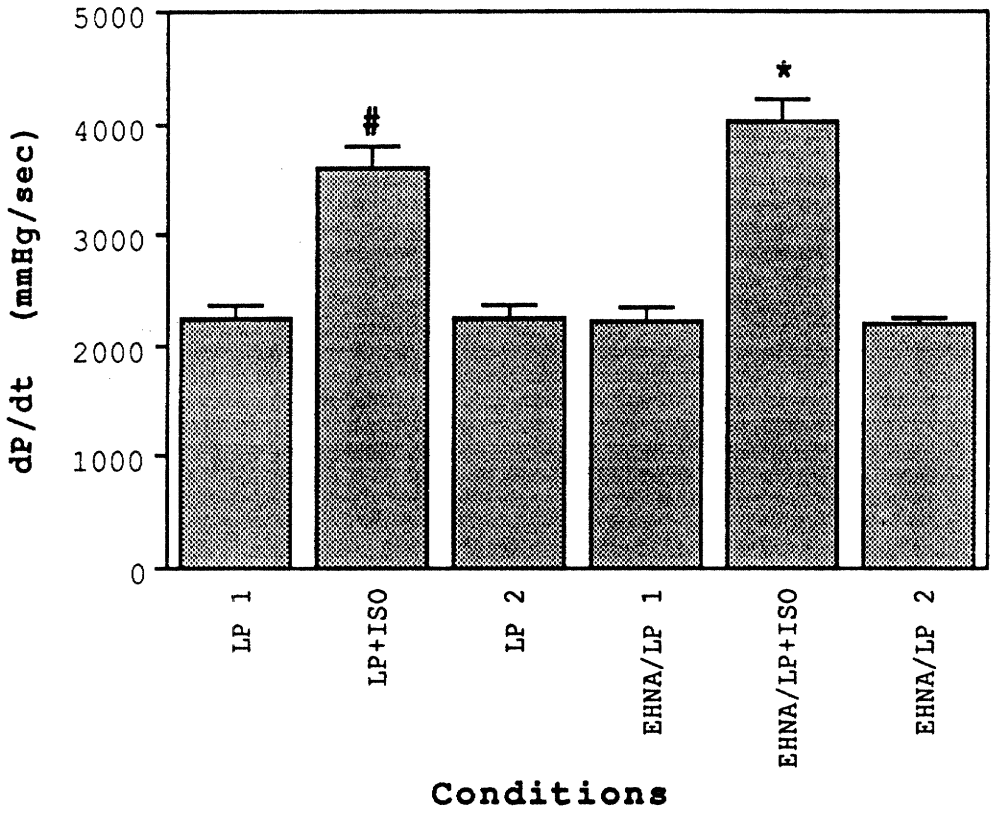

Figure 52. The maximum rate of LVP (dP/dt) response to EHNA infusion (EHNA/LP) and to ISO infusions in absence (LP+ISO) and presence (EHNA/LP+ISO) of EHNA when PP was held at 60 mmHg. n = 6. *, #: different from other values at $P < 0.05$.

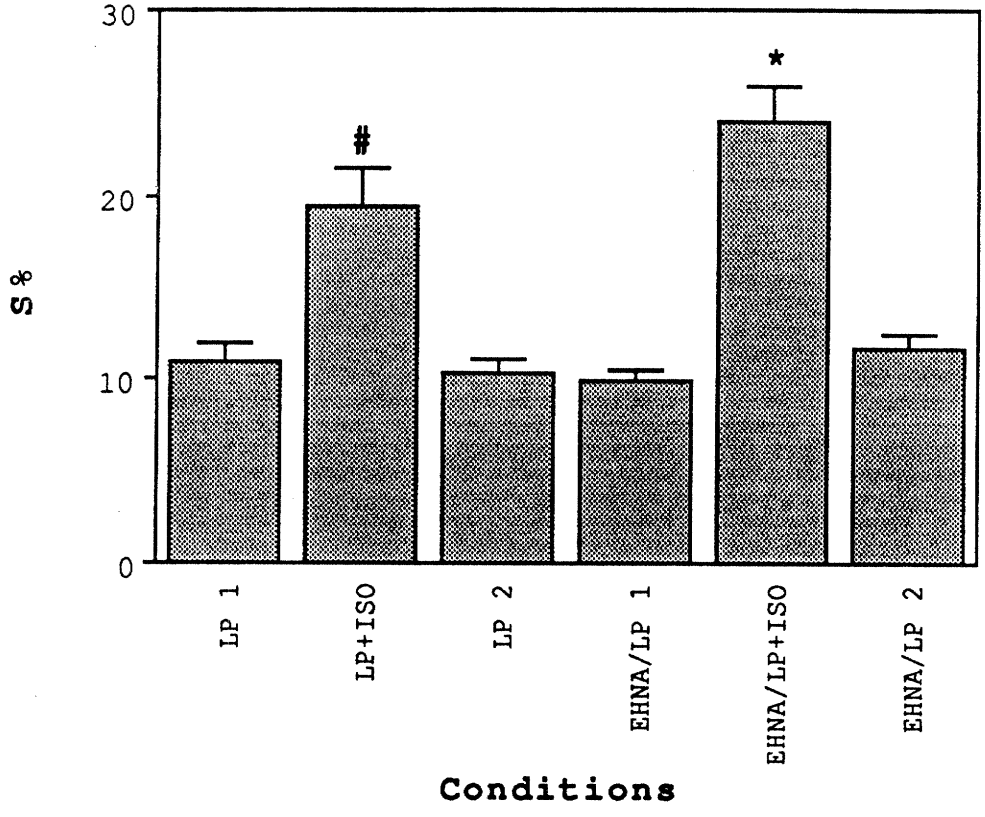

Figure 53. The percent segment shortening (S%) response to EHNA infusion (EHNA/LP) and to ISO infusions in absence (LP+ISO) and presence (EHNA/LP+ISO) of EHNA when PP was held at 60 mmHg. n = 6. *, #: different from other values at $P < 0.05$.

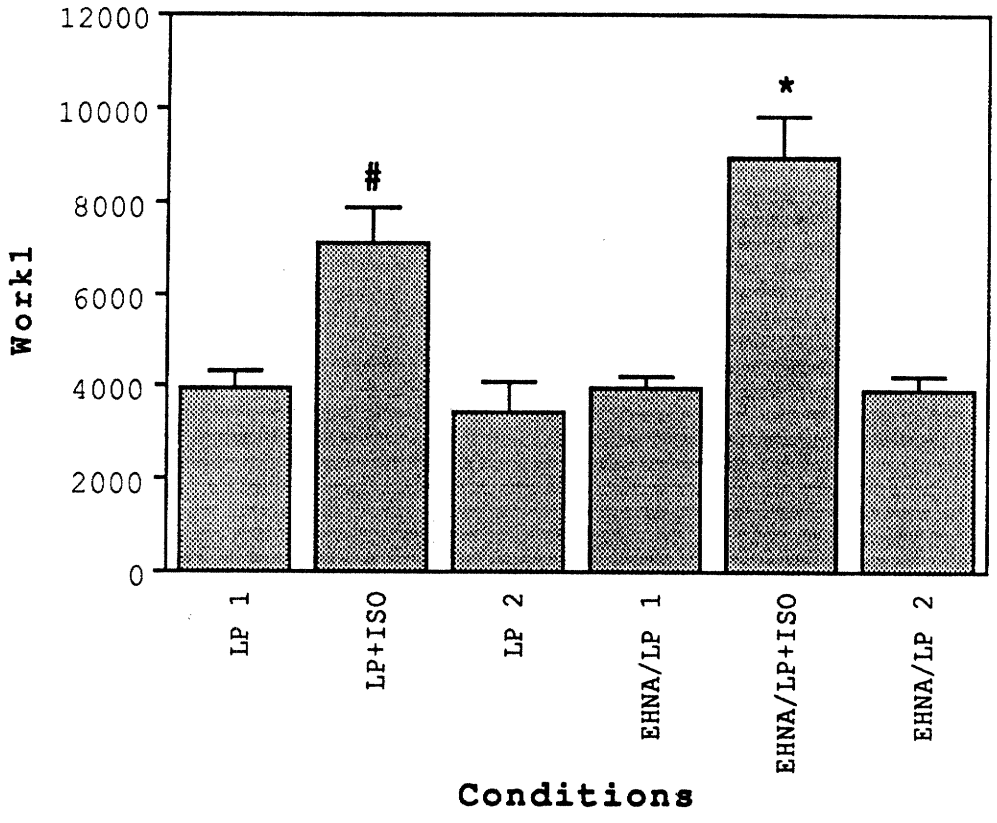

Figure 54. The estimated myocardial work response to EHNA infusion (EHNA/LP) and to ISO infusions in absence (LP+ISO) and presence (EHNA/LP+ISO) of EHNA when PP was held at 60 mmHg. Myocardial work was estimated as Work1 = HR X LVP X dP/dt. n = 6. Unit of Work1: $10^4 \cdot mmHg^2/sec/min$. *, #: different from other values at $P < 0.05$.

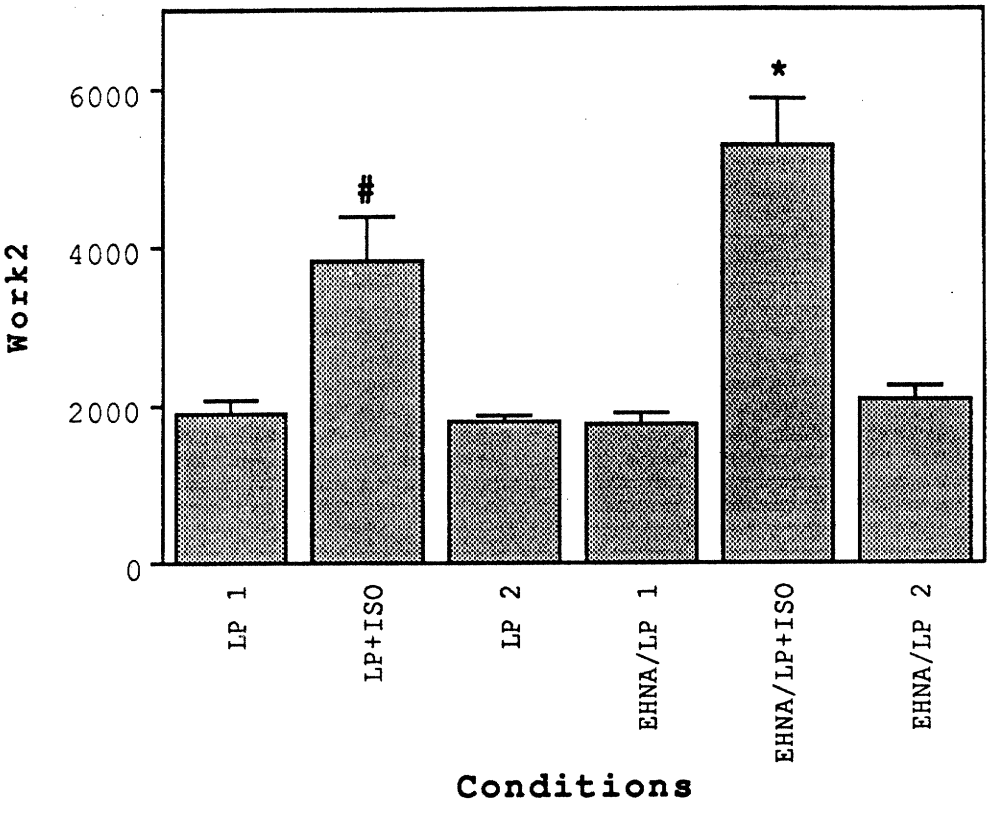

Figure 55. The estimated myocardial work response to EHNA infusion (EHNA/LP) and to ISO infusions in absence (LP+ISO) and presence of EHNA (EHNA/LP+ISO) when PP was held at 60 mmHg. Myocardial work was estimated as Work2 = HR X LVP X S%. n = 6. Unit of Work2: 10^2·mmHg/min. *, #: different from other values at $P < 0.05$.

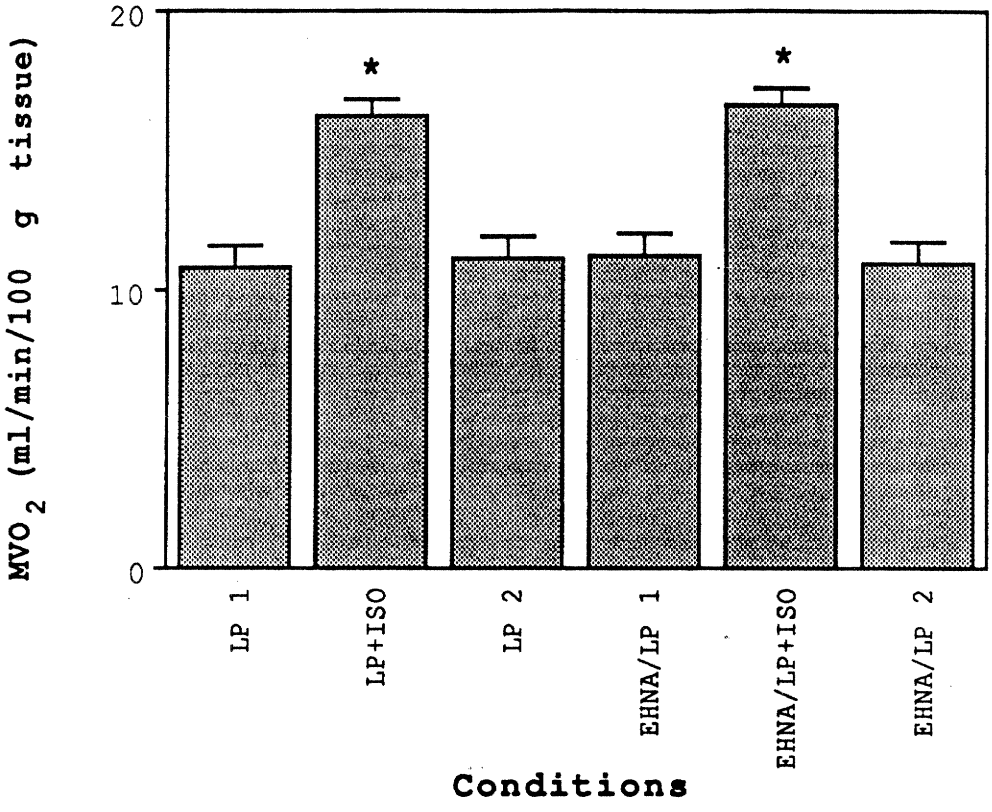

Figure 56. The myocardial oxygen consumption ($\dot{M}VO_2$) response to EHNA infusion (EHNA/LP) and to ISO infusions in absence (LP+ISO) and presence (EHNA/LP+ISO) of EHNA when PP was held at 60 mmHg. n = 6. *: different from other values at $P < 0.05$.

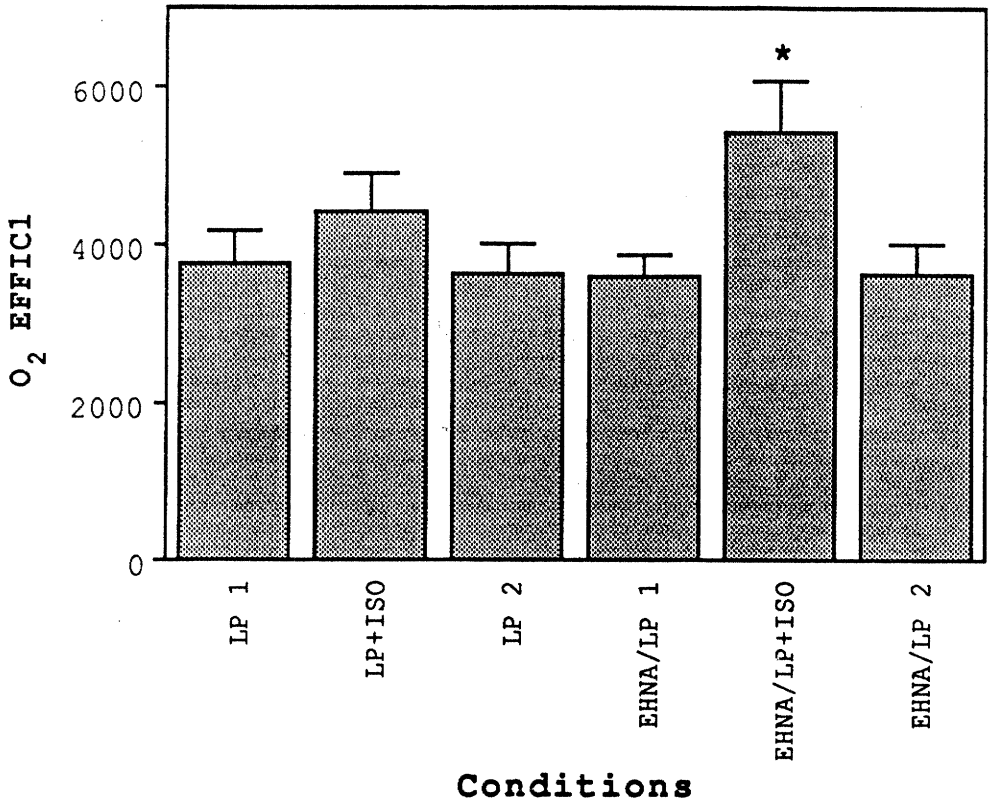

Figure 57. The estimated myocardial oxygen utilization efficiency response to EHNA infusion (EHNA/LP) and to ISO infusions in absence (LP+ISO) and presence (EHNA/LP+ISO) of EHNA when PP was held at 60 mmHg. Myocardial oxygen utilization efficiency was estimated as O_2EFFIC1 = Work1/MVO_2. n = 6. Unit of O_2EFFIC1: 10^6·mmHg^2/sec/ml/g. *: different from other values at $P < 0.05$.

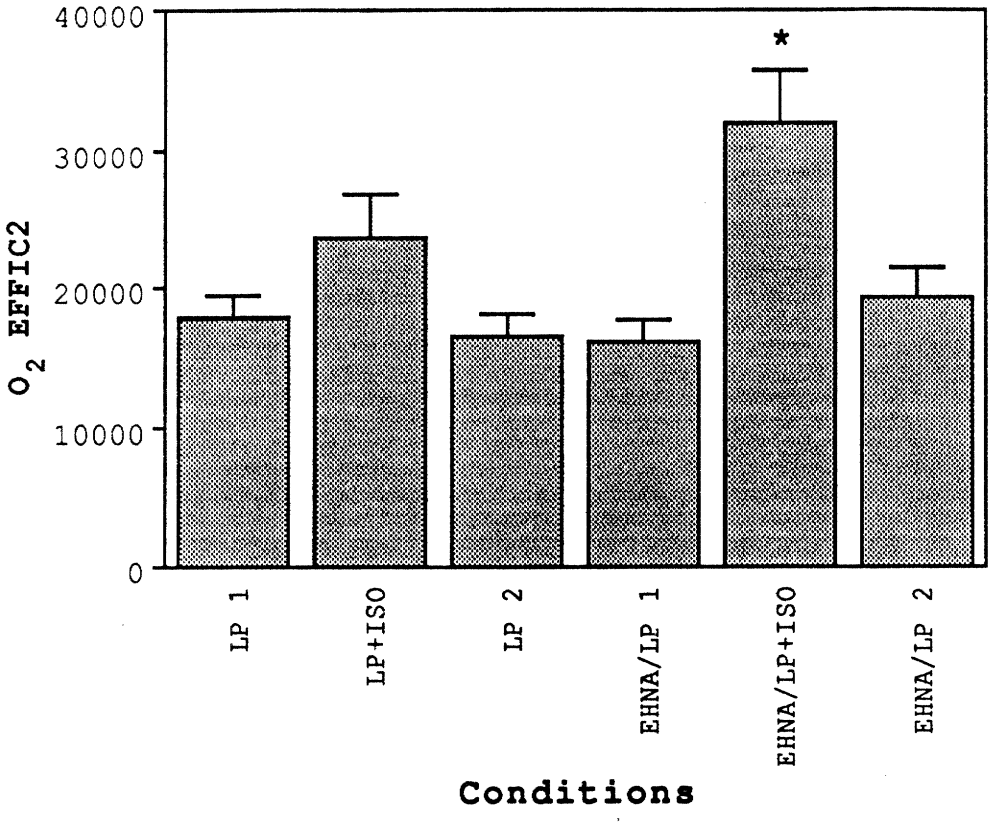

Figure 58. The estimated myocardial oxygen utilization efficiency response to EHNA infusion (EHNA/LP) and to ISO infusions in absence (LP+ISO) and presence (EHNA/LP+ISO) of EHNA when PP was held at 60 mmHg. Myocardial oxygen utilization efficiency was estimated as O_2EFFIC2 = Work2/MVO_2. n = 6. Unit of O_2EFFIC2: 10^3·mmHg/ml/g. *: different from other values at $P < 0.05$.

Hypoxemia, PP = 100 mmHg

7) HYPX & ISO

Table 8 presents systemic hemodynamic, coronary oxygen extraction, coronary venous oxygen tension, coronary lactate extraction, and myocardial function responses to hypoxemia and to ISO infusions during hypoxemia, and normoxemia. AoP, HR, LVP, and La% were essentially constant throughout the protocol. All variables had similar values at Control 1, 2, and 3. Hypoxemia decreased CaO_2 from 21.1 ± 0.9 ml/dl to 9.8 ± 0.2 ml/dl, but did not change dP/dt. O_2% was 69.9 ± 0.9% before and 58.7 ± 2.2% after hypoxemia, and CVR was 79.0 ± 4.6 mmHg·min/ml·g before and 33.3 ± 1.6 mmHg·min/ml·g after hypoxemia. ISO alone increased dP/dt from 2180 ± 120 mmHg/s to 3550 ± 220 mmHg/s ($P < 0.05$). O_2% was 70.3 ± 1.0% before and 71.6 ± 0.8% after ISO, and P_vO_2 was 25.6 ± 1.5 mmHg before and 25.1 ± 2.0 mmHg after ISO. When ISO was infused during hypoxemia, ISO decreased CVR further from 33.3 ± 1.6 mmHg·min/ml·g during HYPX to 20.0 ± 0.6 mmHg·min/ml·g during HYPX+ISO. As shown in the table, the ISO-induced increase in dP/dt was significantly decreased by hypoxemia (3550 ± 220 mmHg/sec during ISO vs. 3180 ± 190 mmHg/sec during HYPX+ISO, $P < 0.05$).

TABLE VIII

The responses to hypoxemia (HYPX) and to ISO infusions in the absence (ISO) and presence (HYPX+ISO) of hypoxemia.

Conditions	AoP	HR	PP	LVP	dP/dt	CaO_2	CvO_2	PvO_2	CVR	La%	O_2%
CONTROL 1	126 ± 5	138 ± 7	100 ± 0	124 ± 4	2180 ±120	20.9 ±1.0	6.2 ±0.4	25.6 ±1.5	79.9 ±5.2	35.1 ±2.0	70.3 ±1.0
ISO	128 ± 4	148 ± 8	100 ± 1	132 ± 5	3550* ±220	20.9 ±1.0	5.9 ±0.3	25.1 ±2.0	48.4 ±2.3	28.4 ±4.9	71.6 ±0.8
CONTROL 2	126 ± 5	137 ± 5	100 ± 1	123 ± 5	2000 ±130	21.1 ±0.9	6.3 ±0.4	25.6 ±1.8	79.0 ±4.6	32.0 ±3.2	69.9 ±0.9
HYPX	127 ± 5	137 ± 6	100 ± 1	125 ± 5	2040 ±120	9.8 ±0.2	4.0 ±0.2	21.4 ±1.4	33.3 ±1.6	33.1 ±8.1	58.7 ±2.2
HYPX+ISO	128 ± 4	138 ± 7	99 ± 1	130 ± 4	3180# ±190	9.8 ±0.3	4.9 ±0.3	23.7 ±1.5	20.0 ±0.6	32.1 ±5.7	49.7 ±2.0
CONTROL 3	125 ± 5	138 ± 6	100 ± 1	125 ± 5	1900 ±100	20.9 ±0.9	6.4 ±0.5	26.0 ±1.8	76.1 ±3.5	28.6 ±3.3	69.7 ±1.1

Values are means ± standard errors of the mean (SEM). n = 6. AoP, mean aortic pressure; HR, heart rate; PP, coronary perfusion pressure; LVP, left ventricular pressure development; dP/dt, maximum rate of LVP; CaO_2, coronary arterial oxygen content; CvO_2, coronary venous oxygen content; PvO_2, coronary venous oxygen tension; CVR, coronary vascular resistance; La%, coronary lactate extraction; O_2%, coronary oxygen extraction. *, #: different from other values at $P < 0.05$.

Figures 59 through 65 illustrate the CBF, dP/dt, S%, Work1, Work2, MVO_2, O_2EFFIC1, and O_2EFFIC2 responses to hypoxemia and to ISO infusions during normoxemia and hypoxemia, respectively. As shown in Figure 59, hypoxemia increased CBF from 1.03 ± 0.06 ml/min/g to 2.45 ± 0.12 ml/min/g ($P < 0.05$), and ISO increased CBF further from 2.45 ± 0.12 ml/min/g to 3.95 ± 0.11 ml/min/g ($P < 0.05$) when infused during hypoxemia. As shown in Figures 60 and 61, the ISO-induced elevations in dP/dt and S% were decreased by 10% and 26%, respectively, in presence of hypoxemia. As shown in Figures 62 and 63, the ISO-induced elevations in Work1 and Work2 were decreased by 18% and 33%, respectively, in presence of hypoxemia. As shown in Figure 64, MVO_2 was not influenced by hypoxemia (14.9 ± 0.3 ml/min/100 g during normoxemia vs. 13.9 ± 0.2 ml/min/100 g during hypoxemia), and the ISO-induced elevation in MVO_2 was decreased by 24% in presence of hypoxemia. This 24% reduction in the MVO_2 and similar percentage decreases in the Work1 and Work2 resulted in a similar oxygen utilization efficiency during ISO alone and during HYPX+ISO, as shown in the Figures 65 and 66. Thus, HYPX+ISO decreased myocardial work and MVO_2 by about 25%, and maintained oxygen utilization efficiency, when compared to ISO alone.

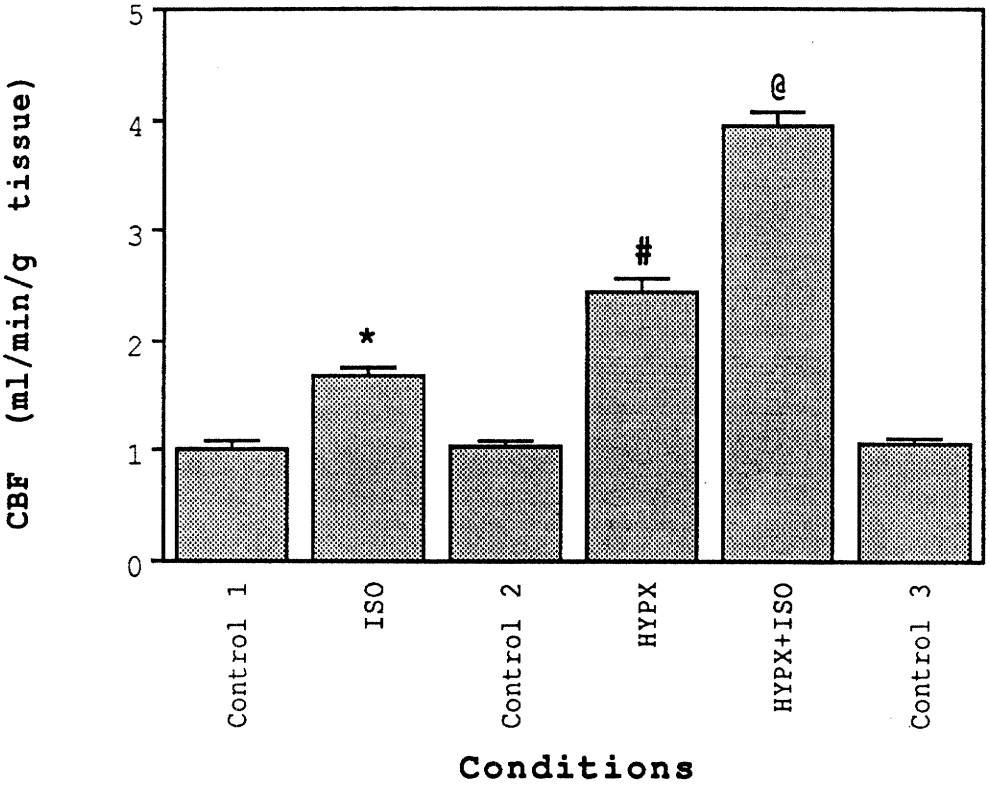

Figure 59. The coronary blood flow (CBF) response to hypoxemia (HYPX) and to ISO infusions in absence (ISO) and presence of hypoxemia (HYPX+ISO). n = 6. *, #, @: different from other values at $P < 0.05$.

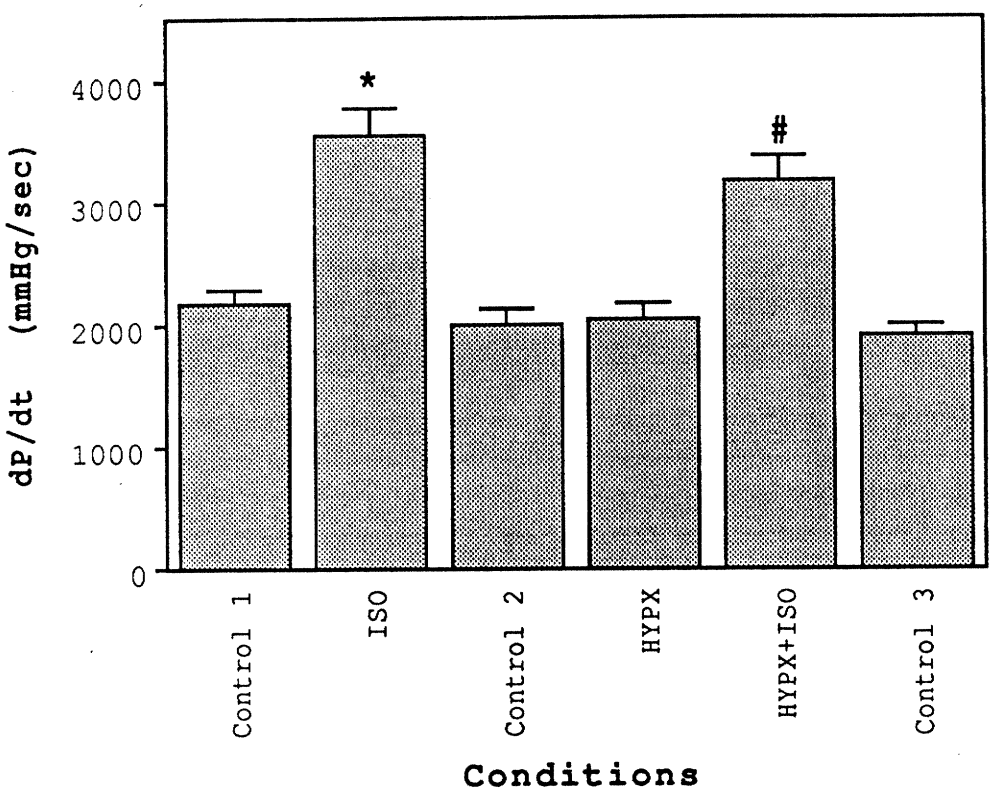

Figure 60. The maximum rate of LVP (dP/dt) response to hypoxemia (HYPX) and to ISO infusions in absence (ISO) and presence of hypoxemia (HYPX+ISO). n = 6. *, #: different from other values at $P < 0.05$.

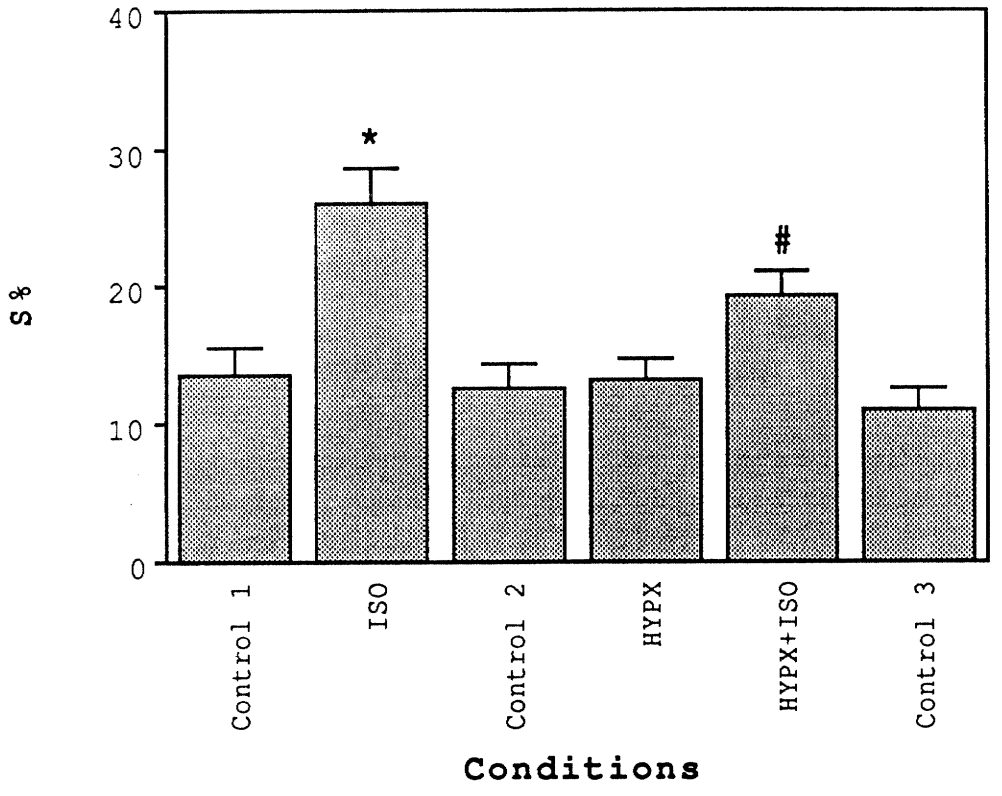

Figure 61. The percent segment shortening (S%) response to hypoxemia (HYPX) and to ISO infusions in absence (ISO) and presence of hypoxemia (HYPX+ISO). n = 6. *, #: different from other values at $P < 0.05$.

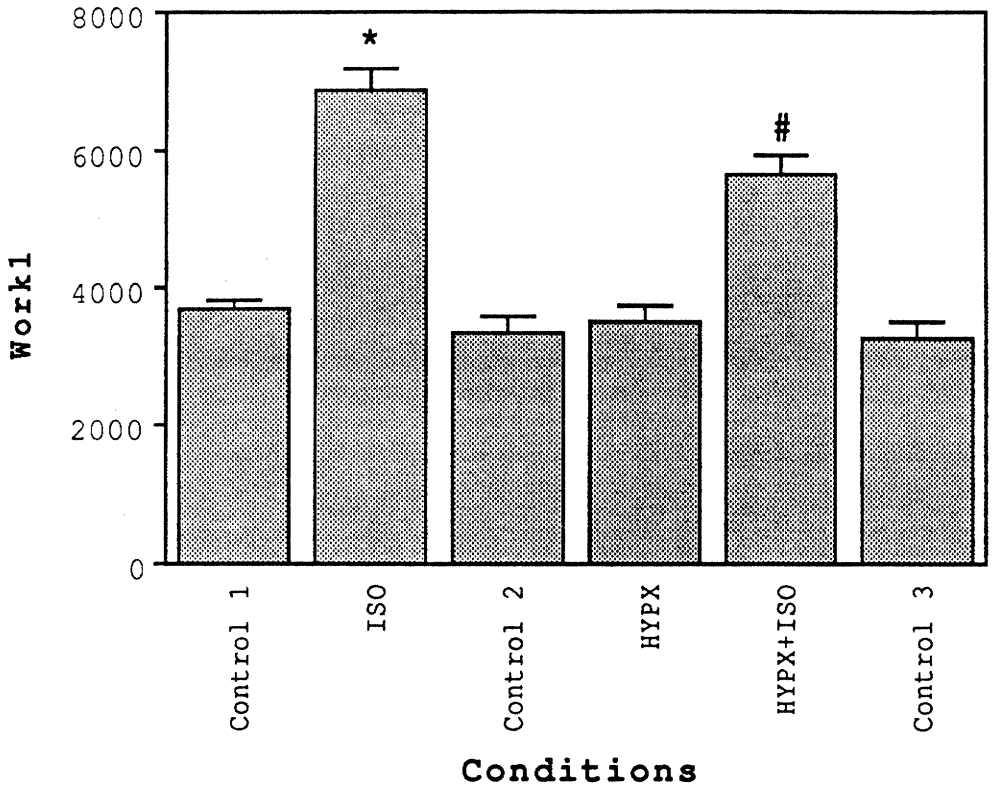

Figure 62. The estimated myocardial work response to hypoxemia (HYPX) and to ISO infusions in absence (ISO) and presence of hypoxemia (HYPX+ISO). Myocardial work was estimated as Work1 = HR X LVP X dP/dt. n = 6. Unit of Work1: $10^4 \cdot mmHg^2/sec/min$. *, #: different from other values at $P < 0.05$.

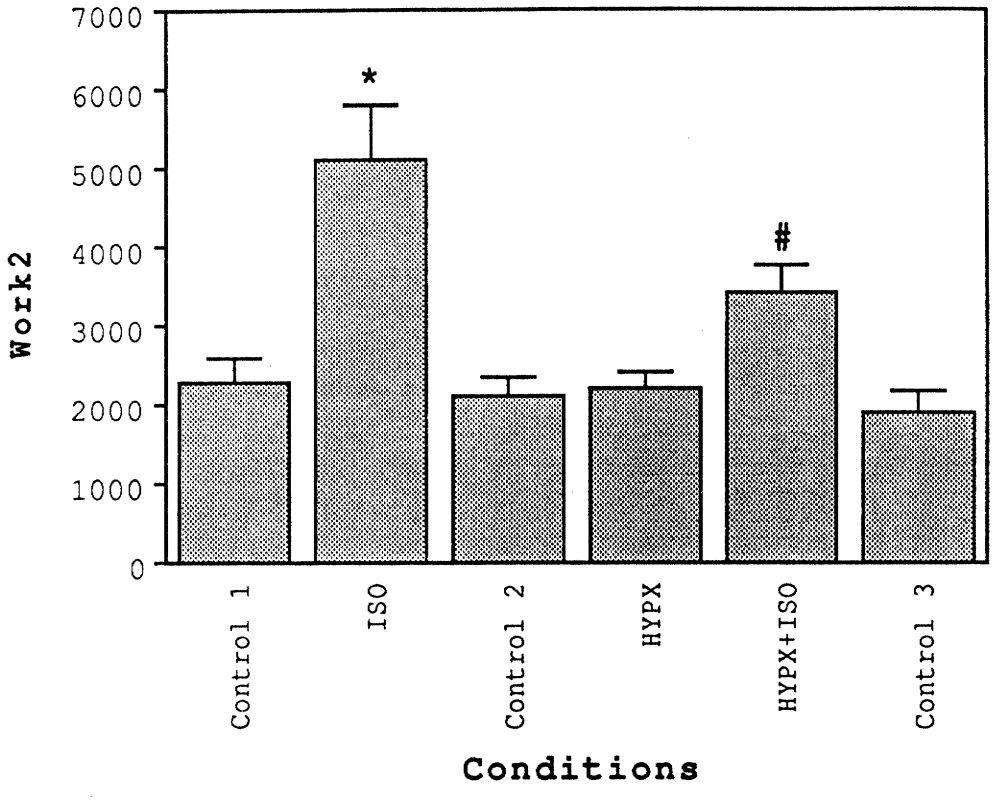

Figure 63. The estimated myocardial work response to hypoxemia (HYPX) and to ISO infusions in absence (ISO) and presence (HYPX+ISO) of hypoxemia. Myocardial work was estimated as Work2 = HR X LVP X S%. n = 6. Unit of Work2: 10^2·mmHg/min. *, #: different from other values at $P < 0.05$.

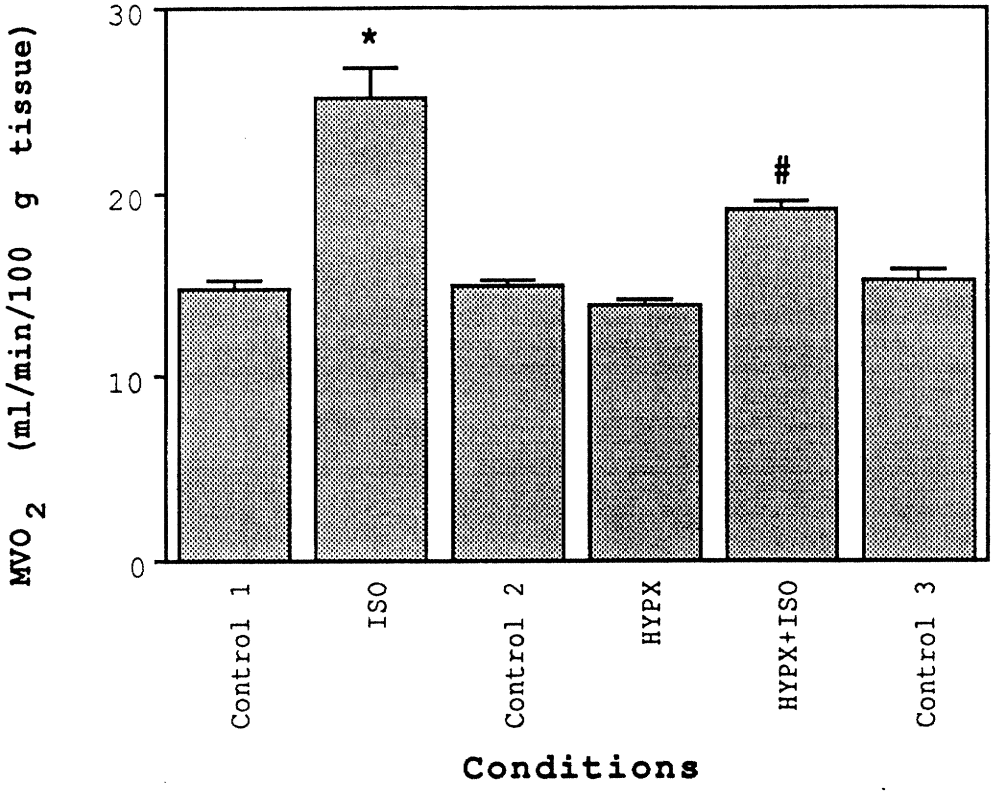

Figure 64. The myocardial oxygen consumption (MVO_2) response to hypoxemia (HYPX) and to ISO infusions in absence (ISO) and presence (HYPX+ISO) of hypoxemia. n = 6. *, #: different from other values at $P < 0.05$.

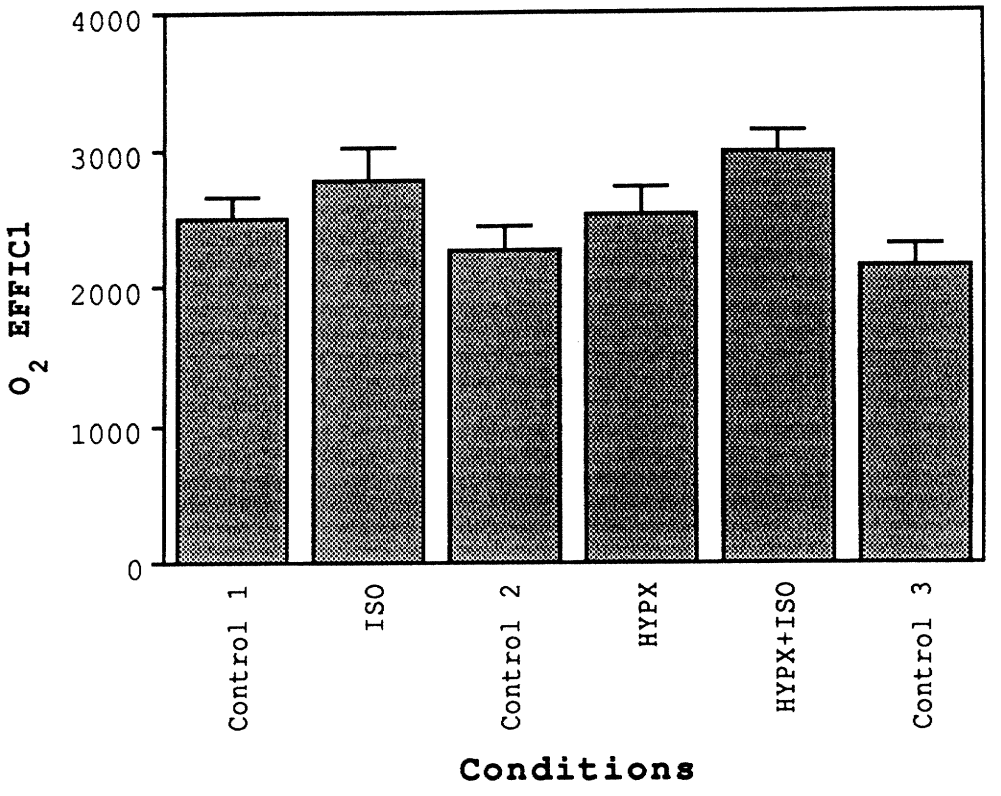

Figure 65. The estimated myocardial oxygen utilization efficiency response to hypoxemia (HYPX) and to ISO infusions in absence (ISO) and presence (HYPX+ISO) of hypoxemia. Myocardial oxygen utilization efficiency was estimated as $O_2EFFIC1 = Work1/MVO_2$. n = 6. Unit of $O_2EFFIC1$: $10^6 \cdot mmHg^2/sec/ml/g$.

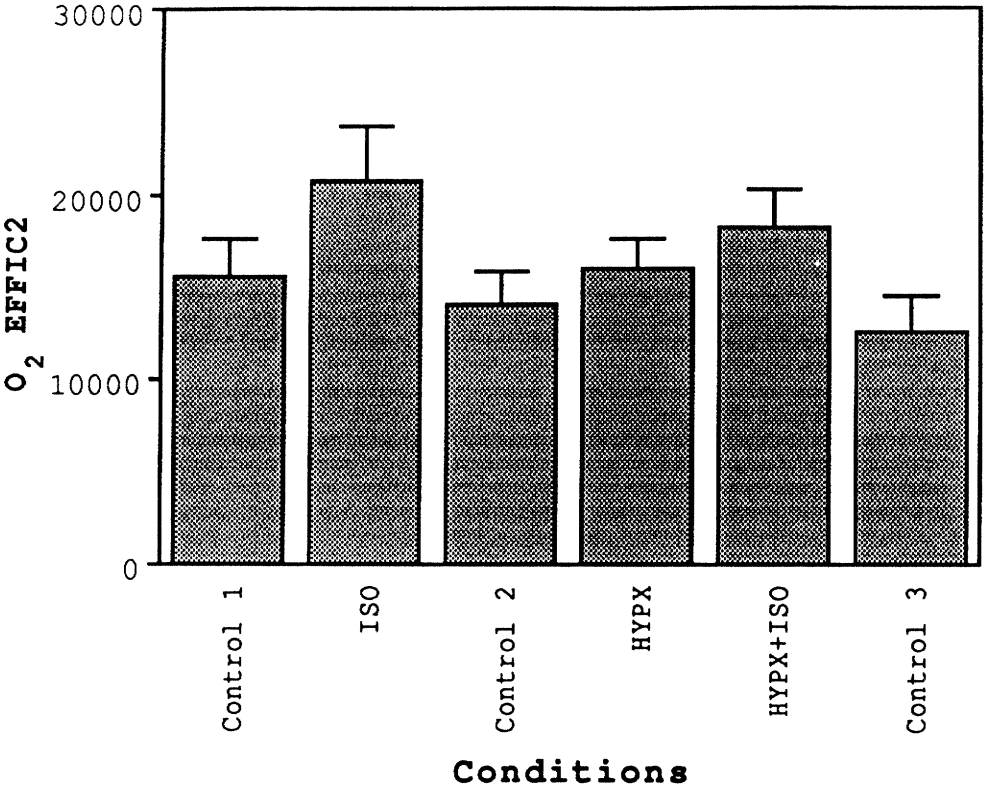

Figure 66. The estimated myocardial oxygen utilization efficiency response to hypoxemia (HYPX) and to ISO infusions in absence (ISO) and presence (HYPX+ISO) of hypoxemia. Myocardial oxygen utilization efficiency was estimated as O_2EFFIC2 = Work2/MVO_2. n = 6. Unit of O_2EFFIC2: 10^3·mmHg/ml/g.

8) ADA/HYPX & ISO

Table 9 presents systemic hemodynamic, coronary oxygen extraction, coronary venous oxygen tension, coronary lactate extraction, and myocardial function responses to hypoxemia and to ISO infusions during hypoxemia and normoxemia in the presence of ADA. ADA infusion was started 15 min before the beginning of the protocol, and continued throughout the protocol. AoP, HR, LVP, and La% were essentially constant throughout the protocol. All variables had similar values at ADA 1, 2, and 3. Hypoxemia decreased CaO_2 from 20.4 ± 0.6 ml/dl to 9.4 ± 0.3 ml/dl, but did not change dP/dt. O_2% was 68.8 ± 1.8% before and 57.7 ± 2.5% after hypoxemia, and CVR was 82.6 ± 4.1 mmHg·min/ml·g before and 33.8 ± 1.5 mmHg·min/ml·g after hypoxemia. ADA+ISO increased dP/dt from 2220 ± 100 mmHg/s to 3220 ± 100 mmHg/s ($P < 0.05$). O_2% was 69.2 ± 1.8% before and 71.3 ± 1.5% after ISO, and P_vO_2 was 25.8 ± 1.4 mmHg before and 25.4 ± 1.9 mmHg after ISO. When ISO was infused during hypoxemia, ISO decreased CVR further from 33.8 ± 1.5 mmHg·min/ml·g during ADA/HYPX to 20.3 ± 0.5 mmHg·min/ml·g during ADA/HYPX+ISO. As shown in the table, the ISO-induced increase in dP/dt was significantly decreased by hypoxemia (3220 ± 100 mmHg/sec during ADA+ISO vs. 2940 ± 80 mmHg/sec during ADA/HYPX+ISO).

TABLE IX

The responses to hypoxemia (ADA/HYPX) and to ISO infusions during normoxemia (ADA+ISO) and hypoxemia (ADA/HYPX+ISO) in the presence of ADA.

Conditions	AoP	HR	PP	LVP	dP/dt	CaO_2	CvO_2	PvO_2	CVR	La%	O_2%
ADA 1	123 ± 4	141 ± 9	100 ± 1	119 ± 4	2220 ±100	20.3 ±0.7	6.2 ±0.3	25.8 ±1.4	84.3 ±5.0	28.1 ±3.6	69.2 ±1.8
ADA+ISO	123 ± 4	143 ± 9	101 ± 1	126 ± 5	3220* ±100	20.4 ±0.7	5.8 ±0.3	25.4 ±1.9	53.5 ±1.9	24.1 ±2.5	71.3 ±1.5
ADA 2	122 ± 4	141 ± 7	100 ± 0	118 ± 7	2180 ± 90	20.4 ±0.6	6.4 ±0.4	26.0 ±1.5	82.6 ±4.1	26.1 ±2.8	68.8 ±1.8
ADA/HYPX	124 ± 4	137 ± 6	99 ± 1	121 ± 3	2170 ±100	9.4 ±0.3	4.0 ±0.2	20.3 ±1.1	33.8 ±1.5	27.6 ±2.7	57.7 ±2.5
ADA/HYPX+ISO	123 ± 5	129 ± 7	99 ± 1	121 ± 3	2940# ± 80	9.2 ±0.2	4.0 ±0.2	20.4 ±1.3	20.3 ±0.5	24.9 ±3.0	56.4 ±2.4
ADA 3	122 ± 5	141 ± 9	100 ± 1	119 ± 4	2000 ± 80	20.3 ±0.6	7.0 ±0.4	28.4 ±1.8	75.5 ±2.4	26.0 ±2.5	65.5 ±2.4

Values are means ± standard errors of the mean (SEM). n = 7. AoP, mean aortic pressure; HR, heart rate; PP, coronary perfusion pressure; LVP, left ventricular pressure development; dP/dt, maximum rate of LVP; CaO_2, coronary arterial oxygen content; CvO_2, coronary venous oxygen content; PvO_2, coronary venous oxygen tension; CVR, coronary vascular resistance; La%, coronary lactate extraction; O_2%, coronary oxygen extraction. *, #: different from other values at $P < 0.05$.

Figures 67 through 74 illustrate the CBF, dP/dt, S%, Work1, Work2, MVO_2, O_2EFFIC1, and O_2EFFIC2 responses to hypoxemia and to ISO infusions during normoxemia and hypoxemia in presence of ADA, respectively. As shown in Figure 67, hypoxemia increased CBF from 0.98 ± 0.04 ml/min/g to 2.35 ± 0.10 ml/min/g ($P < 0.05$), and ISO increased CBF further from 2.35 ± 0.10 ml/min/g to 4.03 ± 0.08 ml/min/g ($P < 0.05$) when infused during hypoxemia. As shown in Figures 68 and 69, the ISO-induced elevations in dP/dt and S% were decreased by 9% and 11%, respectively, in the presence of hypoxemia. As shown in Figures 70 and 71, the ISO-induced elevations in Work1 and Work2 were decreased by 20% and 23%, respectively, in the presence of hypoxemia. As shown in Figure 72, MVO_2 was not influenced by hypoxemia (13.7 ± 0.4 ml/min/100 g during normoxemia vs. 12.7 ± 0.4 ml/min/100 g during hypoxemia), and the ISO-induced elevation in MVO_2 was not influenced by the presence of hypoxemia. This similar increase in the MVO_2 and 20% to 23% decrease in myocardial work resulted in a significant decrease in O_2EFFIC1 by 15%, as shown in Figure 73. As shown in Figure 74, O_2EFFIC2 during ADA/HYPX+ISO was 19% less than O_2EFFIC2 during ADA+ISO ($P < 0.08$). Thus, ADA/HYPX+ISO decreased myocardial work and oxygen utilization efficiency by about 20%, but maintained myocardial MVO_2, when compared to ADA+ISO.

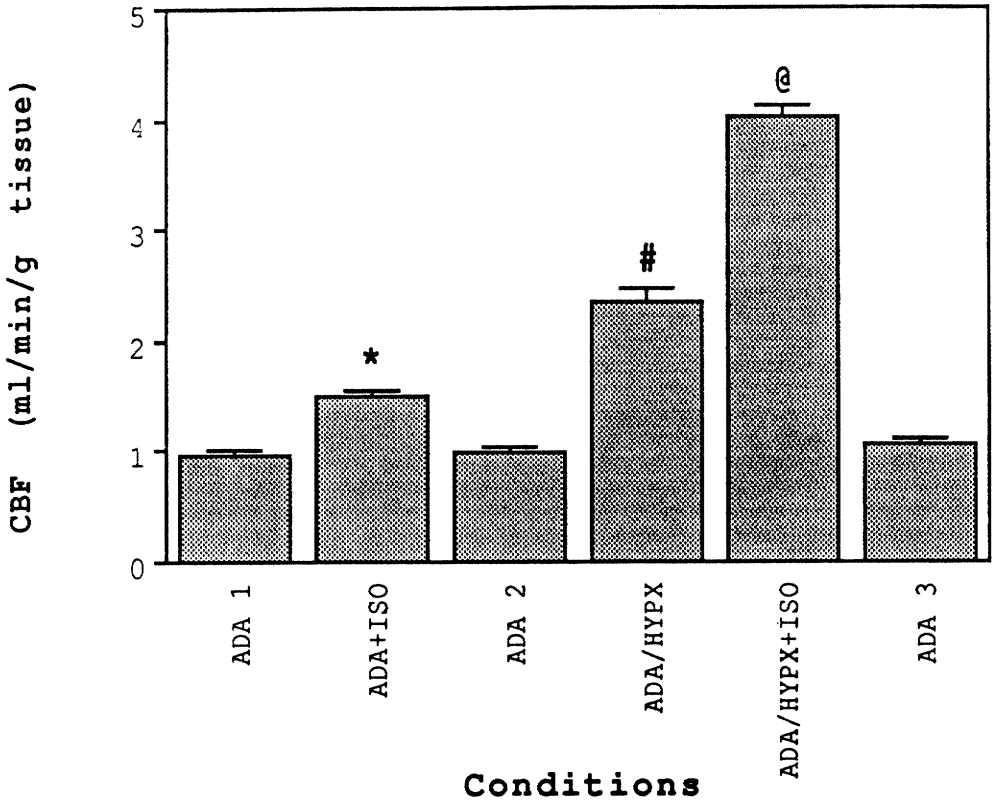

Figure 67. The coronary blood flow (CBF) response to hypoxemia (ADA/HYPX) and to ISO infusions during normoxemia (ADA+ISO) and hypoxemia (ADA/HYPX+ISO) in the presence of ADA. n = 7. *, #, @: different from other values at $P < 0.05$.

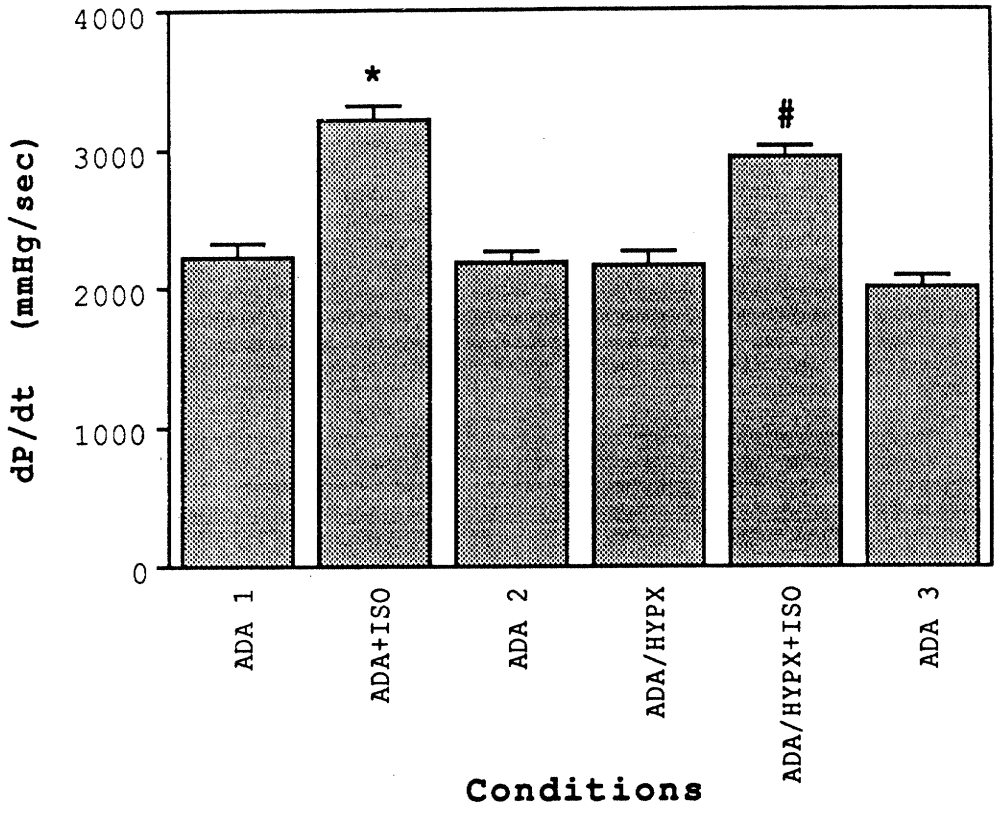

Figure 68. The maximum rate of LVP (dP/dt) response to hypoxemia (ADA/HYPX) and to ISO infusions during normoxemia (ADA+ISO) and hypoxemia (ADA/HYPX+ISO) in the presence of ADA. n = 7. *, #: different from other values at $P < 0.05$.

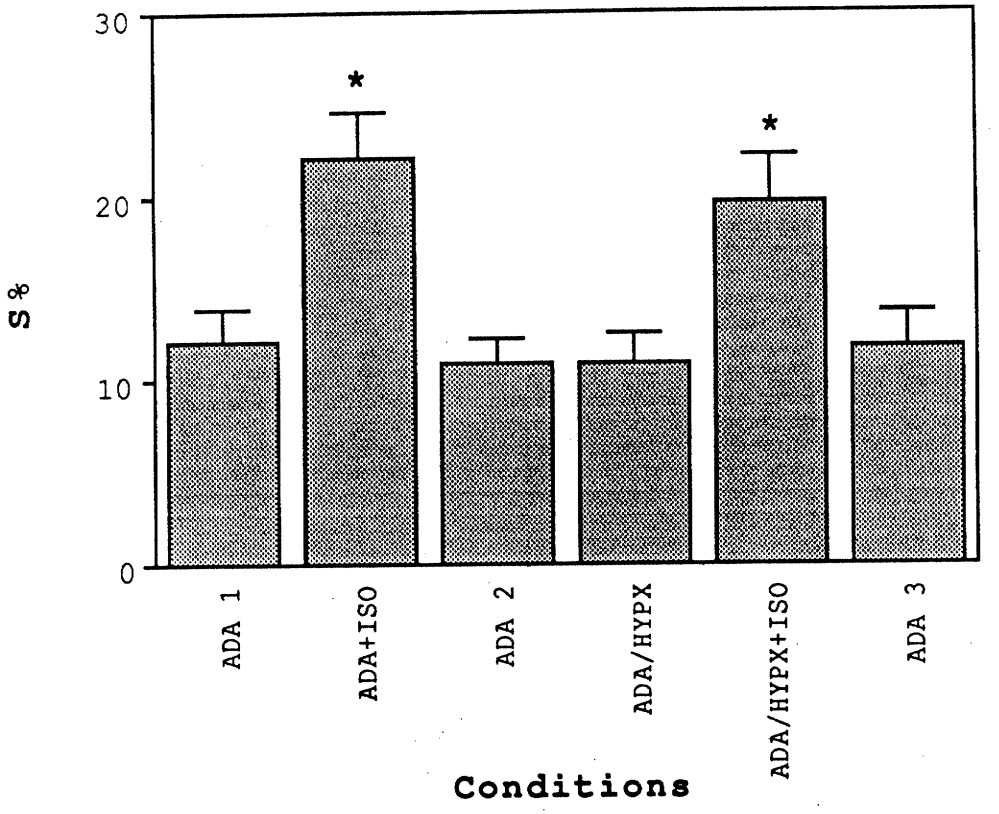

Figure 69. The percent segment shortening (S%) response to hypoxemia (ADA/HYPX) and to ISO infusions to normoxemia (ADA+ISO) and hypoxemia (ADA/HYPX+ISO) in the presence of ADA. n = 7. *: different from other values at $P < 0.05$.

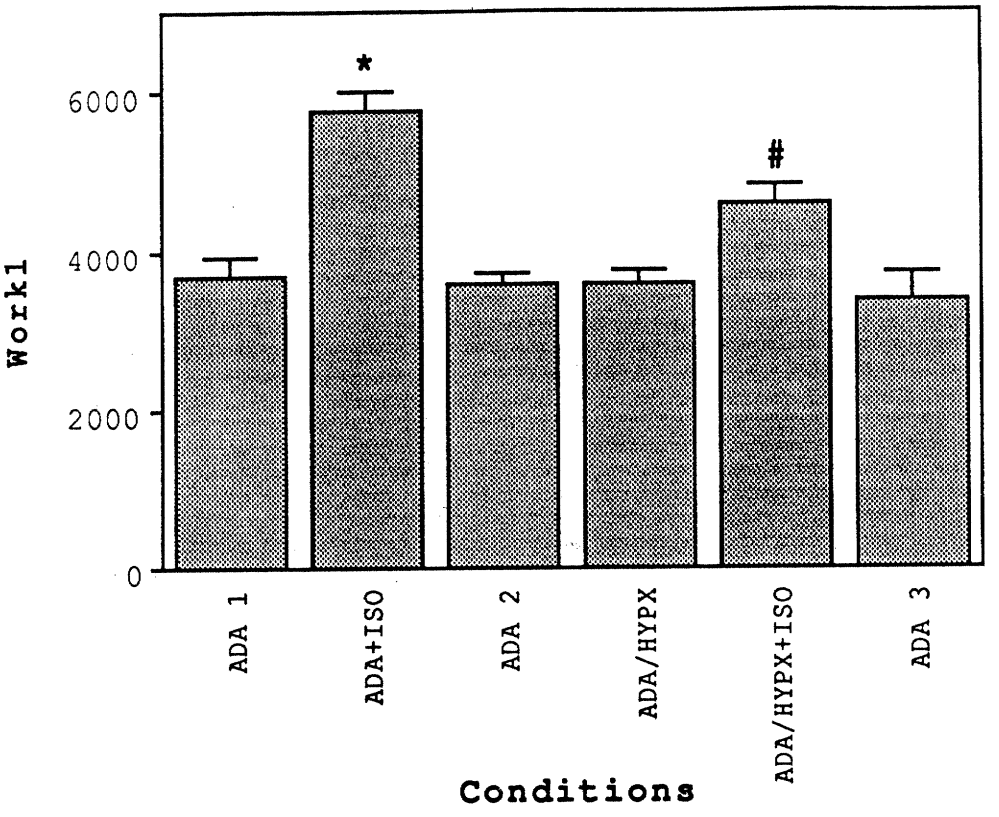

Figure 70. The estimated myocardial work response to hypoxemia (ADA/HYPX) and to ISO infusions during normoxemia (ADA+ISO) and hypoxemia (ADA/HYPX+ISO) in the presence of ADA. Myocardial work was estimated as Work1 = HR X LVP X dP/dt. n = 7. Unit of Work1: $10^4 \cdot mmHg^2/sec/min$. *, #: different from other values at $P < 0.05$.

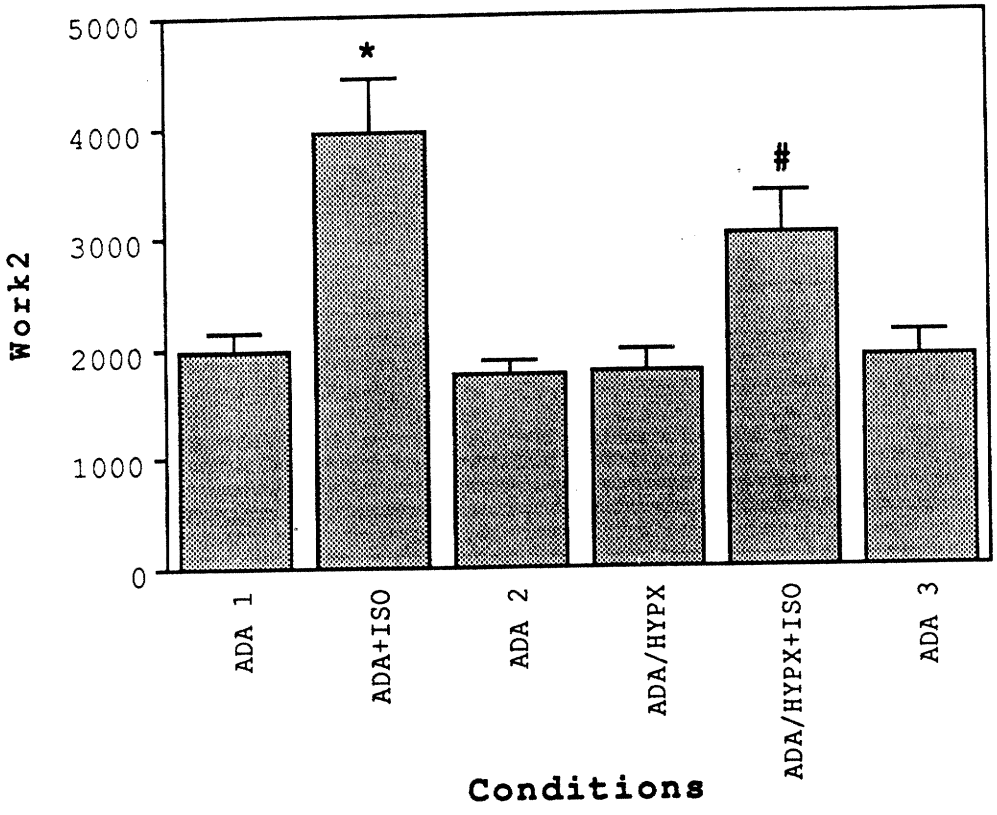

Figure 71. The estimated myocardial work response to hypoxemia (ADA/HYPX) and to ISO infusions during normoxemia (ADA+ISO) and hypoxemia (ADA/HYPX+ISO) in the presence of ADA. Myocardial work was estimated as Work2 = HR X LVP X S%. n = 7. Unit of Work2: 10^2·mmHg/min. *, #: different from other values at $P < 0.05$.

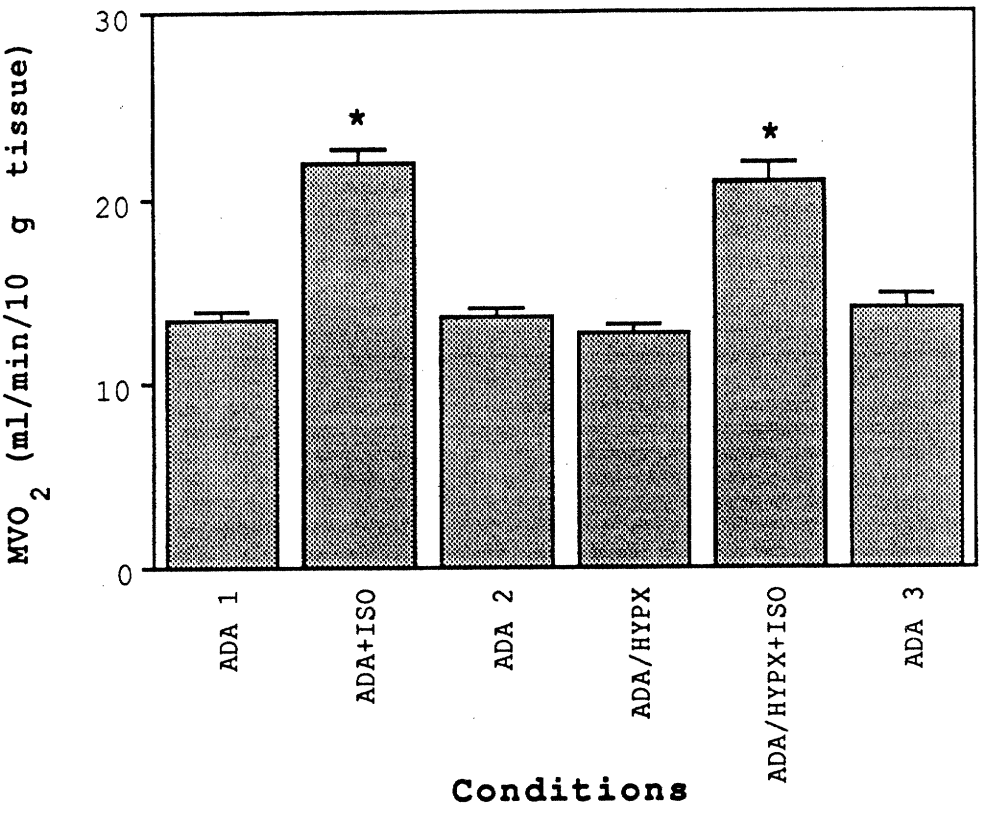

Figure 72. The myocardial oxygen consumption (MVO_2) response to hypoxemia (ADA/HYPX) and to ISO infusions during normoxemia (ADA+ISO) and hypoxemia (ADA/HYPX+ISO) in the presence of ADA. n = 7. *: different from other values at $P < 0.05$.

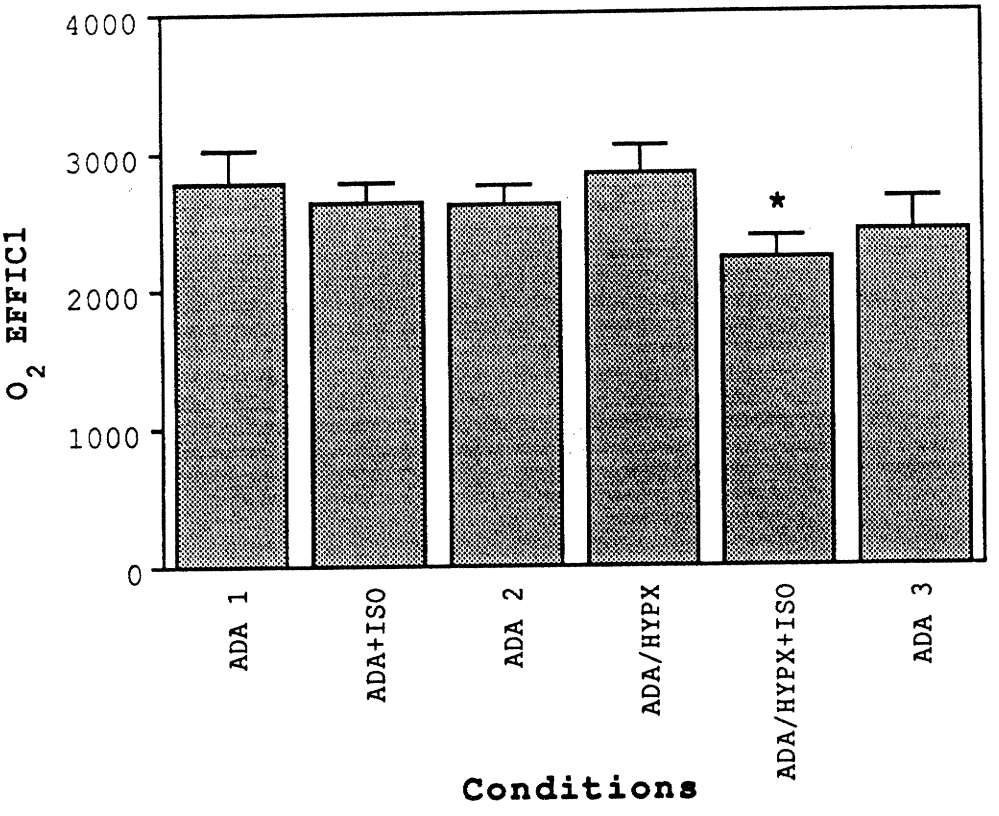

Figure 73. The estimated myocardial oxygen utilization efficiency response to hypoxemia (ADA/HYPX) and to ISO infusions during normoxemia (ADA+ISO) and hypoxemia (ADA/HYPX+ISO) in the presence of ADA. Myocardial oxygen utilization efficiency was estimated as O_2EFFIC1 = Work1/MVO_2. n = 7. Unit of O_2EFFIC1: 10^6·mmHg^2/sec/ml/g. *: different from other values at $P < 0.05$.

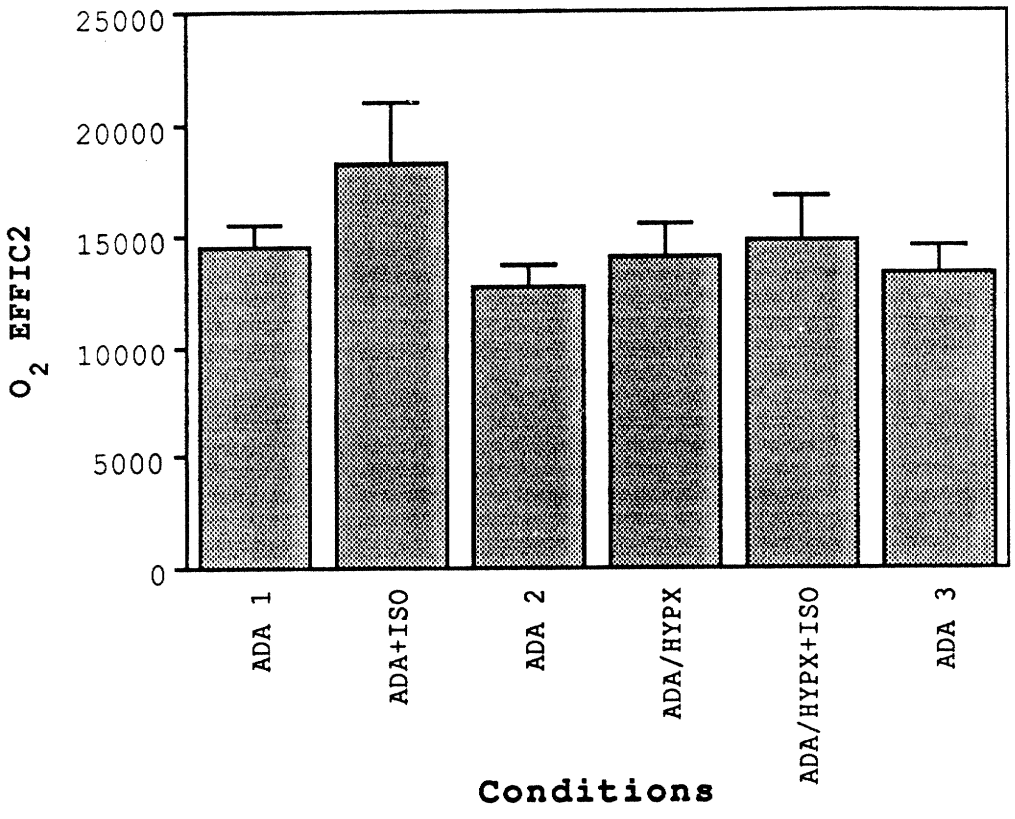

Figure 74. The estimated myocardial oxygen utilization efficiency response to hypoxemia (ADA/HYPX) and to ISO infusions during normoxemia (ADA+ISO) and hypoxemia (ADA/HYPX+ISO) in the presence of ADA. Myocardial oxygen utilization efficiency was estimated as O_2EFFIC2 = Work2/MVO_2. n = 7. Unit of O_2EFFIC2: 10^3·mmHg/ml/g.

9) EHNA/HYPX & ISO

Table 10 presents systemic hemodynamic, coronary oxygen extraction, coronary venous oxygen tension, coronary lactate extraction, and myocardial function responses to hypoxemia and to ISO infusions during hypoxemia, and normoxemia in the presence of EHNA. EHNA infusion was started 15 min before the begin of the protocol, and continued throughout the protocol. AoP, LVP, and La% were essentially constant throughout the protocol. All variables had similar values at EHNA 1, 2, and 3. Hypoxemia decreased CaO_2 from 20.5 ± 0.8 ml/dl to 9.2 ± 0.3 ml/dl, but did not change dP/dt. O_2% was 69.2 ± 1.5% before and 51.0 ± 1.6% after hypoxemia, and CVR was 72.3 ± 1.6 mmHg·min/ml·g before and 32.1 ± 1.6 mmHg·min/ml·g after hypoxemia. EHNA+ISO increased dP/dt from 2210 ± 120 mmHg/s to 3610 ± 130 mmHg/s ($P < 0.05$). O_2% was 70.4 ± 1.7% before and 69.9 ± 1.2% after ISO, and P_vO_2 was 25.4 ± 1.2 mmHg before and 25.7 ± 1.1 mmHg after ISO. When ISO was infused during hypoxemia, ISO decreased CVR further from 32.1 ± 1.6 mmHg·min/ml·g during EHNA/HYPX to 20.4 ± 0.5 mmHg·min/ml·g during EHNA/HYPX+ISO. Heart rate was 151 ± 8 beats/min during EHNA+ISO and 129 ± 7 beats/min during EHNA/HYPX+ISO ($P < 0.05$). As shown in the table, the ISO-induced elevation in dP/dt was significantly increased by hypoxemia (3610 ± 130 mmHg/sec during EHNA+ISO vs. 3950 ± 170 mmHg/sec during EHNA/HYPX+ISO).

TABLE X

The responses to hypoxemia (EHNA/HYPX) and to ISO infusions during normoxemia (EHNA+ISO) and hypoxemia (EHNA/HYPX+ISO) in the presence of EHNA.

Conditions	AoP	HR	PP	LVP	dP/dt	CaO_2	CvO_2	PvO_2	CVR	La%	O_2%
EHNA 1	122 ± 5	146 ± 7	101 ± 1	120 ± 5	2210 ±120	20.4 ±0.9	6.0 ±0.4	25.4 ±1.2	74.1 ±3.4	33.1 ±2.3	70.4 ±1.7
EHNA+ISO	123 ± 4	151 ± 8	100 ± 1	130 ± 3	3610* ±130	20.5 ±0.9	6.1 ±0.3	25.7 ±1.1	41.6 ±1.5	32.4 ±3.4	69.9 ±1.2
EHNA 2	117 ± 6	145 ± 6	101 ± 1	119 ± 6	2140 ± 60	20.5 ±0.8	6.3 ±0.3	26.1 ±0.9	72.3 ±1.6	33.2 ±2.5	69.2 ±1.5
EHNA/HYPX	120 ± 6	143 ± 5	99 ± 1	121 ± 5	2180 ± 60	9.2 ±0.3	4.5 ±0.2	20.8 ±1.1	32.1 ±1.6	22.5 ±1.7	51.0 ±1.6
EHNA/HYPX+ISO	121 ± 6	129* ± 7	98 ± 1	139 ± 4	3950# ±170	9.1 ±0.3	4.6 ±0.2	21.1 ±1.3	20.4 ±0.5	19.0 ±3.9	49.3 ±1.3
EHNA 3	116 ± 6	138 ± 6	100 ± 1	118 ± 6	2170 ± 70	20.4 ±0.8	7.3 ±0.3	28.9 ±1.8	68.6 ±1.6	27.8 ±3.0	64.2 ±2.0

Values are means ± standard errors of the mean (SEM). n = 6. AoP, mean aortic pressure; HR, heart rate; PP, coronary perfusion pressure; LVP, left ventricular pressure development; dP/dt, maximum rate of LVP; CaO_2, coronary arterial oxygen content; CvO_2, coronary venous oxygen content; PvO_2, coronary venous oxygen tension; CVR, coronary vascular resistance; La%, coronary lactate extraction; O_2%, coronary oxygen extraction. *, #: different from other values at $P < 0.05$.

Figures 75 through 82 illustrate the CBF, dP/dt, S%, Work1, Work2, MVO_2, O_2EFFIC1 and O_2EFFIC2 responses to hypoxemia and to ISO infusions during normoxemia and hypoxemia in presence of EHNA, respectively. As shown in Figure 75, hypoxemia increased CBF from 1.12 ± 0.02 ml/min/g to 2.78 ± 0.08 ml/min/g ($P < 0.05$), and ISO, when infused during hypoxemia, increased CBF further from 2.78 ± 0.08 ml/min/g to 4.11 ± 0.07 ml/min/g ($P < 0.05$). As shown in Figure 76, the ISO-induced elevation in dP/dt was increased by 10% in presence of hypoxemia ($P < 0.05$). As shown in Figure 77, the ISO-induced elevation in S% was not influenced by the presence of hypoxemia. As shown in Figures 78 and 79, the ISO-induced elevations in Work1 and Work2 were not influenced by the presence of hypoxemia. As shown in Figure 80, MVO_2 was not influenced by hypoxemia, and ISO-induced elevation in MVO_2 was decreased by 33% in the presence of hypoxemia ($P < 0.05$). This 33% decrease in the MVO_2 and no change in myocardial work resulted in a significant increase in oxygen utilization efficiency. As shown in Figures 81 and 82, O_2EFFIC1 and O_2EFFIC2 during EHNA/HYPX+ISO was 48% and 42% higher than those during EHNA+ISO ($P < 0.05$), respectively. Thus, EHNA/HYPX+ISO decreased MVO_2, increased oxygen utilization efficiency, and maintained myocardial work, when compared to EHNA+ISO.

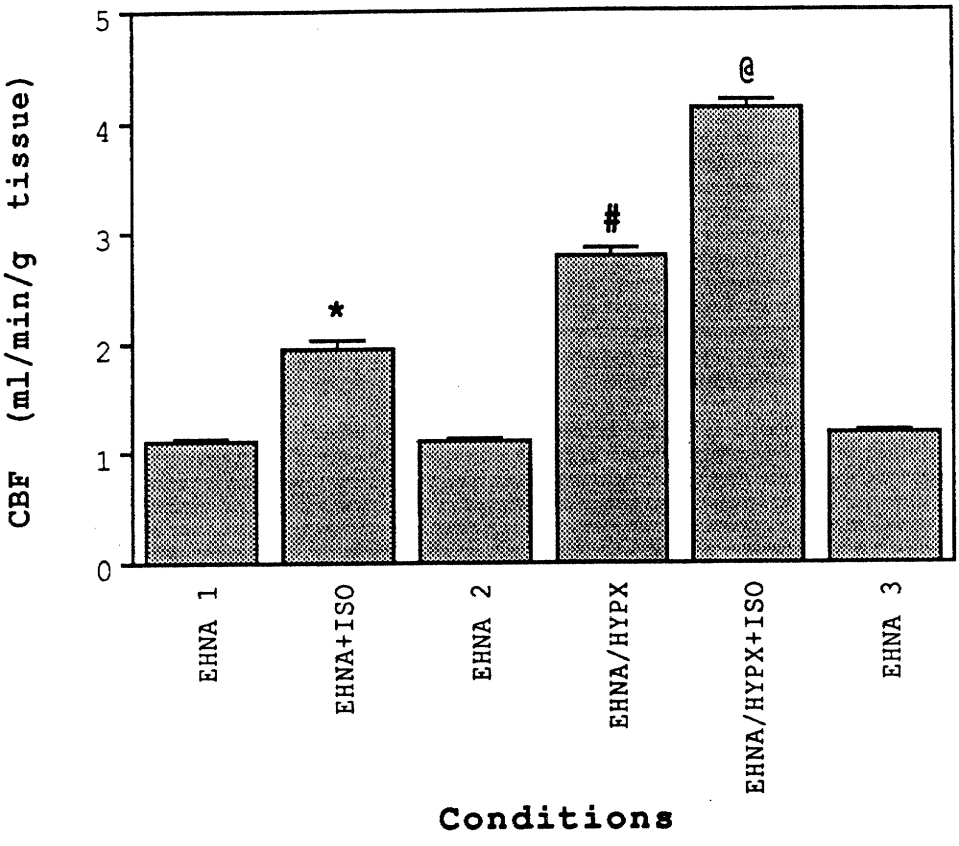

Figure 75. The coronary blood flow (CBF) response to hypoxemia (EHNA/HYPX) and to ISO infusions during normoxemia (EHNA+ISO) and hypoxemia (EHNA/HYPX+ISO) in the presence of EHNA. n = 6. *, #, @: different from other values at $P < 0.05$.

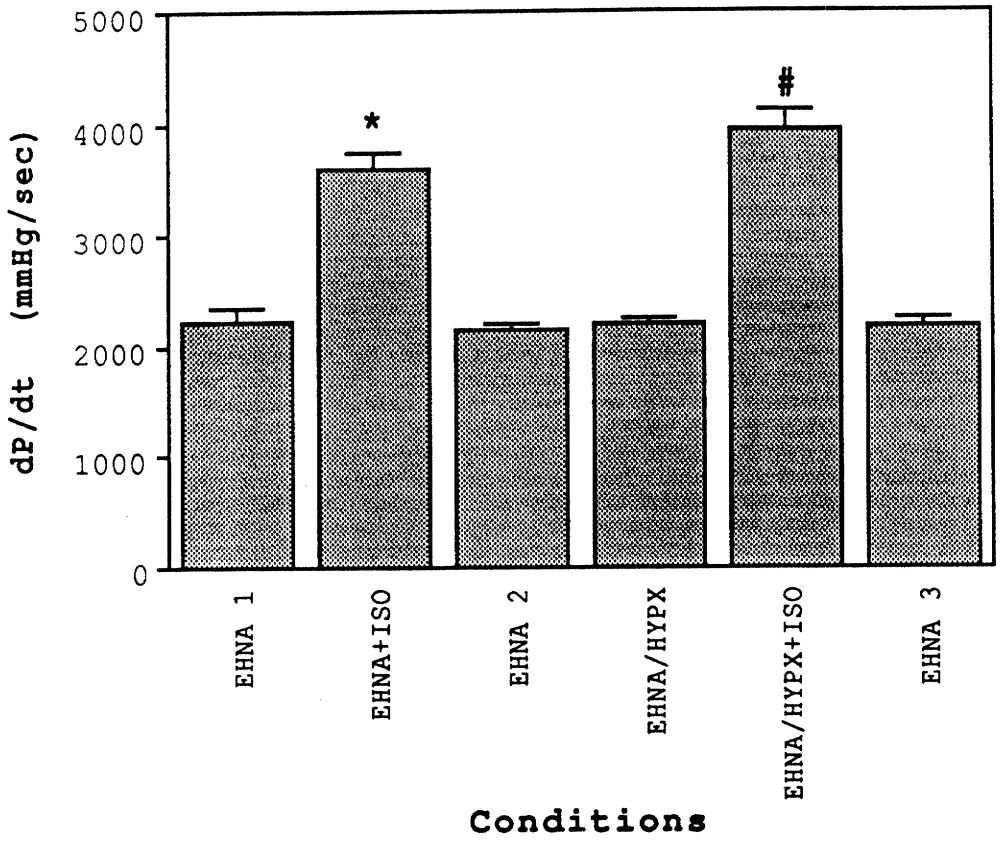

Figure 76. The maximum rate of LVP (dP/dt) response to hypoxemia (EHNA/HYPX) and to ISO infusions during normoxemia (EHNA+ISO) and hypoxemia (EHNA/HYPX+ISO) in the presence of EHNA. n = 6. *, #: different from other values at $P < 0.05$.

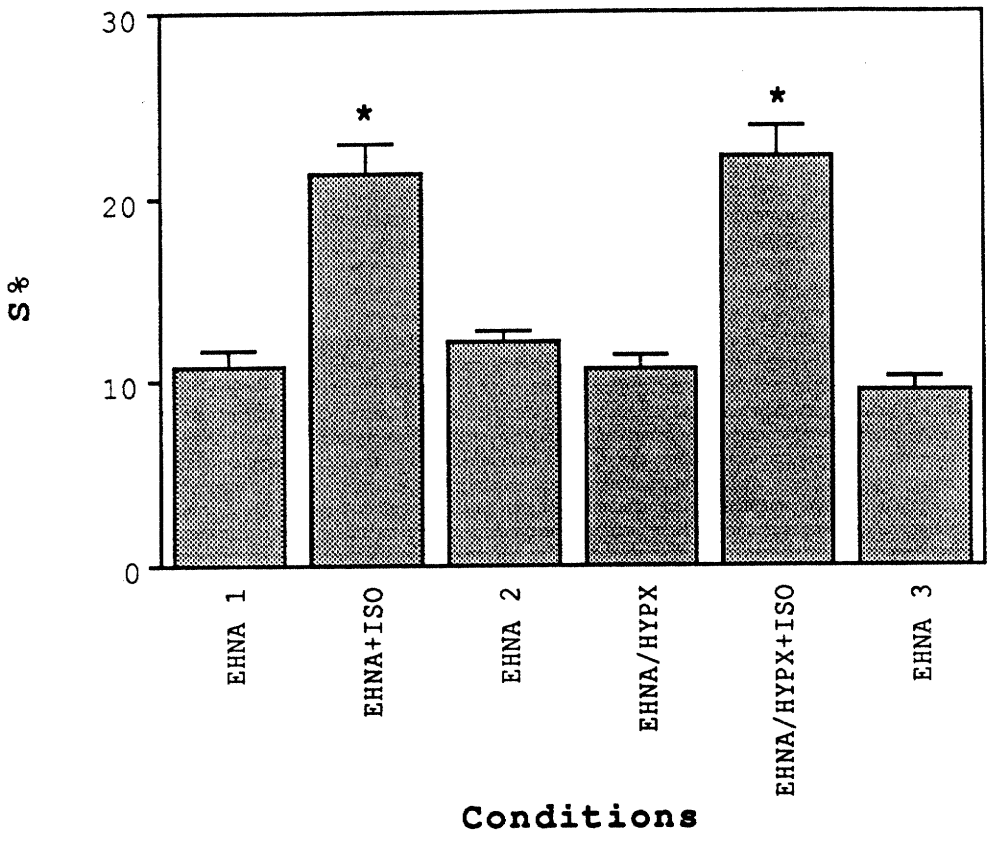

Figure 77. The percent segment shortening (S%) response to hypoxemia (EHNA/HYPX) and to ISO infusions to normoxemia (EHNA+ISO) and hypoxemia (EHNA/HYPX+ISO) in the presence of EHNA. n = 6. *: different from other values at $P < 0.05$.

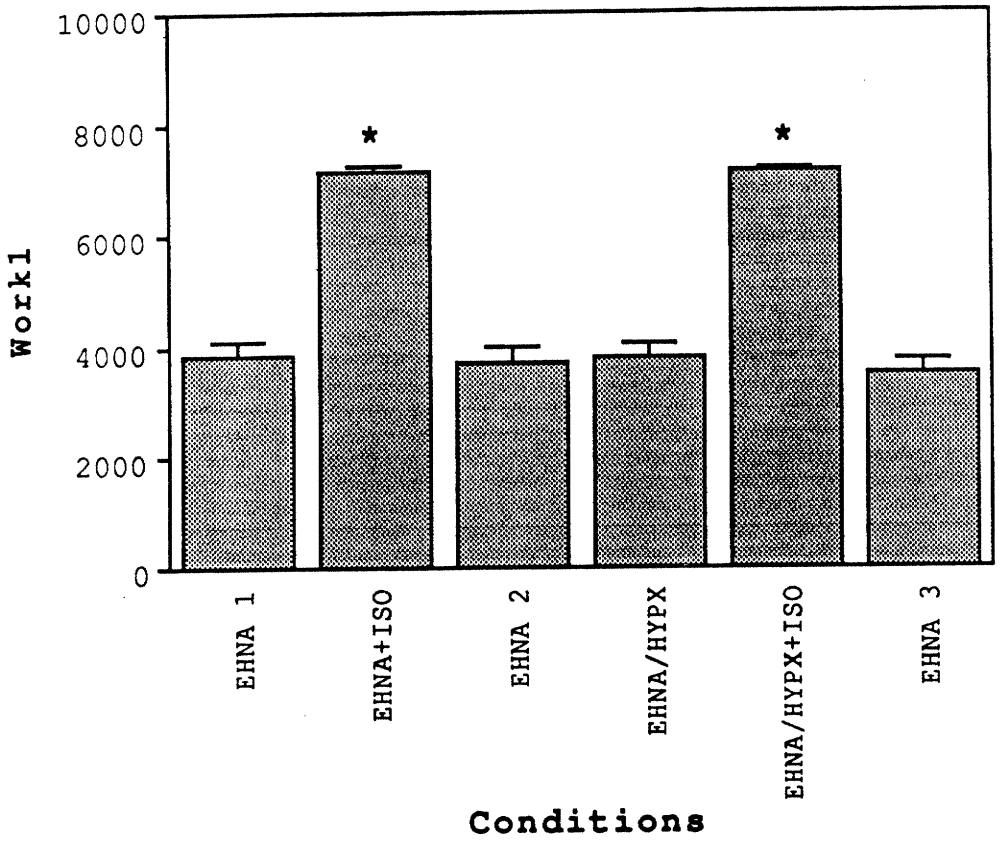

Figure 78. The estimated myocardial work response to hypoxemia (EHNA/HYPX) and to ISO infusions during normoxemia (EHNA+ISO) and hypoxemia (EHNA/HYPX+ISO) in the presence of EHNA. Myocardial work was estimated as Work1 = HR X LVP X dP/dt. n = 6. Unit of Work1: $10^4 \cdot mmHg^2/sec/min$. *: different from other values at $P < 0.05$.

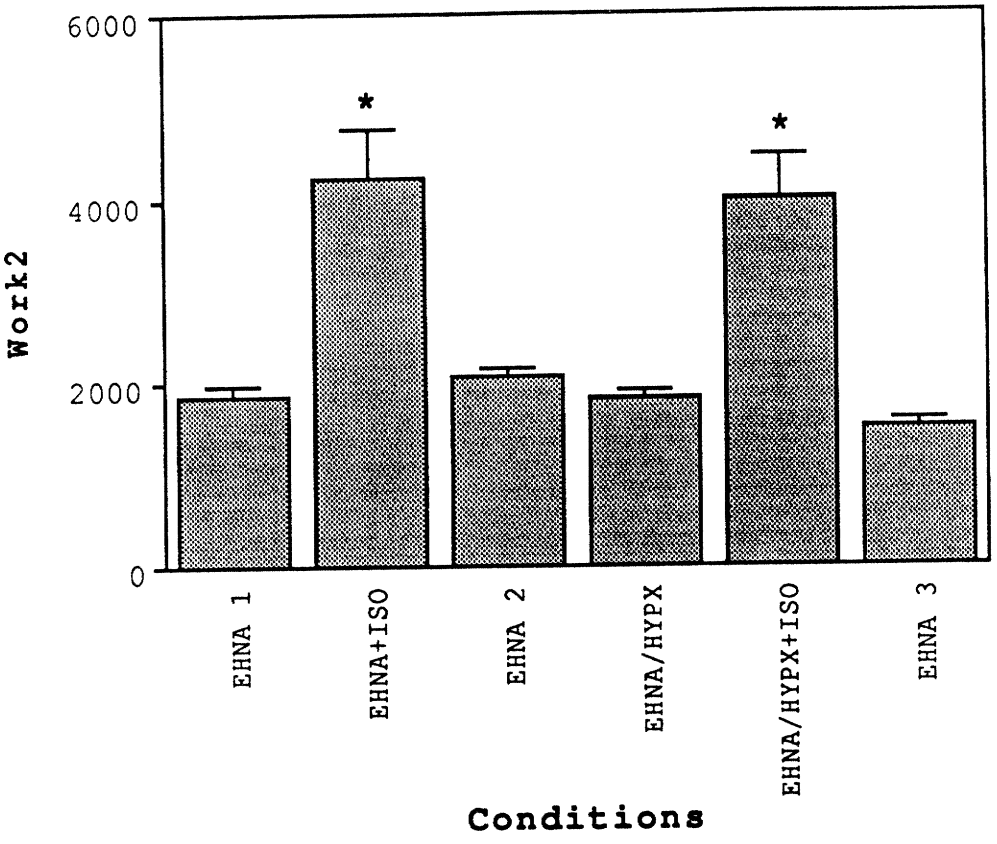

Figure 79. The estimated myocardial work response to hypoxemia (EHNA/HYPX) and to ISO infusions during normoxemia (EHNA+ISO) and hypoxemia (EHNA/HYPX+ISO) in the presence of EHNA. Myocardial work was estimated as Work2 = HR X LVP X S%. n = 6. Unit of Work2: 10^2·mmHg/min. *: different from other values at $P < 0.05$.

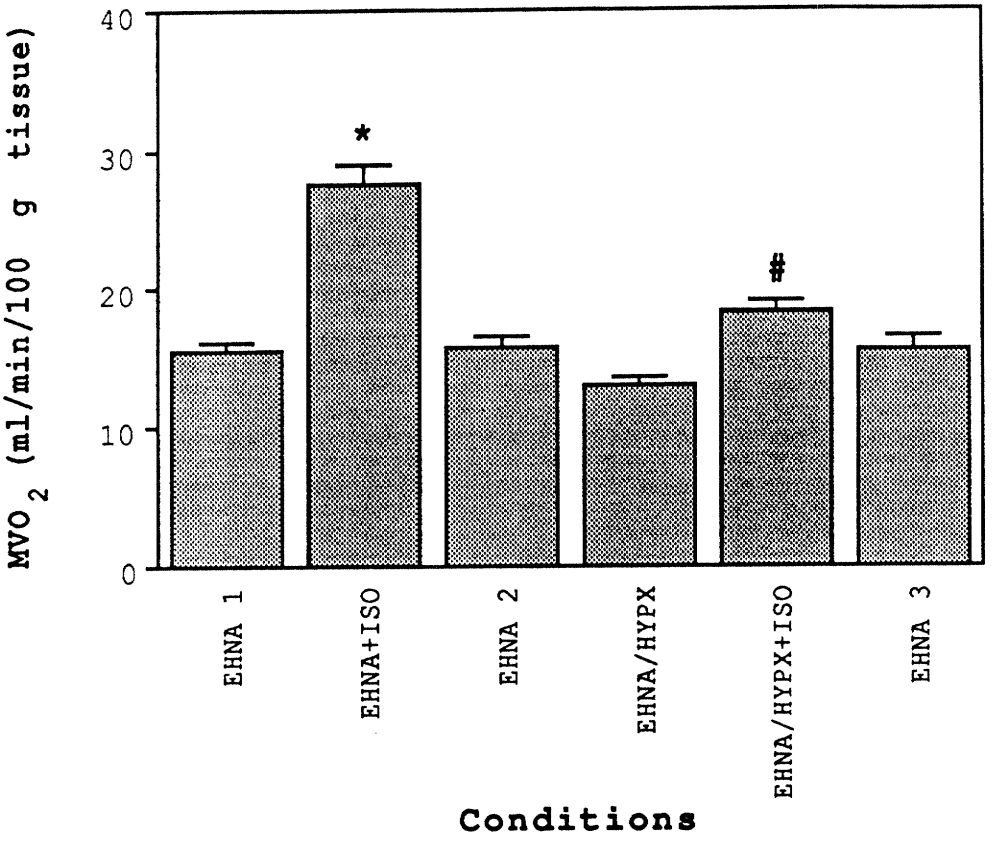

Figure 80. The myocardial oxygen consumption (MVO_2) response to hypoxemia (EHNA/HYPX) and to ISO infusions during normoxemia (EHNA+ISO) and hypoxemia (EHNA/HYPX+ISO) in the presence of EHNA. n = 6. *, #: different from other values at $P < 0.05$.

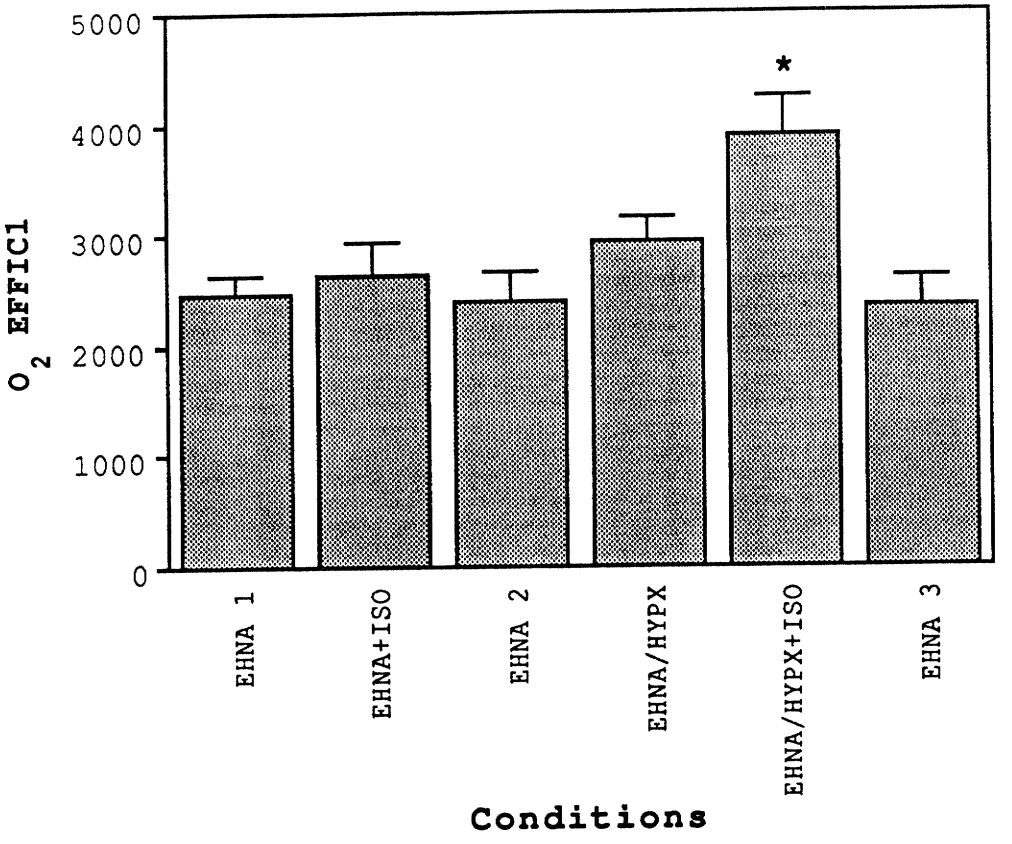

Figure 81. The estimated myocardial oxygen utilization efficiency response to hypoxemia (EHNA/HYPX) and to ISO infusions during normoxemia (EHNA+ISO) and hypoxemia (EHNA/HYPX+ISO) in the presence of EHNA. Myocardial oxygen utilization efficiency was estimated as $O_2EFFIC1 = Work1/MVO_2$. n = 6. Unit of $O_2EFFIC1$: $10^6 \cdot mmHg^2/sec/ml/g$. *: different from other values at $P < 0.05$.

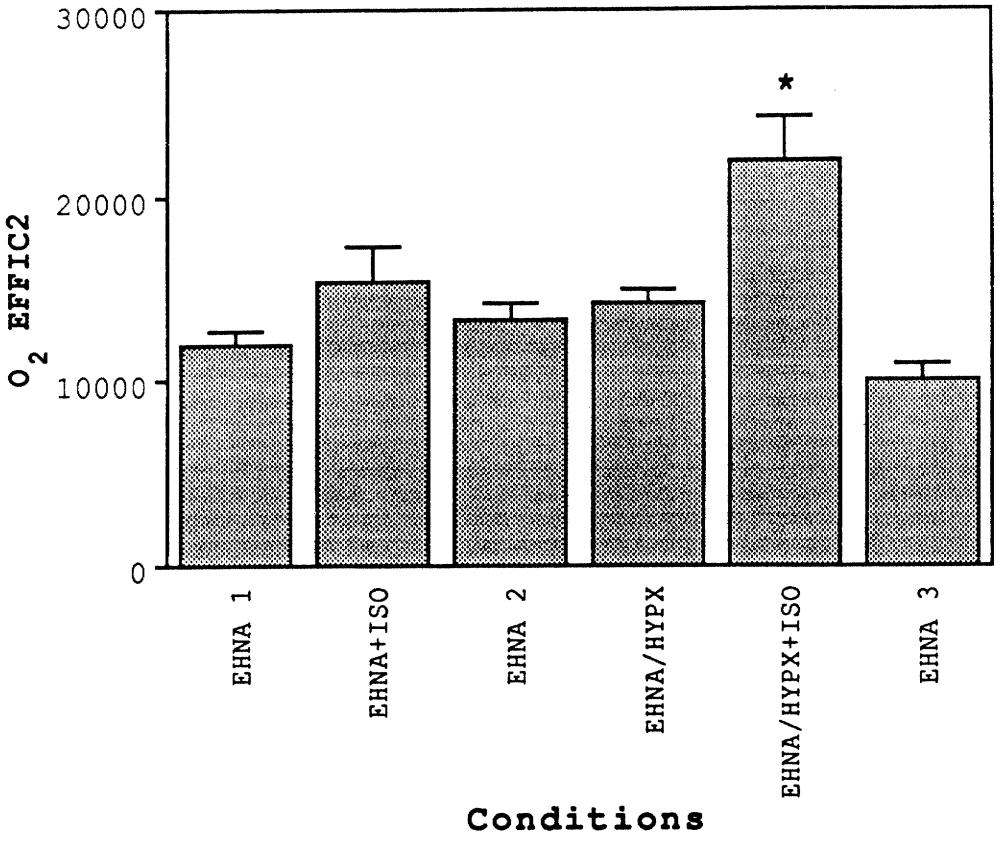

Figure 82. The estimated myocardial oxygen utilization efficiency response to hypoxemia (EHNA/HYPX) and to ISO infusions during normoxemia (EHNA+ISO) and hypoxemia (EHNA/HYPX+ISO) in the presence of EHNA. Myocardial oxygen utilization efficiency was estimated as O_2EFFIC2 = Work2/MVO_2. n = 6. Unit of O_2EFFIC2: 10^3·mmHg/ml/g. *: different from other values at $P < 0.05$.

CHAPTER IV

DISCUSSION

This investigation examined physiological means by which the myocardium can survive and function effectively when oxygen supply is limited and oxygen demand is increased. As summarized in Table 11, there are nine major findings in this investigation.

Normoxemia, PP = 100 mmHg

1) ISO-induced elevation in MVO_2 but not work was decreased in the presence of exogenous adenosine. Exogenous adenosine, thus, increased oxygen utilization efficiency of ISO-stimulated myocardium.

2) At PP = 100 mmHg, ISO-induced elevations in MVO_2 and work were both decreased in the presence of ADA. ADA, thus, did not influence oxygen utilization efficiency at PP = 100 mmHg.

3) At PP = 100 mmHg, ISO-induced elevations in MVO_2 and work were not influenced by EHNA. EHNA, however, increased oxygen utilization efficiency slightly.

Normoxemia, PP = 60 mmHg

4) ISO-induced elevation in MVO_2 but not work was decreased. Low perfusion pressure and reduced oxygen

TABLE XI

Summary of results.

	Normoxemia						Hypoxemia		
	PP = 100 mmHg			PP = 60 mmHg			PP = 100 mmHg		
	ADO	ADA	EHNA	No drug	ADA	EHNA	No drug	ADA	EHNA
Work	=	↓	=	=	↓	↑	↓	↓	=
MVO_2	↓	↓	=	↓	=	=	↓	=	↓
O2 Efficency	↑	=	= or ↑	↑	↓	↑	=	↓	↑

=, ↑, and ↓ represent no change, increase, and decrease, respectively, compared to respective control value at $P < 0.05$.

delivery, thus, increased oxygen utilization efficiency.

5) At PP = 60 mmHg, ISO-induced elevation in work but not MVO_2 was decreased in the presence of ADA. ADA, thus, decreased oxygen utilization efficiency at PP = 60 mmHg.

6) At PP = 60 mmHg, ISO-induced elevation in work but not MVO_2 was increased in the presence of EHNA. EHNA, thus, increased oxygen utilization efficiency at PP = 60 mmHg.

Hypoxemia, PP = 100 mmHg

7) ISO-induced elevations in MVO_2 and work were decreased in the presence of hypoxemia. Hypoxemia, thus, did not influence oxygen utilization efficiency.

8) In the presence of ADA, ISO-induced elevation in work but not MVO_2 was decreased in the presence of hypoxemia. Hypoxemia, thus, decreased oxygen utilization efficiency in the presence of ADA.

9) In the presence of EHNA, ISO-induced elevation in MVO_2 but not work was decreased in the presence of hypoxemia. Hypoxemia, thus, increased oxygen utilization efficiency in the presence of EHNA.

Can Myocardium Down-regulate Oxygen Demand?

Results from Group Ia LP & ISO showed that lowering perfusion pressure from 100 mmHg to 60 mmHg decreased flow and MVO_2 by 32% and 29%, respectively. The failure of flow to

remain constant suggests, at first, that the coronary circulation was not capable of autoregulation (25). However, autoregulation of flow is expected only if MVO_2 is constant (25). In these experiments MVO_2 fell along with flow. Since lactate extraction was not decreased by low perfusion pressure, the fall in MVO_2 was apparently not due directly to limited oxygen supply, but rather to a fall in oxygen demand.

A fall in MVO_2 associated with a decrease in coronary perfusion was described by Gregg and is known as the Gregg phenomenon (29). The mechanism of the Gregg phenomenon is unknown. In Gregg's original experiments, increasing the perfusion pressure in a cannulated left coronary artery resulted in an increase in 1) coronary artery transmural pressure, 2) coronary blood flow, and 3) oxygen delivery. Among them, the coronary distension (garden-hose) hypothesis has been the explanation most extensively studied. Lochner et al. (49) reported that coronary distension altered cardiac fiber length, and, thus, increased myocardial contractility and MVO_2 (the garden-hose effect of Lochner and co-workers). Scharf and Bromberger-Barnera (71) also observed Gregg's phenomenon when they changed coronary distention pressure by increasing the rate of flow pumping into the coronary artery or increasing coronary sinus outflow resistance with a clamp while holding coronary flow constant. The coronary distention hypothesis (garden-hose effect) for Gregg's

phenomenon is further supported by the observation that increasing coronary perfusion pressure in an arrested heart induces an increase in sarcomere length estimated with electron microscopy (64).

However, Zborowska-Sluis et al. (91) observed no Gregg's phenomenon when the flow was held constant and coronary distention pressure was increased or decreased by angiotensin or dipyridamole, respectively. Similar results were reported by Abel and Reis (1), who used nitroglycerin to decrease coronary distention pressure while holding the flow constant. The absence of Gregg's phenomenon in the constant-flow experiments of Zborowska-Sluis and Abel and Reis argues against the garden-hose hypothesis. In addition, Bacaner et al. (5) employed oxygen desaturation and hemodilution experiments and concluded that oxygen delivery is the most important variable for eliciting Gregg's phenomenon.

In the present study, contractility as well as end-diastolic length, estimated from the piezoelectric crystals, were not changed by low perfusion pressure, but MVO_2 was decreased by low perfusion pressure. This seems to contradict the garden-hose hypothesis. However, it is possible that the signal from the piezoelectric crystals was not sensitive enough to detect the small changes of sarcomere length resulting from changing coronary distention pressure. The focus of the present study is not on Gregg's phenomenon,

but rather on the responses to ISO under conditions of restricted oxygen supply. However, since lowering perfusion pressure might increase the rate of adenosine release by myocardium (25), the increased myocardial oxygen utilization efficiency observed under this condition was likely mediated by adenosine. This view is consistent with our observed effects of ADA and EHNA, which were used to modulate endogenous adenosine during ISO. Furthermore, most studies of Gregg's phenomenon have examined effects of increasing perfusion pressure. Normally the coronary perfusion pressure is essentially the aortic pressure. The aortic pressure is generated by the left ventricle, which is perfused by the coronary circulation. Considering the powerful feedback control of aortic pressure and direct effects of autonomic nervous system on the myocardium, the modest increase in MVO_2 observed in experiments with increased perfusion pressure probably has little physiological importance. In contrast, the physiological importance of Gregg's phenomenon may be in decreasing oxygen demand and, thus, protecting myocardium from ischemia, when oxygen supply is limited.

ISO-induced elevation in MVO_2 was reduced by 40% in the presence of low perfusion pressure, which decreased oxygen delivery. Since there was no decrease in lactate extraction or local contractile function throughout the protocol, myocardial ischemia did not occur. Thus, it is reasonable to

use MVO_2 as an index of myocardial oxygen demand, and we concluded that ISO-stimulated myocardium down-regulated its oxygen demand, when its oxygen supply was limited. This is consistent with a study of Marshall et al. (50). They found that the myocardium did not increase its rate•pressure product in response to rapid paired electrical stimulation in the presence of restricted flow. In their experiment, MVO_2 increased only 40% in response to the stimulation when flow was held constant at basal flow, compared to 148% in response to the same stimulation when the flow was allowed to increase. Thus, one can say that the stimulation-induced elevation in MVO_2 was decreased due to the restricted flow. However, although the tissue lactate content remained unchanged, the lactate extraction was negative during rapid paired stimulation in the studies of Marshall et al. (50). Thus, the heart used in the studies of Marshall et al. probably was ischemic. If so, the decrease of the stimulation-induced elevation in MVO_2 was due to ischemia-induced heart failure rather than down-regulation of myocardial oxygen demand.

Myocardial ischemia has been defined as an imbalance between the supply of oxygenated blood and the oxygen requirements of the myocardium; major hallmarks of ischemia include reduction in contractile function and the presence of anaerobic glycolysis, as evidenced by accumulation of its

metabolic products, including lactate (65). It has been known for a long time that ischemia decreases myocardial work and MVO_2, and this is simply due to lack of oxygen for aerobic energy production and inability of anaerobic glycolysis to maintain myocardial work. It is very important to point out that this ischemia-induced inability to increase MVO_2 and myocardial work in response to positive inotropic or chronotropic stimulation is different from the myocardial down-regulation of oxygen demand observed in the present study. In Group Ia LP & ISO, lactate extraction was above 30% throughout the protocol, and contractile function was maintained during LP and increased to the same degree by ISO as during HP. Thus, we can say that the ISO-induced elevation in myocardial oxygen utilization was reduced deliberately rather than obligatorily when oxygen supply was limited. This explanation is consistent with the observed decrease in MVO_2 when PP was reduced.

The difference of coronary lactate extraction between the results of Marshall et al. (50) and Group Ia LP & ISO might be due to different animal models. They used an isolated, isovolumic, bovine-blood perfused rabbit heart, whereas we used an in vivo canine heart. However, it is more likely that the difference is due to the method of controlling CBF. Marshall et al. kept CBF constant at the basal rate throughout the protocol. We held perfusion

pressure instead of CBF constant throughout the protocol. We consider the constant pressure approach more physiological since CBF could increase, making the heart more resistant to ischemia. This was apparent from the ISO-induced vasodilation in the constant pressure protocol, which increased flow 70% and 45% when the perfusion pressure was held at 100 mmHg and 60 mmHg, respectively. Thus, coronary flow reserve was still available at constant perfusion pressure of 60 mmHg.

Results from Group IIa Hypoxemia & ISO showed that ISO-induced elevations in MVO_2 and myocardial work were decreased by 24% and 25% in the presence of hypoxemia (CaO_2 = 9.8 ± 0.3 ml/dl), respectively. Since there was again no evidence of myocardial ischemia, this decrease in MVO_2 was interpreted as a decrease in myocardial oxygen demand.

We chose CaO_2 = 9.8 ± 0.3 ml/dl to avoid a hypoxemia-induced decrease in contractile function. In preliminary experiments, we found that contractile function was decreased when CaO_2 fell below 5 ml/dl, although there was a four-fold increase in flow and, thus, normal oxygen delivery. In Group IIa hypoxemia & ISO, hypoxemia alone did not change contractile function, MVO_2 or lactate extraction. In addition, when ISO was infused during hypoxemia, flow increased further from 240% of control to 390% of control.

Consequently, oxygen supply to the ISO-stimulated myocardium during hypoxemia was not different from oxygen supply to the ISO-stimulated myocardium during normoxemia.

The reductions of ISO-induced elevations in MVO_2 and myocardial work in the presence of hypoxemia were consistent with the results reported by Kammermeier et al. (41). In their study, the heart rate was increased up to 400 beats/min by electrical stimulation. During high heart rate, both myocardial work, estimated by pressure•rate product, and oxygen demand, estimated from MVO_2, were decreased when the oxygen tension of buffer perfusate was decreased from 95% to 45%. The animal model they employed was an isolated isometrically working rat heart. Since lactate extraction was not measured, and the buffer-perfused isolated heart is always akin to ischemia (25), especially under the condition of hypoxemia and elevated oxygen demand, one must wonder if the hearts in their experiments were ischemic.

From the results of Group Ia LP & ISO and Group IIa Hypoxemia & ISO, we concluded that the ISO-stimulated myocardial demand can be down-regulated by the presence of low oxygen supply and low CaO_2, respectively. In addition, ISO-stimulated myocardium seems to have different strategies available to down-regulate its oxygen demand in response to the different stresses, for instance, low oxygen supply or low CaO_2.

Despite the similar oxygen down-regulations observed in low perfusion pressure (Group Ia) and hypoxemia (Group IIa), it is apparent that different strategies were used to decrease the oxygen demand. By definition, myocardial oxygen demand can be decreased by either decreasing myocardial work and/or increasing oxygen utilization efficiency. In Group Ia, ISO-induced elevation in myocardial work was not influenced by low perfusion pressure, and oxygen utilization efficiency in the ISO-stimulated heart was increased in the presence of low perfusion pressure. In Group IIa, ISO-induced elevation in myocardial work was decreased in the presence of hypoxemia, and oxygen utilization efficiency was not influenced by hypoxemia. Thus, in the ISO-stimulated myocardium, oxygen down-regulation was achieved by increasing oxygen utilization efficiency in the presence of low perfusion pressure and by decreasing myocardial work in the presence of hypoxemia.

The different strategies may be due to the different oxygen supplies during LP+ISO in Group Ia and during HYPX+ISO in Group IIa, since myocardial oxygen supply during LP+ISO in Group Ia was 40% less than ISO at PP = 100 mmHg, and myocardial oxygen supply during HYPX+ISO in Group IIa was not different during ISO at PP = 100 mmHg. However, results from Group Ia ADO & ISO contradict this approach. Although myocardial oxygen supply during ADO+ISO was 190% higher than ISO, myocardial oxygen down-regulation during ADO+ISO was

accompanied by increasing oxygen utilization efficiency, as during LP+ISO, instead of decreasing myocardial work, as during HYPX+ISO. To explain why Group Ia and Group IIa apparently used different strategies to achieve oxygen down-regulation, it is helpful to discuss the possible mechanisms of myocardial oxygen down-regulation.

What Is the Mechanism of Myocardial Oxygen Down-Regulation?

ADO & ISO

There is controversy regarding the effects of adenosine on ISO-stimulated myocardium. Schrader et al. (73) reported that adenosine attenuated the positive inotropic effects of catecholamines in isolated perfused guinea pig heart by inhibiting the ISO-induced stimulation of cardiac adenylate cyclase. On the basis of this finding, it was assumed that endogenously released adenosine, like exogenous adenosine, can act in the myocardium as a negative feedback inhibitor of myocardial adenylate cyclase, and prevent sympathetic overstimulation. However, Schutz and Tuisl (75) demonstrated that neither the adenosine analogue PIA (N^6-phenyl-isopropyl-adenosine) nor the adenosine analogue NECA (5'-N-ethylcarboxamide-adenosine) had any inhibitory effect on the ISO-induced stimulation of adenylate cyclase activity in guinea pig heart. In an isolated rat heart preparation, Dobson (21) reported that 10 μM of adenosine caused a 62% reduction in the ISO-induced elevation in left ventricular

dP/dt. Similarly, Schmitz et al. (72) found that adenosine eliminated the positive inotropic effect induced by ISO in guinea pig auricles but did not affect the ISO-induced elevation in c-AMP. In contrast to those findings in buffer-perfused, isolated hearts, Seitelberger et al. (77) reported that the dose dependent positive inotropic and chronotropic effect of intracoronary infusions or bolus injections of isoproterenol were neither antagonized by adenosine nor by PIA in the in vivo canine heart. Based on this finding, the authors suggested that an adenosine-catecholamine antagonism is of no physiological relevance in dogs. In the present study, ISO-induced increases in dP/dt and S% were apparently not influenced by the presence of adenosine as long as O_2 delivery was uncompromised, as shown in Group Ia ADO & ISO. This confirms the conclusion of Seitelberger et al. (77) that adenosine does not decrease ISO-induced elevation in myocardial contractility in the in vivo canine heart. However, as also shown in Group Ia ADO & ISO, ISO-induced elevation in MVO_2 was decreased in the presence of ADO. The concept that adenosine can act in the myocardium to prevent the imbalance between O_2 supply and demand during sympathetic overstimulation, as proposed by Schrader (73) and Dobson (21), is substantiated by the present study.

Thus, we conclude that adenosine protects myocardium from sympathetic overstimulation by decreasing myocardial

oxygen demand as proposed by Schrader(73) and Dobson (21), but, unlike their proposal, myocardial oxygen demand is decreased by increasing oxygen utilization efficiency rather than antagonizing ISO-induced positive inotropy.

ADO as a mediator

In this investigation, we tested the hypothesis that myocardial down-regulation of oxygen demand is mediated by adenosine. Results from Group Ia ADO & ISO demonstrated that ISO-induced elevation in MVO_2 was reduced in the presence of exogenous adenosine, but ISO-induced elevation in myocardial work was not influenced by exogenous adenosine. As a result, exogenous adenosine increased oxygen utilization efficiency of ISO-stimulated myocardium. This ADO-induced myocardial oxygen down-regulation was similar to the LP-induced myocardial down-regulation of oxygen demand. As shown in Group Ia LP & ISO, low perfusion pressure increased oxygen utilization efficiency of ISO-stimulated myocardium by 60%, and ISO-induced elevation in myocardial work was not influenced by low perfusion pressure. The similarities of the strategy with which ISO-stimulated myocardium down-regulated its oxygen demand during exogenous adenosine administration and during low perfusion pressure suggest that a similar mechanism was employed in these two conditions.

The hypothesis that myocardial down-regulation of oxygen demand is mediated by adenosine is further supported by the

results of ADA and EHNA studies. As shown in the results of Group Ib ADA/LP+ISO, the oxygen utilization efficiency of ISO-stimulated myocardium during low perfusion pressure was reduced in the presence of ADA. This reduction in the oxygen utilization efficiency resulted from the unchanged ISO-induced elevation in MVO_2 and the significant decrease in ISO-induced elevation in myocardial work. It is rather surprising that the lactate extraction during ADA/LP+ISO was not decreased. This suggests that ISO-stimulated myocardium in the presence of ADA during low perfusion pressure can still down-regulate its oxygen demand, but employs a different strategy, i.e., decreasing myocardial work instead of increasing myocardial oxygen utilization efficiency as during low perfusion pressure in the absence of ADA. It also suggests that there may be other mechanisms in addition to adenosine to protect the myocardium from being ischemic in the absence of adenosine during ADA/LP+ISO. Results from Group Ic EHNA/LP+ISO further support the role of adenosine in the myocardial down-regulation of oxygen demand. As shown in the results, the oxygen utilization efficiency of ISO-stimulated myocardium during low perfusion pressure was increased in the presence of EHNA.

Thus, from the results of Group Ib ADA/LP+ISO and Group Ic EHNA+ISO, we propose the following mechanism for the myocardial down-regulation of oxygen demand during LP+ISO.

During LP+ISO, adenosine is released from the myocardium. In addition to acting as a coronary dilator (13), released adenosine supports an ISO-induced elevation in myocardial work by increasing myocardial oxygen utilization efficiency. This increase in oxygen efficiency limits the need for further coronary reserve. In the presence of ADA, less adenosine is available to increase myocardial oxygen utilization efficiency during ADA/LP+ISO, and consequently, oxygen utilization efficiency decreases and ISO-induced elevation in myocardial work can no longer be maintained. In contrast, in the presence of EHNA, more adenosine is available to increase myocardial oxygen utilization efficiency during EHNA/LP+ISO, and, consequently, oxygen utilization efficiency increases and ISO-induced elevation in myocardial work also increases.

ADO release during ISO

In the present investigation, we did not measure adenosine concentration in myocardial interstitial fluid. Consequently, we do not know adenosine concentrations in the different experimental conditions, i.e., LP, LP+ISO, HYPX, HYPX+ISO, ADA/LP+ISO etc. However, the relative changes of adenosine concentrations can be predicted in the present experimental design (see below).

ADO release

There is a great deal of controversy about how myocardium releases adenosine. In the original proposal of

Berne (13), the production of adenosine in the heart was due to an imbalance in the supply of oxygen to the myocardium relative to the myocardial oxygen demand. Thereafter, several studies (22, 45, 53) showed a general correlation between adenosine formation and MVO_2 under a range of conditions. Based on those observations (22, 45, 53), it was postulated that adenosine formation was regulated by cardiac energy expenditure or MVO_2. However, further studies have revealed that the relation between adenosine formation and MVO_2 is not always consistent, especially during cardiac pacing and elevated afterload (34, 90). Also inconsistent with the proposal that MVO_2 is the determinant of adenosine formation is the observation of Bardenheuer and Schrader (6) that enhancement of oxygen supply by overperfusion of the coronary arteries in ISO-stimulated hearts was associated with a significant reduction of nucleoside release into the effluent. In addition, the original oxygen supply-demand model seems to explain the inability of elevated afterload, and, the resulting increase in MVO_2 to increase adenosine formation (6, 7) since an increased afterload results in increased oxygen delivery via elevated coronary flows. As a result, the ratio of oxygen supply to demand is not significantly decreased under these conditions. Consequently, Bardenheuer and Schroder (7) concluded that the oxygen supply to demand ratio appears to provide a good index

for ADO release over variety of conditions. More recently, Headrick and Willis (33) observed that adenosine release was approximately four fold greater in the hearts perfused at 12 ml/min/g than in similar hearts perfused at 20 ml/min/g, although MVO_2 was the same in both cases. Since they observed adenosine release independent of MVO_2, they also proposed that the oxygen supply to demand ratio and not MVO_2 is the major determinant of adenosine release, as described originally by Berne (12) in 1963.

In 1986, Bunger and Soboll (17) proposed a model to explain how the adenosine is released from the myocardium. In accordance with the linkage between the ATP and AMP shown in Fig. 1, an increase in $[ADP]_f$, whether induced by an increase in ATP utilization or by a decrease in the resynthesis of ATP from ADP, will increase adenosine production. This is due to a proportional relationship between ATP utilization and $[ADP]_f$ and a close relationship between $[ADP]_f$ and $[AMP]_f$, as predicted from the myokinase reaction, and a proportional relationship between adenosine production and $[AMP]_f$, as predicted from the 5'-nucleotidase reaction. In fact, Bunger and Soboll (17) showed a proportional relationship among MVO_2, $[AMP]_f$, and adenosine production in the isolated guinea pig heart.

Thus, what controls adenosine releasing from myocardium

is still controversial. Fortunately, ISO-induced elevation in adenosine release has been consistently reported in the literature. Firstly, since ISO is a positive inotropic agent, it increases MVO_2, and, as predicted by the MVO_2 theory of adenosine release, increased MVO_2 will increase adenosine release. Secondly, even in the studies of Bardenheuer and Schrader (6, 7) and Headrick and Willis (33), an ISO dose-related increase in adenosine release was observed, although the slope between MVO_2 and adenosine was changed by oxygen supply.

Van Wylen et al. (87) using an in vivo cardiac microdialysis technique demonstrated a 2.5 fold increase in dialysate adenosine concentration during dobutamine infusion in canine heart. Gidday et al. (28), using an epicardial well technique, also showed a two fold increase in interstitial fluid adenosine concentration in catecholamine-stimulated canine heart. Consequently, in the present study, it is reasonable to assume that adenosine in myocardial interstitial fluid was increased by ISO infusion during both normal perfusion pressure and low perfusion pressure. Thus, it is not surprising that the effects of ADA and EHNA infusions on the ISO-stimulated myocardium during normal perfusion pressure are similar to the effects of ADA and EHNA infusions during low perfusion pressure. As shown in the results of Group Ib ADA & ISO, under normal perfusion

pressure, ISO-induced elevations in the MVO_2 and myocardial work were reduced by 17% and 20% in the presence of ADA, respectively. As shown in the results of Group Ic EHNA & ISO, under normal perfusion pressure, oxygen utilization efficiency of ISO-stimulated myocardium was increased by EHNA. Therefore, like low perfusion pressure, endogenous adenosine released from the ISO-stimulated myocardium during normal perfusion pressure also supports ISO-induced elevation in myocardial work and increased oxygen utilization efficiency.

Since low perfusion pressure limits oxygen supply, and increases the rate of adenosine release by myocardium (25), adenosine concentration in myocardial interstitial fluid was probably higher during LP+ISO than during ISO. Thus, the more pronounced effects of ADA and EHNA on the ISO-stimulated myocardium during low perfusion pressure compared to the effects during normal perfusion pressure were probably due to more adenosine available to down-regulate oxygen demand during LP+ISO than during ISO.

Hypoxemia & ADO Release

In the present investigation, we produced non-ischemic hypoxia in a restricted region of left ventricular myocardium by perfusing selectively the LAD with partially deoxygenated blood (CaO_2 = 9.8 ± 0.2 ml/dl). This approach precluded the hemodynamic instabilities that accompany cardiac failure and

arousal of the chemoreceptor reflex caused by systemic hypoxemia. The stability of our systemic and cardiac hemodynamic data is evident in Table VIII. This stable preparation provided a satisfactory model to study myocardial oxygen demand during hypoxemia.

Unfortunately, the adenosine concentration in myocardial interstitial fluid during moderate hypoxemia is not known. However, adenosine has been shown to be released into venous effluent and into pericardial fluid during nonischemic myocardium hypoxemia (25). Consequently, interstitial fluid adenosine concentration was likely higher than control value during HYPX+ISO but lower than the value during LP+ISO.

Results of Group IIb and Group IIc supported the role of adenosine in down-regulating myocardial demand during hypoxemia. As shown in the results of Group IIb ADA/HYPX+ISO, in the presence of ADA, the oxygen utilization efficiency of ISO-stimulated myocardium was decreased in the presence of hypoxemia compared to normoxemia. In contrast, in the absence of ADA, the oxygen utilization efficiency was not influenced by the presence of hypoxemia, as shown in the results of Group IIa. In addition, in the presence of EHNA, the oxygen utilization efficiency of ISO-stimulated myocardium was increased by 44% in the presence of hypoxemia. Thus, we postulate that adenosine, released from the ISO-stimulated myocardium during hypoxemia, was not completely washed out and remained relatively high concentration during

hypoxemia. However, there was some additional washout of adenosine during HYPX+ISO. As a result, adenosine concentration in myocardial interstitial fluid was high enough to maintain oxygen utilization efficiency, but not high enough to increase the oxygen utilization efficiency and maintain myocardial work during HYPX+ISO.

How Does Adenosine Down-Regulate Myocardial Oxygen Demand?

The mechanism by which adenosine improves myocardial utilization efficiency is currently unknown. One possibility is that adenosine improves the oxygen utilization efficiency by modulating the type of work the myocardium performs, since oxygen demand is determined not only by the amount of work, but also by the type of work performed by the myocardium. By type of work we refer to the work due to heart rate or the work due to pressure development. Berglund et al. (11) reported that increased myocardial work due to increased heart rate results in a disproportionally large increase in MVO_2, whereas increased myocardial work due to increased developed pressure results in a relatively small increase in MVO_2. More recently, Tanaka et al. (82) demonstrated that, within the tested heart rate range of 100-200 beats/min, MVO_2 monotonically increased with increases in heart rate despite constant cardiac work. In the study of Tanaka et al., cardiac output was kept constant with a constant-flow bypass

pump, and mean aortic pressure was also kept constant by inflation or deflation of an intra-aortic balloon. As a result, cardiac work was kept constant. Consequently, they concluded that an optimal heart rate which minimized myocardial oxygen consumption for a constant cardiac work was at the lowest tested heart rate, i.e., 100 beats/min. Tanaka et al. thought an increase in the MVO_2 component for the excitation-contraction coupling is responsible for the lower oxygen utilization efficiency during high heart rate. In the present study, heart rate was relatively constant throughout the protocols in all groups except Group IIc EHNA/HYPX+ISO. Thus, it is unlikely that oxygen down-regulation observed in this investigation is due to the negative chronotropic effect of released adenosine on the heart. In the Group IIc, however, there was a 15% decrease in heart rate accompanied by a 44% increase in oxygen utilization efficiency during EHNA/HYPX+ISO, when compared to EHNA+ISO. But since our calculation of myocardial work includes heart rate, the decrease in heart rate also proportionally decreased estimated myocardial work. Thus, the 44% increase in oxygen utilization efficiency observed during EHNA/HYPX+ISO was unlikely due completely to the ADO-induced decrease in heart rate.

Another possibility is the ability of adenosine to switch the primary energy-yielding substrate from lipid to

carbohydrate. Kahles et al. (40) studied the effects of N^6-alkyl-N^6-cyclohexyl-adenosine, an adenosine analogue, on myocardial metabolism and ischemic stress following coronary occlusion. They demonstrated that the adenosine analogue promotes glucose uptake, and thereby possibly protects the heart with a compromised coronary circulation.

The fatty acid molecules contain little oxygen and, therefore, can yield more ATP for each carbon atom, when compared to carbohydrate. The disadvantage of fatty acids as fuel is that for each molecule of ATP produced, they need relatively more oxygen. Experimentally, a heart using fatty acid alone would need about 12% more oxygen to produce the same amount of ATP than when using only glucose (62). This is because that each turn of the fatty acid spiral yields equal amounts of $FADH_2$ and $NADH_2$. $FADH_2$ enters the respiratory chain at a lower energy level than $NADH_2$ and yields less ATP. This accounts for part of the oxygen-wasting capacity of fatty acids. In addition, excess FFA may be oxidized by mechanisms not coupled to ATP formation (18).

In the human heart, Simonsen and Kjekshus (78) studied the effect of myocardial uptake of free fatty acids on myocardial oxygen consumption in relation to increased heart rate and inotropic stimulation. They found that inhibition of lipolysis with ß-pyridyl carbinol almost abolished myocardial uptake of free fatty acids and reduced MVO_2

significantly. They also found that augmentation of myocardium uptake of free fatty acid by triglyceride infusion increased MVO_2 significantly above control level in the ISO-stimulated heart. In canine heart, an inhibitor of lipolysis has a protective effect on the acutely ischemic myocardium (43, 61). Free fatty acids released by catecholamine stimulation have also been shown to augment MVO_2 in anesthetized dogs above the changes induced by the increase in myocardial work (54). Thus, it is likely that ISO-induced release of adenosine switches the primary energy-yielding substrate from fatty acid to carbohydrate, and increases oxygen utilization efficiency of the myocardium.

Although our data suggest that endogenous adenosine is a mediator of myocardial oxygen down-regulation, they should not be construed as evidence that adenosine is the exclusive mechanism. As mentioned above, lactate extraction during ADA/LP+ISO and ADA/HYPX+ISO was not decreased. This suggests that ISO-stimulated myocardium has other mechanisms in addition to adenosine to protect itself from ischemia. Metabolites released from cardiomyocytes other than adenosine such as carbon dioxide, hydrogen and potassium ions, factors released from coronary endothelium such as endothelium-derived relaxing factor or prostaglandin, and direct effect of reduced PO_2 during both low perfusion pressure and hypoxemia perfusion might have unknown effects on myocardial

oxygen demand and thus promote down-regulation or even serve as redundant mechanisms other than adenosine to down-regulate myocardium oxygen demand. These other possible mechanisms can not be excluded at the current stage.

Future Investigations

This investigation has addressed the question "Can myocardium down-regulate its oxygen demand?", and also studied the role of adenosine in down-regulation. However, it has brought forth other questions which could be clarified by future investigations:

1. What is the clinical relevance and functional significance of the observed myocardial oxygen down-regulation in obstructive coronary disease and pulmonary disease? Myocardial oxygen down-regulation is apparently an intrinsic mechanism to protect the myocardium from ischemia and resulting dysfunction in canine heart. Does myocardial oxygen down-regulation exist in human heart? Is myocardial oxygen down-regulation related to myocardial stunning, which has been thought to be a mechanism to protect myocardium from infarction (74)? Is myocardial oxygen down-regulation related to myocardial ischemic preconditioning, which has been shown to be a mechanism to protect myocardium from infarction (56), and might adenosine be responsible for this phenomena (47)?

2. Myocardial interstitial adenosine concentration during conditions such as LP+ISO, ADA/LP+ISO, EHNA/LP+ISO, HYPX+ISO, ADA/HYPX+ISO, and EHNA/HYPX+ISO should be measured to confirm the role of adenosine in the myocardial oxygen down-regulation.
3. In the present study, oxygen demand is elevated by ISO stimulation. Other interventions to increase oxygen demand such as sympathetic nerve stimulation, pacing-induced tachycardia, and increase of Ca^{++} concentration should be examined to show that the observed oxygen down-regulation is or is not specific to ISO-stimulation.

CHAPTER V

SUMMARY AND CONCLUSIONS

Summary

1. ISO-induced elevation in MVO_2 was reduced by lowering perfusion pressure from 100 mmHg to 60 mmHg while ISO-induced elevation in the myocardial work was not significantly changed. Exogenous adenosine mimicked the effects of low perfusion pressure on the ISO-stimulated myocardium.

2. ADA decreased and EHNA increased the oxygen utilization efficiency of ISO-stimulated myocardium during low perfusion pressure.

3. Hypoxemia decreased ISO-induced elevations in MVO_2 and myocardial work, but did not change oxygen utilization efficiency.

4. ADA decreased and EHNA increased the oxygen utilization efficiency of ISO-stimulated myocardium during hypoxemia.

Conclusion

1. ISO-stimulated myocardium can down-regulate its oxygen demand when oxygen delivery is limited or when arterial oxygen content is decreased.

2. Endogenous adenosine released from ISO-stimulated myocardium can increase oxygen utilization efficiency and, thus, down-regulate myocardial oxygen demand.

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