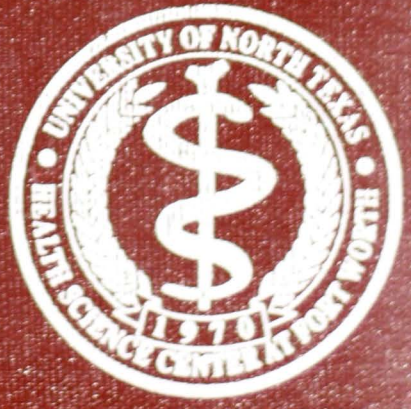
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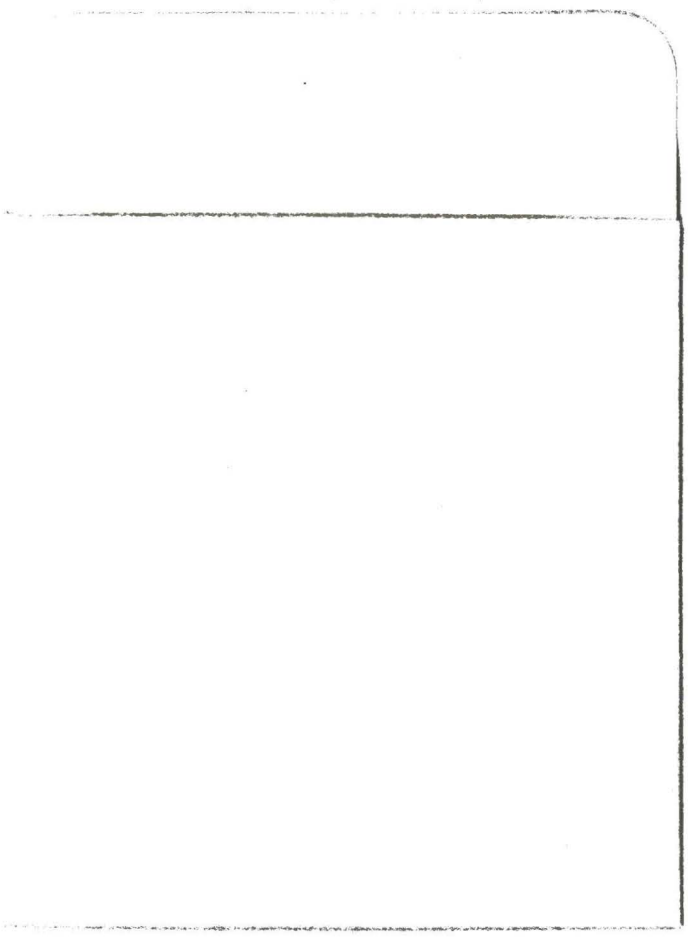

REGULATION OF MYOCARDIAL BLOOD FLOW AND FUNCTION

DURING EXERCISE IN DOGS

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DISSERTATION

Presented to the Graduate Council of the

Graduate School of Biomedical Sciences

University of North Texas Health Science Center at Fort Worth

in Partial Fulfillment of the Requirements

For the Degree of

DOCTOR OF PHILOSOPHY

By

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June, 1995

ACKNOWLEDGMENTS

This investigation was supported by NIH Grant HL-29232 and HL-34172. Education and research training was supported by the Department of Physiology at the University of North Texas Health Science Center at Fort Worth. Five original articles and manuscripts, and four abstracts are the result of this support and training. The first article was selected as the second place in Upjohn Research Achevement Award. The second article was selected as a National Student Research Award by the 40th annual meeting of American College of Sport Medicine in Indianapolis, Indiana, and also first place in poster presentation in American College of Sports Medicine's Texas Regional Chapter, 1994.

I express my sincere appreciation to Dr. Patricia A. Gwirtz for guidance and friendship, and Dr. Peter B. Raven for encouragement and leadership during the past two years. I would also like to thank Drs. James L. Caffrey, Thomas Yorio, Eugene Quist, and Edward L. Orr for their assistance and interest in serving as members of my committee. I will surely miss the outstanding technical expertise of Abraham Heymann and Linda Legorreta.

To my wife, Son-Hui, daughter, Sarah, and son, Timothy, who have provided a unwavering emotional support and patience, I love you. To my mother who is not with

me, I miss you and would like to give you this achievement and glory. Heartfully, to my Lord, I praise you!

The following original publications and abstracts are the result of this support and training.

PUBLICATIONS:

1. **Song-Jung Kim**, Patricia A. Gwirtz. Limitation of cardiac output by a coronary α_1- constrictor tone during exercise in dogs. *Am. J. Physiol.* (in revision).
2. **Song-Jung Kim**, Patricia A. Gwirtz. Effect of intracoronary nitric oxide blockade on coronary blood flow and dp/dt_{max} during exercise. *Am. J. Physiol.* (submitted).
3. **Song-Jung Kim**, Patricia A. Gwirtz. Interaction between α-adrenergic vasoconstriction and endothelial-dependent vasodilation during exercise. *Am. J. Physiol.* (in preparation).
4. **Song-Jung Kim**, Patricia A. Gwirtz. α_1-adrenergic vasoconstriction does not limit coronary vasodilation and cardiac output during maximal exercise. *Am. J. Physiol.* (in preparation).

5. Gwirtz, Patricia A., **Song-Jung Kim**. Effect of coronary α_1-adrenergic constrictor tone on transmural flow distribution. *J. Appl. Physiol.* (in preparation).

ABSTRACTS:

1. **Song-Jung Kim**, Patricia A. Gwirtz. α_1-adrenergic vasoconstriction does not limit coronary vasodilation and cardiac output during maximal exercise. *FASEB J.* 8:A853, 1994.
2. **Song-Jung Kim**, Patricia A. Gwirtz. Intracoronary EDRF blockade limits coronary blood flow and dp/dt_{max} during submaximal exercise. *Med. Sci. Sports Exerc.* 26:S130, 1994.
3. Patricia A. Gwirtz, **Song-Jung Kim**. Intracoronary blockade NO synthase limits coronary vasodilation during submaximal exercise. *Eur. J. Appl. Physiol.* 69 (suppl):S22, 1994.
4. **Song-Jung Kim**, Patricia A. Gwirtz. Competition between nitric oxide-mediated coronary dilation and an α_1-adrenergic vasoconstrictor tone during exercise. *FASEB J.* (submitted)

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ACh	acetylcholine
Cao_2	arterial oxygen content
CBF	circumflex blood flow
CFV	main coronary flow velocity
cGMP	cylic guanosine momophosphate
CO	cardiac output
Cvo_2	venous oxygen content
dP/dt_{max}	maximum rate of rise of left ventricular pressure
EDRF	endothelium-derived relaxing factor
GC	guanylate cyclase
HR	heart rate
L-NMMA	N^G-monomethyl-L-arginine
LVEDP	left ventricular end diastolic pressure
LVP	left ventricular pressure
LVSP	left ventricular systolic pressure
MAP	mean aortic pressure
MVO_2	myocardial oxygen consumption
NLA	N-nitro-L-arginine
NO	nitric oxide

SNP	sodium nitroprusside
SV	stroke volume
SVR	systemic vascular resistance

CHAPTER I

INTRODUCTION

BACKGROUND

Coronary circulation during exercise

Coronary blood flow is regulated primarily by local metabolic mechanisms according to the oxygen and nutrient needs of the heart (2, 4, 19). The local "metabolic signal" involves vasoactive metabolites, such as adenosine, released from myocytes in direct proportion to myocardial work (Figure 1). However, other external factors are superimposed on local regulatory mechanisms and can substantially modulate coronary blood flow. One of these modulatory factors is the sympathetic nervous system. Sympathetic vasoconstriction mediated by α-adrenergic receptors in the coronary circulation has been shown to oppose metabolic vasodilation and limit oxygen supply to the myocardium during physiologic and pathophysiological cardiac stresses, such as exercise and myocardial hypoperfusion (1, 6, 7, 9, 10-14, 17, 18, 21). This limitation on myocardial oxygenation appears to impose a restriction on the increase in regional left ventricular subendocardial contractile function during submaximal exercise (7). In this regard, studies have shown that removing this α_1-constrictor tone leads to an increase in coronary blood flow and, as

a result, regional contractile function (8). This adrenergic coronary constriction during exercise is mediated by neurally released norepinephrine, not by circulating catecholamines (8).

Endothelial-mediated control of coronary vascular tone

Recent investigations indicate that another factor involved in modulating coronary blood flow is the vascular endothelium. The endothelium exerts an influence on vascular smooth muscle vasomotor tone by releasing an endothelium-derived relaxing factor (EDRF) or nitric oxide (NO), which is derived from the amino acid L-arginine by nitric oxide synthase (5, 22). Synthesized NO diffuses into the underlying vascular smooth muscle to activate cytosolic guanylate cyclase (GC), thereby stimulating the intracellular accumulation of cyclic GMP (cGMP). This is illustrated in Figure 2. NO is released by the stimulation of muscarinic receptors on endothelial cells by acetylcholine, as well as by other agonists or physical stimuli (e.g., shear stress) at the interface between blood and endothelial cell surface (15).

During exercise, for example, the work output of the normal heart may increase several-fold by the stimulation of sympathetic nerves to heart. The increased work output of the heart increases myocardial oxygen demand. Consequently, the coronary circulation undergoes vasodilation due to local metabolic mechanisms. The elevation in shear stress caused by increases in coronary blood flow triggers release of NO from the endothelium because of the extremely pulsatile nature of the flow.

Therefore, it is likely that during exercise, release of NO by shear stress and by neurohormonal stimuli, concomitant with local release of metabolites, contributes to coronary dilation. These vasodilatory influences counteract a sympathetic α-adrenergic coronary constriction, which limits the increase in coronary blood flow and cardiac performance. Accordingly, coronary vascular smooth muscle tone during exercise is modulated by the endothelium, which responds to the increased shear stress and adrenergic stimulation, which provides the major extrinsic input.

SPECIFIC AIMS

Dynamic exercise results in activation of the sympathetic nervous system which increases both the inotropic and chronotropic states of the myocardium. While the increased work output of the heart causes increased coronary blood flow in proportion to the increased metabolic demand, it has been shown that the direct result of sympathetic activation of coronary vessels is vasoconstriction mediated though α-adrenergic receptors. During exercise in conscious dogs, this coronary vasoconstriction, mediated by α_1-adrenergic receptors, places a limitation on myocardial oxygenation and imposes a restriction on regional left ventricular contractile function. Removing this coronary α_1-constrictor tone with intracoronary prazosin leads to an increase in coronary blood flow and, as a result, regional contractile function. However, no study to date has examined whether the restriction of coronary blood flow by an α_1-constrictor tone during submaximal exercise, a state

of increased oxygen demand, imposes a significant limitation on global cardiac performance which is related to the simple pumping function of the heart, as reflected in the cardiac output. Therefore, the first aim of the proposed series of studies is to address this possibility.

Since Furchgott and Zawadzki (5) first described that the vascular relaxation induced by acetylcholine was dependent on the presence of the endothelium, many studies have been conducted in isolated perfused vessels *in vitro* to investigate the role of the endothelium. While these findings show that NO plays an important role in modulating vasomotor tone, the nonphysiologic characteristics of the preparation, such as lack of basal neurogenic and humoral influences, raise important questions about the applicability of the *in vitro* findings to the normal physiological condition. Thus, several studies attempted to investigate the role of endothelium in dilation of the coronary artery during resting and exercise conditions in conscious dogs with intravenous administration of N^G-monomethyl-L-arginine (L-NMMA) or nitro-L-arginine (NLA), specific inhibitors of NO formation (3, 16, 20, 23). However, intravenous administration of L-NMMA or NLA induced alterations in hemodynamic parameters, including aortic blood pressure, heart rate, and systemic vascular resistance. Alterations in these parameters have been demonstrated by themselves to have profound effects on coronary hemodynamics and myocardial oxygen consumption, making it difficult to identify the direct effects of NO on coronary circulation. Accordingly, the primary objective of the current study is to evaluate

coronary blood flow control mechanisms during exercise by using the selective intracoronary infusion of drugs in chronically instrumented conscious dogs. This approach avoids the pronounced systemic hemodynamic responses that confounded interpretation of previous studies because of concurrent changes in myocardial work demand and activation of neural and hormonal reflex pathways.

The proposed studies using selective intracoronary infusion of drugs will test the following hypotheses:

i) the restriction of coronary blood flow by an α_1-constrictor tone during exercise imposes a significant limitation on global cardiac performance (i.e., cardiac output);

ii) NO, in addition to metabolic vasodilation, released from the endothelium contributes to the coronary vasodilation during exercise;

EXPERIMENTAL DESIGN AND METHODS

Series 1. Effect of an α_1-constrictor tone on cardiac performance.

Rationale: Sympathetic vasoconstriction in the coronary circulation has been shown to oppose metabolic vasodilation and limit oxygen supply to the myocardium during exercise. Removing an coronary α_1-constrictor tone leads to an increase in coronary blood flow and, as a result, regional conrtractile function. However, it has not been addressed whether the restriction of coronary hyperemia by an α_1-constrictor tone during exercise imposes a significant limitation on global cardiac performance. Thus,

Series 1 experiments will test the hypothesis that *during submaximal exercise, a state of increased oxygen demand, an α_1-constrictor tone limits coronary blood flow and, thus, limits overall cardiac performance measured as cardiac output.*

Experimental Preparation: Studies will be performed in healthy mongrel dogs (25 - 35 kg) of either sex on the basis of being heartworm free, and willing to run on a motor-deriven treadmill. A chronically instrumented dog model will be used since it permits repeated measurements of global cardiac function and coronary blood flow in the absence of anesthesia. All dogs will be premedicated with acepromazine (0.03 mg/kg, s.c.), anesthetized with surital (0.2 mg/kg, i.v.) and intubated. A surgical level of anesthesia will be maintained using gaseous anesthesia with isoflurane (1-3 %) with nitrous oxide (0.6 l) and oxygen (1.0 l). The tidal volume and rate of the ventilation will be set at approximately 15 ml/kg and 15 - 20 breaths/min, respectively.

Using sterile techniques, a left thoracotomy will be performed in the fifth intercostal space, and the heart will be suspended in a pericardial cradle and instrumented as illustrated in Figure 3. A noncannulating Transonic transit time Doppler flow probe (Transonic Systems Inc., Ithaca, NY) will placed around the root of the aorta to measure cardiac output (CO). The heart will then be exposed and suspended in a pericardial cradle. Global left ventricular function (left ventricular pressure, dP/dt, and heart rate) will also be measured by implanting a Konigsberg P

6.5 transducer and a fluid-filled Tygon (1.25 mm ID and 1.27 mm OD) catheter in the apex of the ventricle. The Tygon catheter will be used to calibrate the Konigsberg transducer at the beginning of each experiment. The maximum rate of rise of the left ventricular pressure (dP/dt_{max}) will be obtained from the left ventricular pressure signal of Konigsberg transducer by electronic differentiation. Catheters will be placed into the proximal descending thoracic aorta for measurement of arterial blood pressure, and into the circumflex coronary artery for measurement of coronary perfusion pressure and intracoronary administration of prazosin.

The circumflex coronary artery will dissected free for a distance of approximately 3 cm, taking care not to damage the vessel adventia and surrounding nerves. A 10 mHz Doppler flow probe will be implanted around the base of the circumflex artery for measurement of circumflex inflow. A hydraulic occluder will be placed distal to the flow probe, and will be used to totally occlude the circumflex artery in order to establish a zero-flow baseline for the Doppler flow probe.

In all animals, when the surgical preparation is complete, all catheters, occluder, and lead wires will be tunneled under the skin to exit between the scapula. The ribs will be approximated, the incision will be closed in layers, and the thoracic cavity will be evacuated. Antibiotics and analgesics will be given as deemed necessary by the Animal Care Facility veterinarian for 5 days or as needed. The indwelling catheters will be flushed daily with heparinized saline to maintain patency. At least 10-14 days will be allowed before beginning experimentation.

After completion of all experiments, each dog will be anesthetized with sodium pentobarbital (30 mg/kg, i.v.). The circumference of the circumflex artery under the Doppler flow probe will be measured and used to calculate its cross-sectional area for conversion of flow velocity (cm/sec) to volume flow rate (ml/min). Circumflex blood flow (CBF) is calculated as: CBF (ml/min) = (π x D^2 x V)/4, where D is vessel diameter (cm), V is flow velocity (cm/sec).

Experimental Protocol: At least 10-14 days of recovery from surgery will be allowed before beginning experimentation. Resting values for left ventricular pressure (LVP), pulsatile and mean CFV, mean aortic pressure (MAP), CO, and heart rate (HR) will be recorded. After resting data is obtained, CFV and global myocardial function will be measured while the dog is subjected to a standardized submaximal exercise protocol using a motor-driven treadmill (Quinton model 18-65). The submaximal exercise test lasts 21 min and is divided into 3-min time blocks beginning with a warm-up at 4.8 kph, 0 % incline. The speed will be increased to 6.4 kph, and the incline of the treadmill varied at 3-min intervals to encompass 0, 4, 8, 12, and 16 % inclines. An intracoronary administration of prazosin will be used to assess the magnitude of the α_1-coronary constrictor tone during exercise at the highest workload of 6.4 kph/ 16 % incline. According to a previous study, the selective α_1-adrenoreceptor blockade of the circumflex perfusion territory will be accomplished with a bolus injection of prazosin (0.5 mg, i.c.). Gwirtz *et al* (8) demonstrated that this dose of prazosin has been shown to abolish the vasoconstrictor response to an

intracoronary dose of 20 μg phenylephrine without causing secondary peripheral circulator effects, such as changes in heart rate or blood pressure.

The experiment will consist of data collection while the dog is lying quietly, standing, and running on a treadmill. Each animal will be subjected to the standardized submaximal exercise regimen as described above three times on separate days. The first exercise test will be considered a control run with no drugs, and data will be recorded at rest and at each level of exercise. On the second day, the α_1-blocker prazosin (0.5 mg, i.c.) will be given at the highest level of exercise during submaximal exercise test. On the third day, the same dose of prazosin will be administered into the systemic circulation using the aortic catheter at the highest level of exercise (6.4 kph/ 16 % incline). These data will provide information regarding whether the changes in coronary blood flow and cardiac output are due to systemic reflex-induced changes or to significant recirculation of prazosin into systemic circulation. Data will be collected at rest, at the peak of exercise (6.4 kph/ 16 % incline) and 2 min after intracoronary injection of prazosin.

Data Acquisition and Analysis: Arterial and coronary blood pressure will be measured with an Isotec® pressure transducer, which is calibrated using a mercury manometer. During each experiment, hemodynamic variables will be recorded continuously both on a 8-channel strip recorder (Coulbourn Instruments) and on magnetic tape (Hewlett-Packard 8-channel tape recorder). Recorded on-line variables include: a) left ventricular pressure (LVP), c) circumflex flow velocity (CFV), c)

circumflex blood pressure (CBP), d) arterial blood pressure (AP), and e) aortic flow. Data will be analyzed with a computer acquisition program DataFlow by Crystal Biotech (Hopkinton, MA). Data will be obtained from this analysis include: a) left ventricular systolic pressure (LVSP), b) left ventricular end-diastolic pressure (LVEDP), c) dP/dt_{max}, d) CO, e) heart rate (HR), f) mean CFV, g) systolic and diastolic AP and MAP.

An analysis of variance for repeated measures will be used and individual post hoc comparisons will be performed using Tukey's protected t-test to evaluate the effects of the exercise and α_1-blocker prazosin on cardiac function and coronary hemodynamics. A $p < 0.05$ will be considered significant throughout this study.

A minimum sample size of seven (7) dogs will be used for the statistical analyses in these studies based on following requirements. The α (type I error) is 0.05 and ß (type II error) or 1 - ß (referred to as the "power" of the test of significance) is 0.1 or 0.9, respectively. If, for example, the estimated standard deviation of the population is 12.28 and minimum difference to detect as significant is 15 according to data from four preliminary experiments, the sample size shall be eight (see Table). However, since the hypothesis in this study is the directed hypothesis, the minimum required sample size is between 5 and 8 (α=0.1, one-tailed) depending on ß or 1-ß (power).

Interpretation of Results: These studies will provide new information regarding the role of a sympathetic mediated α-adrenergic constrictor tone on the coronary

vasculature on control of blood flow and cardiac output during exercise. We expect to find that removing a coronary α_1-constrictor tone with prazosin during submaximal exercise leads to an increase in myocardial oxygen supply and, as a result, we will observe an increase in stroke volume and cardiac output. Thus, a limitation of coronary flow by α_1-constrictor tone might prove to be beneficial for the delivery and distribution of coronary blood flow in the heart and for the distribution of cardiac output to working skeletal muscle during exercise.

Series 2. The role of NO on coronary vasodilation during exercise.

Rationale: Endothelium-dependent flow-induced dilation has been decribed in conduit arteries and small arteries. In isolated vessel preparations, NO release increases when flow is made pulsatile and the activity of endogenous NO appears to be greastest in large arteries, where resistance and shear stress are also highest. During exercise, the increased work output of the heart increases coronary blood flow by increasing myocardial oxygen demand. The elevation in shear stress caused by increases in coronary blood flow may trigger release of NO from the endothelium. Therefore, this study will test the hypothesis that, *in addition to the metabolic vasodilation, NO released by shear stress (as well as by neurohumoral stimulation) from the endothelium contributes to the coronary vasodilation and, thus, increases cardiac performance during exercise.* Furthermore, this study will determine the mechanism whether this change in vascular tone by NO during exercise will change

myocardial oxygen consumption.

Experimental Preparation: The chronically instrumented dog model described for Series 1 will be used with the following exceptions: a) a noncannulating Transonic transit time Doppler flow probe (Transonic Systems Inc, Ithaca, NY) will not be needed for the measurement CO in these studies, and b) a catheter will be placed into the coronary sinus for myocardial venous blood sampling.

Measurements of oxygen consumption in circumflex-perfused myocardium will provide fundamental information regarding whether changes in coronary blood flow due to the blockade of NO release from the vascular endothelium with L-NMMA will change or maintain MVO_2 by increasing oxygen extraction during exercise. Blood samples will be collected anaerobically from the aortic catheter and coronary sinus catheter. Oxygen saturation and hemoglobin content will be determined using a Radiometer OSM 2 hemoximeter. Arterial and venous oxygen content will be obtained by multiplying the respective oxygen saturation by hemoglobin content and by 1.36 gm/ml (the oxygen carrying capacity of hemoglobin). Oxygen extraction will be obtained by dividing the arterial-venous oxygen content difference by the arterial oxygen content. Myocardial oxygen consumption (MVO_2) will be calculated by Fick equation: MVO_2 (ml O_2/ml/min) = CBF x (Cao_2-Cvo_2), where CBF is circumflex blood flow (ml/min), Cao_2 is arterial oxygen content (ml O_2/ 100 ml blood), and Cvo_2 is coronary sinus oxygen content (ml O_2/ 100 ml blood).

Protocol: On day 1, the effective dose of L-NMMA to block NO synthase will be

evaluated using graded intracoronary infusions of acetylcholine ACh (5, 10, 20 μg/min), an endothelium-dependent vasodilator, and sodium nitroprusside (SNP) (20, 40, 80 μg/min), an endothelium-independent vasodilator. These dose-response relationships will be performed before and after intracoronary infusion of L-NMMA (35 mg) (see below). The concentrations of the vasodilators will be calculated so that an identical range of infusion rates can be used for each (0.25 - 2.0 ml/min).

Experiments will consist of data collection while the dog is lying quietly, standing, and running on a Quinton model 18-65 motor-driven treadmill. Each animal will be subjected to the standardized submaximal exercise regimen as described in Series 1.

On day 2, exercise test will be considered a control run with no drugs and data will be recorded at rest and at each level of exercise. The α_1-blocker prazosin (0.5 mg, i.c.) will be given at the peak level of exercise (6.4 kph/ 16 % incline).

On day 3, According to preveous findings, L-NMMA (35 mg) at dose of 1.75 mg/min for 20 min will be infused into the left circumflex artery to evaluate the role of NO in coronary hyperemia during exercise (3, 16). The submaximal exercise test will be repeated, and data collected as during the control run. The α_1-blocker prazosin (0.5 mg, i.c.) will be given at the highest level of exercise. Data will be collected as during the control run.

Arterial and coronary sinus blood samples will be taken simultaneously at rest, and at the each level of exercise during protocol mentioned above. These samples

will be used for determination of myocardial oxygen extraction and MVO_2.

Data Acquisition and Analysis: Data recorded on magnetic tape will be analyzed as described in Series 1. For all comparisons, data within and among the protocols will be analyzed for statistical significance using an two-way analysis of variance (ANOVA) and the Student-Newman-Kuels test. Student's t-test for paired samples will be used to compare effects of the exercise, and of the intracoronary infusion of α_1-blocker prazosin (0.5 mg, i.c.) on cardiac parameters. A $p < 0.05$ will be considered significant throughout this study.

Interpretation of Results: These studies will provide new information regarding the role of NO, released from the endothelium by shear stress, on coronary circulation during exercise. We expect to find that the blockade of NO synthase with L-NMMA will decrease coronary blood flow during exercise. This finding would indicate that endothelium-dependent flow-induced dilation, in addition to the metabolic vasodilation, is probably a important stimulus to maintain myocardial oxygen supply during exercise. Therefore, the absence of endothelium in damaged coronary arteries may be one explanation for cardiac insufficiency of patients with coronary artery disease such as atherosclerosis.

SIGNIFICANCE

The proposed study will provide fundamental information relating to the role of endothelium and sympathetic vasoconstriction on coronary circulation as well as

cardiac performance during exercise. It will demonstrate for the first time whether NO may contribute to the intrinsic coronary blood flow control mechanisms during exercise in physiological conditions. It will also clearly show the degree of interaction between coronary vasodilating and constricting influences during exercise along with published reports. We will be able to evaluate the contribution of endothelial, metabolic, and sympathetic control system in regulating coronary blood flow and cardiac function during exercise.

POTENTIAL PROBLEMS

Potential problems include movement of or damage caused by the chronically implanted aortic flow probe, Doppler flow probe, and catheters. To avoid the first problem, Merocel Sponse (Transonic Systems Inc, Ithaca, New York) will be used to wrap and cushion the aorta before implanting aortic flow probe around it. Catheters will be filled with a heparinized saline solution and, if necessary, streptokinase will be used to remove clots. Since a catheter positioned in a posterior regional vein (which drains the circumflex perfusion territory) is technically difficult to maintain patent in the chronically instruented dog for several weeks, a catheter will be position in the coronary sinus to allow sampling of coronary venous blood for determination of MVO_2. This might cause influence on calculated values of oxygen extraction and MVO_2 under the limited circumflex blood flow in Series 2 study after intracoronary L-NMMA.

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Table 1. Estimated sample size based on α (type I error) and ß (type II error) or 1-ß (referred to as the "power" of the test of significance).

Estimated standard deviation of the population		12.28			
Minimum difference you wish to detect as significant		15			
		α= 0.10	0.05	0.02	0.01
Power	β	Sample Size			
0.80	0.20	5	6	7	8
0.90	0.10	6	8	9	10
0.95	0.05	8	9	11	12

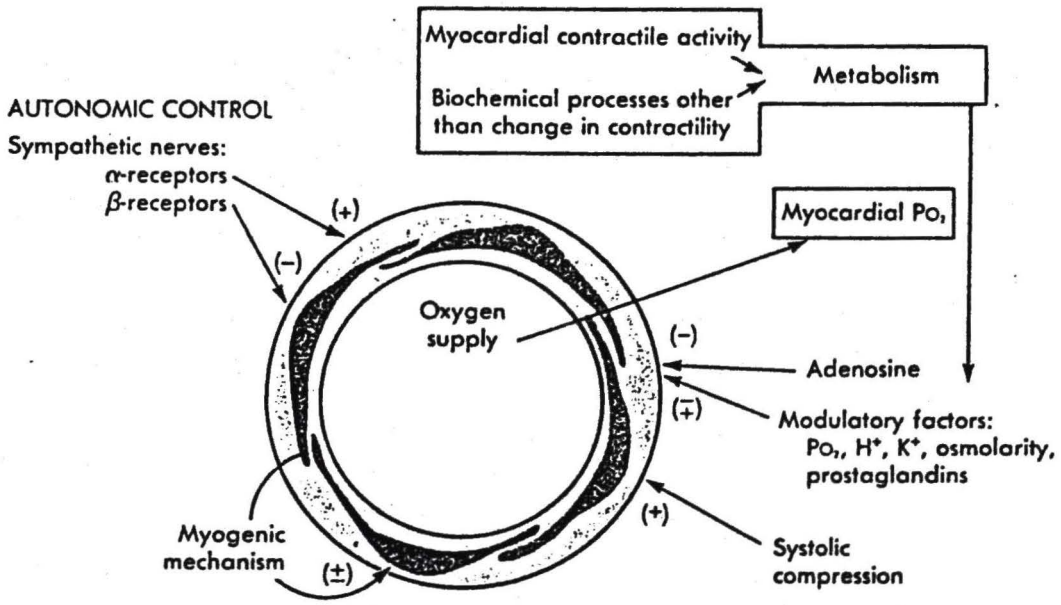

Figure 1. Schematic representation of factors that increase (+) or decrease (-) coronary vascular resistance (from Berne and Levy, Cardiovascular Physiology, Mosby, 1986).

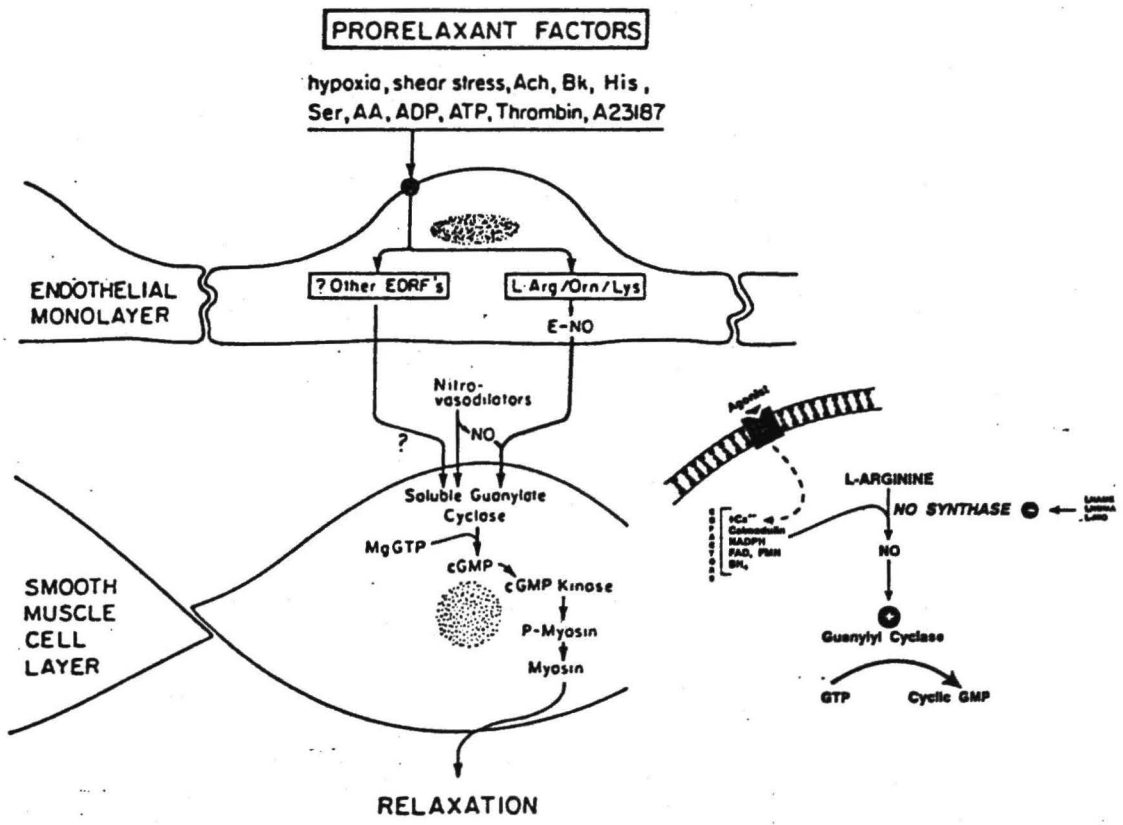

Figure 2. Mechanism of endothelium-dependent vascular smooth muscle relaxation (form Brenner *et al.*, J. Clin. Invest. 1989).

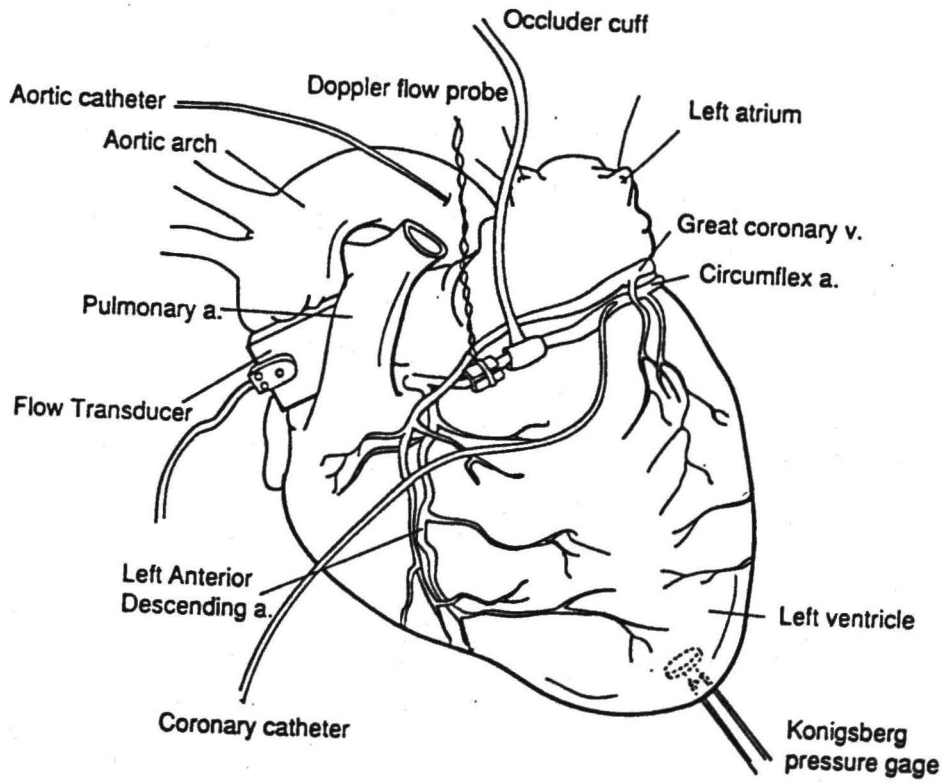

Figure 3. Surgical instrumentation of the heart.

CHAPTER II

LIMITATION OF CARDIAC OUTPUT BY A CORONARY α_1-CONSTRICTOR TONE DURING EXERCISE IN DOGS

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Running Title: Cardiac output limitation during erercise

Submitted to : American Journal of Physiology

ABSTRACT

This study was performed to examine whether an α_1-constrictor tone, which limits coronary functional hyperemia during exercise, imposes a significant limitation on global cardiac performance as determined by cardiac output. Seven dogs were chronically instrumented to measure left ventricular pressure (LVP), dP/dt_{max}, heart rate (HR), mean aortic pressure (MAP), circumflex blood flow velocity (CFV), and cardiac output (CO) at rest and during submaximal exercise. The selective α_1-adrenergic antagonist prazosin (0.5 mg) was administered into the circumflex artery during exercise at 6.4 kph/16 % treadmill incline. Exercise caused significant increase in MAP, HR, LVP, $dP/dt_{max,}$ CFV, stroke volume (SV), and CO, while systemic vascular resistance (SVR) was significantly reduced. After intracoronary α_1-blockade with prazosin, CFV, dP/dt_{max}, SV and CO increased further (17 $\pm$ 3 %, 18 $\pm$ 6 %, 15 $\pm$ 2 %, and 15 $\pm$ 2 %, respectively) without changing MAP, HR, or SVR. These results indicate that removing a coronary α_1-constrictor tone with prazosin during submaximal exercise leads to an increase in myocardial oxygen supply and, as a result, cardiac pump performance (stroke volume and cardiac output).

KEY WORDS: cardiac output, coronary blood flow, left ventricular function, exercise, prazosin, α-adrenergic receptor

INTRODUCTION

Although coronary blood flow is regulated primarily by local metabolic mechanisms according to the myocardial metabolic demand (6), a sympathetic mediated constrictor tone can substantially modulate coronary blood flow. For example, sympathetic stimulation of the heart during exercise results in increases in heart rate and contractility by myocardial ß-adrenergic receptor stimulation. While this leads to coronary vasodilation through local metabolic mechanisms, the direct result of sympathetic activation on coronary vessels is a vasoconstriction mediated by α-adrenergic receptor stimulation (6, 7, 24, 27). Sympathetic vasoconstriction in the coronary circulation has been shown to oppose metabolic vasodilation and limits oxygen supply to the myocardium during physiological and pathophysiological cardiac stresses, such as exercise and myocardial hypoperfusion (10, 21). Although the precise physiological function of a coronary α_1-constrictor tone is unknown, this limitation on myocardial oxygenation appears to impose a restriction on the degree of increase in regional left ventricular subendocardial contractile function during submaximal exercise, since studies have shown that removing coronary α_1-constrictor tone leads to an increase in coronary blood flow and, as a result, regional contractile function (12, 28).

A more important question that must be addressed is whether the restriction of coronary hyperemia by an α_1-constrictor tone during exercise imposes a significant limitation on global cardiac performance. Thus, the present study tests the hypothesis

that during submaximal exercise, an α_1-constrictor tone limits coronary blood flow and, as a result imposes a limit on cardiac performance (i.e., cardiac output).

MATERIAL AND METHODS

Surgical Preparation

Experiments were performed on seven (7) healthy, mongrel dogs of either sex (weight range 25-35 kg). All dogs were premedicated with acepromazine (0.03 mg/kg, s.c.), anesthetized with surital (5 mg/kg, i.v.), and the trachea intubated. Anesthesia was maintained with isoflurane gas (1-3 %) and nitrous oxide (0.6 l) with equal offset of O_2 (1 l).

Using sterile technique, a thoracotomy was performed through the left fifth intercostal space and the heart instrumented as illustrated in Figure 1. The aorta was exposed and a fluid-filled Tygon catheter (1.27 mm OD) was inserted just distal to the aortic arch to monitor aortic pressure (AP). A Transonic transit time Doppler flow probe (Transonic Systems Inc, Ithaca, NY) was placed around the root of the aorta to measure cardiac output (CO). The heart was then exposed and suspended in a pericardial cradle. For the measurement of left ventricular pressure (LVP) a Konigsberg P-6.5 transducer solid state micromanometer (Konigsberg Instruments Inc, Pasadena, CA) and a fluid-filled Tygon catheter (1.27 mm OD) was inserted into the left ventricle through a stab-wound in the apex. The Tygon catheter was used to calibrate the Konigsberg transducer. At the beginning of each experiment the Tygon

catheter was connected to an Isotec® pressure transducer (Cardiovascular Concepts, Arlington, TX) and calibrated using a mercury manometer. The micromanometer was calibrated against the pressure measured in the catheter. The circumflex artery was dissected free of the surrounding tissue for a distance of approximately 3 cm beginning at the origin of the vessel. A 10 MHz Doppler ultrasonic flow probe (4 mm ID) was positioned around the circumflex artery for measurement of circumflex blood flow velocity (CFV). To check the zero flow reference, a pneumatic occluder was placed around the circumflex artery immediately distal to the Doppler ultrasonic flow probe such that there was no vessel branch between the two. A heparin-filled Silastic catheter (0.12 mm ID and 0.6 mm OD) was inserted into the circumflex artery distal to the occluder for injection of the solutions into the circumflex artery.

After instrumentation was completed, a chest tube was placed in the thoracic cavity to evacuate the pneumothorax and any post-surgical intrathoracic exudate accumulation. All wires and catheters were tunneled subcutaneously to exit between the scapula. The indwelling catheters were flushed daily with heparinized saline to maintain patency. Post-operative analgesics, antibiotics, and antipyretics were given as specified by the veterinarian.

Experimental Protocol

Experimentation began 10-14 days following surgery. For each experiment, electrical leads were connected to appropriate instruments. Resting values for LVP,

pulsatile and mean CFV, CO, mean aortic pressure (MAP), and heart rate (HR) were recorded. After resting data were obtained, CFV and global myocardial function were evaluated while the dog was subjected to a standardized submaximal exercise protocol using a motor-driven treadmill (Quinton model 18-60) described by Tipton *et al* (30). Briefly, this protocol lasted 21 min and consisted of six 3-min intervals of increasing workload, beginning with a warm-up at 4.8 kph, 0 % incline. The treadmill speed was increased to 6.4 kph, and the incline was varied at 3-min intervals to include 0,4,8,12, and 16 % inclines. An intracoronary administration of prazosin was used to assess the magnitude of the α_1-coronary constrictor tone during exercise at the highest workload of 6.4 kph/ 16 % incline. The selective α_1-adrenoceptor blockade of the circumflex perfusion territory was accomplished with a bolus injection of prazosin (0.5 mg, i.c.). This dose of prazosin has been shown to abolishe the vasoconstrictor response to an intracoronary dose of 20 μg phenylephrine without causing secondary peripheral circulatory effects, such as changes in HR or MAP (11). This was confirmed in the present study by intracoronary injection of 20 μg phenylephrine before and after the submaximal exercise test in all dogs. Data were collected at the control period, at the peak of exercise (6.4 kph/ 16 % incline) and 2 min after intracoronary injection of prazosin.

In four (4) dogs, an equal dose of prazosin (0.5 mg) was given systemically via injection into the aortic catheter at the peak level of exercise to evaluate whether the increase in cardiac function may be due to a systemic effect of the prazosin.

Data Collection and Analysis

On-line variables were recorded on a Coulbourn 8-channel chart recorder (Allentown, PA) and on an 8-channel Hewlett-Packard model 3968A tape recorder (San Diego, CA) for subsequent computer analysis. Computer analysis was done by using a custom software package (Dataflow, Crystal Biotech, Hopkinton MA). The program samples recorded data at 2 msec intervals over 10 consecutive beats. The following data were analyzed from the recorded variables: left ventricular systolic pressure (LVSP) and end-diastolic pressure (LVEDP), $+dP/dt_{max}$, HR, MAP, CO, and CFV. Stroke volume (SV) was calculated by dividing CO by HR. Systemic vascular resistance (SVR) was calculated by dividing MAP by CO.

Data are reported as mean $\pm$ standard error of the mean (SEM), and differences between means were considered statistically significant if the probability of their occurring by chance was less than 5 % ($p < 0.05$). An analysis of variance for repeated measures was used, and when a significant result was found, individual post hoc comparisons were performed using Tukey's protected t-test to evaluate the effects of the exercise and α_1-adrenergic blockade on cardiac function and coronary hemodynamics.

RESULTS

Figure 2 shows representive tracings of LVP, dP/dt, AP, CFV, CO, and HR during rest and submaximal exercise (6.4 kph/16 % incline) before and after

intracoronary injection of prazosin from one of the dogs. The left panel depicts the resting state while the dog was standing quietly on the treadmill. The right panel illustrates the cardiac and coronary response to exercise at 6.4 kph/16 % incline before and after prazosin (injection indicated by the arrow). As expected, exercise caused a substantial increase in left ventricular function (LVP and $+dP/dt_{max}$), MAP, CFV, CO, and HR. After administration of prazosin during exercise, CFV increased further by 16 %, which was associated with a 15 % increase in CO in this dog.

Data obtained from all dogs are shown in Table 1. During exercise conditions, significant increases in LVSP, dP/dt_{max} , HR, CFV, CO and SVR were observed. The increase in CO during exercise was due to an increase in both HR and SV. Prazosin was given by intracoronary injection during exercise to remove an α_1-adrenergic coronary constrictor tone. As shown in Table 1 and graphically in Figure 3, CFV significantly increased by 17 $\pm$ 3% after prazosin. Similar to previous reports, the increase in CFV was associated with a significant 18 $\pm$ 6 % increase in dP/dt_{max} during exercise after intracoronary prazosin (12). The primary purpose of this study was to examine whether this apparent increase in left ventricular contractility was related to an increase in cardiac pump performance. Indeed, CO increased an additional 15 $\pm$ 2 % ($p < 0.05$), and was related to a significant 15 $\pm$ 2 % increase in SV, since no further changes in LVSP, LVEDP, MAP, SVR or HR were observed after intracoronary injection of prazosin.

Figure 4 depicts typical results from one experiment to evaluate whether the

increase in cardiac function may be due to a systemic effect of the prazosin. Similar results were observed in all 4 dogs and these data are given in Table 2. It is noteworthy that systemic administration of this dose of prazosin had no effect on CFV, LVP, dP/dt_{max} , MAP, HR, or CO. These results indicate that the results observed following intracoronary prazosin were due to removal of a coronary α_1-constrictor tone and not to systemic vascular effects of the antagonist.

DISCUSSION

The major new finding of this study is that removing a coronary α_1-constrictor tone with prazosin during strenuous exercise leads to an increase in myocardial oxygen supply and, as a result, stroke volume and cardiac output.

Evidence indicates that in the quiescent, conscious dog, sympathetic stimulation of the heart and coronary vasculature is low or nonexistent such that there is no measurable coronary α-adrenergic mediated constrictor tone (3, 12). Although there may be little sympathetic constriction of the coronary circulation at rest, many conditions which cause increased sympathetic stimulation of the heart are associated with increased adrenergic coronary constriction, such as submaximal exercise (2, 4, 5, 8, 11-18, 20, 25, 26, 28).

During exercise the work output of the normal heart can increase several fold. Intimately involved in the increased pumping capacity of the heart is a sympathetic stimulation, which increases both heart rate and inotropic state of the myocardium.

The increased work output of the heart causes increased oxygen and blood flow demands, and due to local metabolic influences on the coronary vasculature, there is a substantial vasodilation and increase in coronary blood flow. It has also been realized that during exercise, adrenergic constrictor influences also increase, and many laboratories report that an α_1-adrenergic coronary constrictor tone limits the increase in blood flow during moderate to strenuous exercise (2, 4, 5, 11-20, 25, 28). Murray and Vatner (25) and Gwirtz and Stone (11) both noticed that coronary flow was greater during exercise after systemic injection of a nonselective α-adrenergic antagonist. Gwirtz and Stone (11) and Heyndrickx *et al* (16-18) also observed an increase in dP/dt_{max} following α-blockade. They observed that after α-blockade with phentolamine, changes in myocardial oxygen delivery was more proportionate to the changes in myocardial oxygen consumption. To overcome difficulties associated with systemic administration of α-blockers, Gwirtz and co-workers administered α-antagonists directly into the coronary circulation during exercise (12-14, 28). Selective α_1-blockade with prazosin resulted in increases in coronary flow and, as a result, regional contractile function. Selective α_2-blockade with yohimbine had no effect on either myocardial flow or function (28). These data, which are supported by other investigators, (2, 5, 16) indicate an important role of α_1-receptors in mediating a coronary constrictor tone in exercise with a resulting limitation of the increase in myocardial oxygen delivery and a possible limitation of the increase in cardiac

contractile function. The present study was designed to more precisely examine this latter possibility.

In the present experiments, intracoronary injection of prazosin during exercise resulted in a significant increase in coronary blood flow in the circumflex bed as well as a significant increase in global left ventricular contractility ($+dP/dt_{max}$). These observations are in agreement with previous studies (2, 5, 10, 12, 13, 16-18, 28). In addition, we found a significant increase in cardiac output due to an increase in stroke volume. These results indicate an important role of α_1-receptors in mediating a coronary constrictor tone in exercise with a resulting limitation on myocardial oxygen delivery and, as a result, cardiac contractile or pump function. It is important to note that the intracoronary infusion of prazosin during exercise did not induce changes in arterial pressure or heart rate. Furthermore, administration of the same dose of prazosin (0.5 mg) into the systemic circulation did not change coronary blood flow or any hemodynamic variable, including arterial pressure and heart rate. These data indicate that the change in coronary blood flow observed in the circumflex perfusion territory was due to a local effect of intracoronary prazosin infusion and not to a systemic reflex or significant recirculation of prazosin as observed in early studies which used systemic injections of α-antagonists (12-14).

Cardiac output is normally determined by the needs of the body, and not by the heart. The heart appears to serve a permissive role in cardiac output regulation, permitting the output to be regulated at any level up to the limit of its pumping

capability (9, 22). Consequently, the factor that normally determines how much blood will be pumped by the heart is the amount of blood that flows into the heart from the systemic circulation (venous return) - not the pumping capacity of the heart. On the other hand, stimulation of the heart by the sympathetic nervous system, such as occurring during exercise, increases the permissive level of heart pumping by increasing both heart rate and contractility within a few seconds after exercise begins (9, 22). Cardiac output is determined by two factors: arterial pressure, and total peripheral resistance. Since venous return to the heart is the sum of all the local blood flows through all the individual tissues of the body, the local blood flow regulatory mechanisms in the peripheral circulation are the true controllers of cardiac output. Therefore, cardiac output is normally controlled in relation to the level of metabolism of the body. In the present study, cardiac output increased four-fold during submaximal exercise, which was caused by both sympathetic stimulation to heart (which increases both contractility and heart rate) and by reduced systemic vascular resistance, which is primarily the result of metabolites that are released from active skeletal muscles in proportion to the metabolic rate. Interestingly, in the present study the increase in coronary blood flow after intracoronary infusion of prazosin during exercise resulted in a significant increase in cardiac output, and was associated with a significant increase in stroke volume. However, arterial pressure and systemic vascular resistance were not changed after intracoronary infusion of prazosin during exercise, indicating that cardiac output was increased not by a

systemic effect, but by a local effect of prazosin on the myocardium. If recirculation of prazosin occurred, then the effect of prazosin on the systemic circulation should be minimal because sympathetic activation on peripheral circulation during exercise is powerful enough to counter any change in systemic vascular resistance caused by such a small quantity of prazosin. Our results strongly support our hypothesis that removing a coronary α_1-constrictor tone during submaximal exercise increased myocardial oxygen delivery and, thus, increased global myocardial contractile function and cardiac output.

The precise basis for the increased myocardial contractile performance associated with the prazosin-induced increase in coronary flow is not certain. This response cannot be attributed to increased adrenergic stimulation of myocardial ß-receptors, since the dose of prazosin used in this study does not appear to cause a substantial increase in norepinephrine release from sympathetic nerve terminals (1, 12, 17, 19, 25). It is noteworthy that the increase in coronary flow and contractile function after α-blockade was not affected by either $ß_1$- or $ß_2$-adrenergic blockade (12, 25), implying that the results cannot be attributed to a presynaptic α_2-adrenergic blockade with increased release of norepinephrine from sympathetic nerve terminals (1, 16, 17, 25). Such increase in norepinephrine release would be expected to cause an myocardial $ß_1$-recepter stimulation with secondary metabolic vasodilation, or an increased coronary $ß_2$-receptor stimulation with direct vasodilation. The lack of response of coronary flow and myocardial function to selective α_2-blockade support

this supposition (2, 3, 28). Furthermore, the increased cardiac pump function cannot be due to inhibition of post-junctional myocardial α-adrenergic receptors, since if these receptors are of functional importance, their inhibition would be expected to cause myocardial depression. The increased myocardial performance after prazosin cannot be due to a direct inotropic effect of the agent, but is dependent on a high sympathetic outflow to the heart since prazosin does not elicit an increase in cardiac function in the resting dog (12). We believe the increase in function is a direct result of the increase in blood flow, since similar results were observed when coronary flow was increased by intracoronary administration of the vasodilator adenosine (13).

Our observations imply that during strenuous exercise, contractile performance (at least in the endocardium) becomes somewhat flow-limited. A contributing factor to the flow limitation is a coronary α_1-constrictor tone. Abolition of this vasoconstriction increased myocardial perfusion and oxygen delivery and permits an increase in myocardial contractile function. However, we also recognize that the increased myocardial function may be explained by the "Garden-Hose" or "Gregg Effect" by which an increase in coronary flow or pressure influences ventricular function by distending the heart from within its walls, thus invoking Starling's law of the heart. This phenomenon has also been shown to be associated with increased calcium release during excitation-contraction coupling (23).

The present study demonstrated that removing coronary α_1-constrictor tone with prazosin during submaximal exercise leads to an increase in myocardial oxygen

supply and, as a result, stroke volume and cardiac output. This limited flow by α_1-coronary constriction in coronary circulation might be beneficial for the delivery and distribution of cardiac output to the heart and working skeletal muscle during exercise.

ACKNOWLEDGEMENTS

We wish to acknowledge the generosity of Pfizer, Inc. for providing Prazosin HCl. These studies were supported by NIH grants HL-34172 and HL-29232. This research was performed as partial fulfillment of the dissertation requirements for the Ph.D. degree for Song-Jung Kim. We thank Abraham Heymann and Linda Legorreta for their outstanding technical assistance.

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Table 1. Cardiac and Coronary Flow Response to Intracoronary Prazosin During Submaximal Exercise.

	Control	Exercise	Exercise + i.c. Prazosin
LVSP (mmHg)	120 ± 5	161 ± 6*	175 ± 10*
LVEDP (mmHg)	3 ± 2	5 ± 2	6 ± 2
dP/dt_{max} (mmHg/sec)	3,078 ± 222	7,328 ± 666*	9,092 ± 498*†
HR (beats/min)	107 ± 5	234 ± 8*	230 ± 7*
MAP (mmHg)	95 ± 3	107 ± 4	104 ± 3
CFV (kHz)	1.93 ± 0.13	4.43 ± 0.44*	5.24 ± 0.44*†
CO (l/min)	2.5 ± 0.2	7.5 ± 0.8*	8.6 ± 0.8*†
SV (ml/min)	24.0 ± 1.6	32.5 ± 3.1*	37.0 ± 3.4*†
SVR (mmHg/ml/min)	0.034 ± 0.005	0.016 ± 0.003*	0.014 ± 0.002*

Values are means ± SEM for 7 dogs. * $P < 0.05$ vs. Control. † $P < 0.05$ vs. Exercise. Abbreviations: LVSP=left ventricular systolic pressure; LVEDP=left ventricular end diastolic pressure; dP/dt_{max}=maximal rate of rise of left ventricular pressure; HR=heart rate; MAP=mean aortic pressure; CFV=mean coronary flow velocity; CO=cardiac output; SV=stroke volume; SVR=index of systemic vascular resistance; and i.c.=intracoronary.

Table 2. Lack of Effect of Intra-aortic Prazosin on Hemodynamic Variables and Coronary Flow During Submaximal Exercise.

	Exercise	Exercise + i.a. Prazosin
LVSP (mmHg)	160 ± 6	162 ± 7
LVEDP (mmHg)	7 ± 2	7 ± 2
dP/dt_{max} (mmHg/sec)	6,388 ± 381	6,575 ± 433
HR (beats/min)	231 ± 16	238 ± 17
MAP (mmHg)	114 ± 2	115 ± 4
CFV (kHz)	4.28 ± 0.29	4.39 ± 0.26

Values are means ± SEM for 4 dogs. Abbreviations: LVSP=left ventricular systolic pressure; LVEDP=left ventricular end diastolic pressure; dP/dt_{max}=maximal rate of rise of left ventricular pressure; HR=heart rate; MAP=mean aortic pressure; CFV=mean coronary flow velocity; and i.a.=intra-aortic.

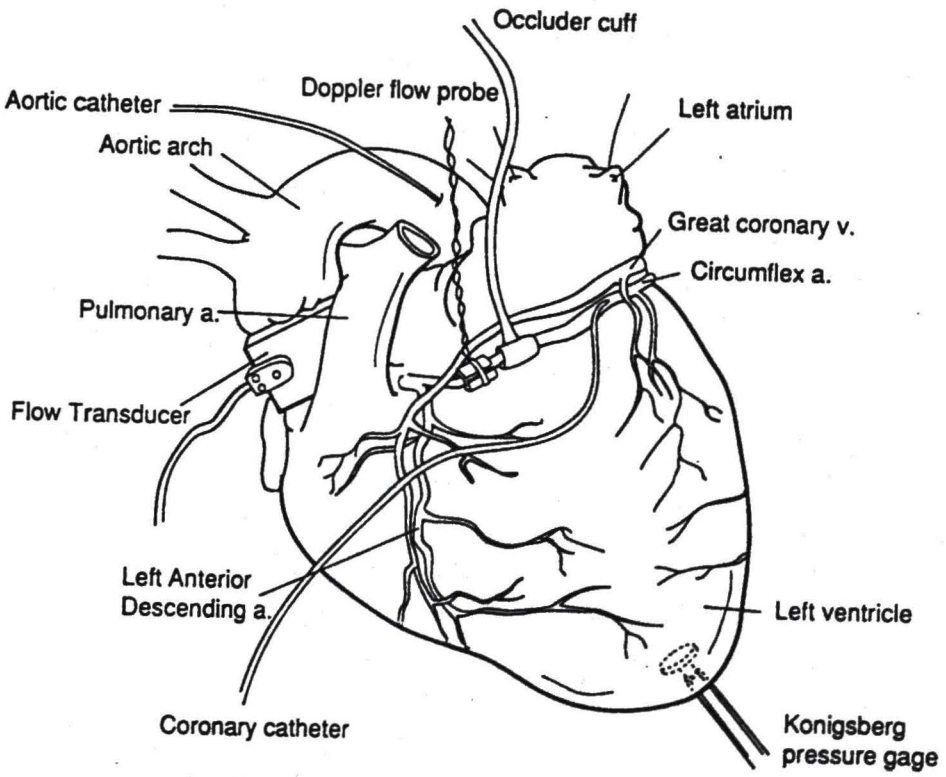

Figure 1. Illustration of the surgical instrumentation of the heart used for the measurement of chronic cardiovascular parameters.

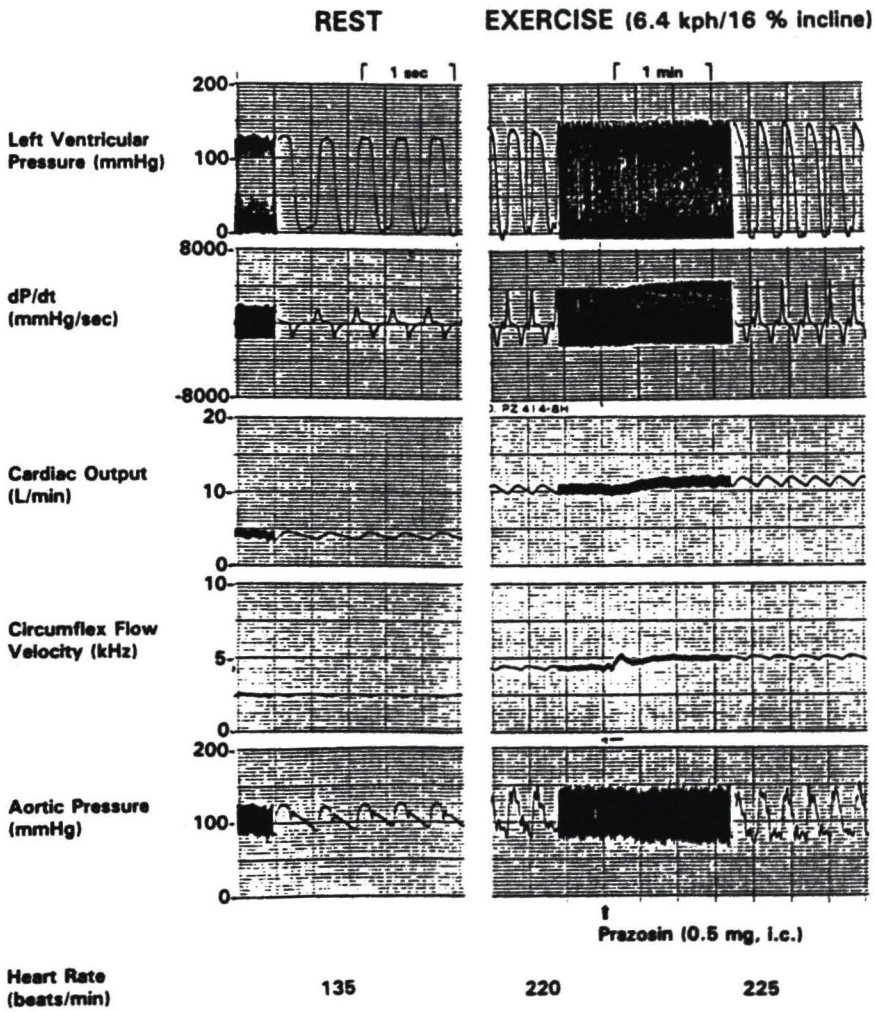

Figure 2. Tracing from a representative experiment showing the response to submaximal exercise before and after intracoronary injection of prazosin (0.5 mg,i.c.) (indicated by the arrow).

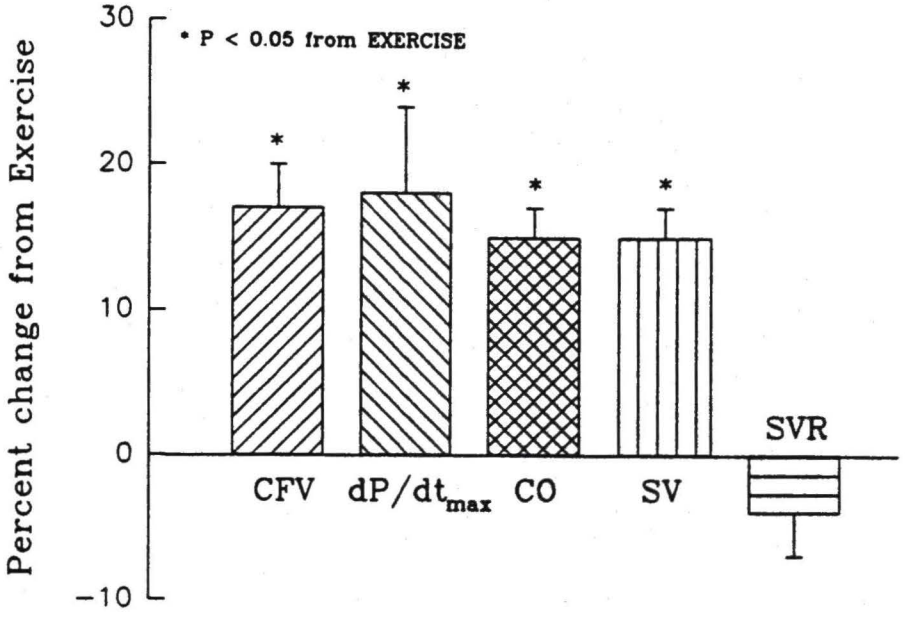

Figure 3. Changes from exercise before prazosin to exercise after intracoronary injection of prazosin (0.5 mg). Values are means ± SEM for 7 dogs. CFV=mean coronary flow velocity; dP/dt_{max}=maximal rate of rise of left ventricular pressure; CO=cardiac output; SV=stroke volume; SVR=index of systemic vascular resistance.

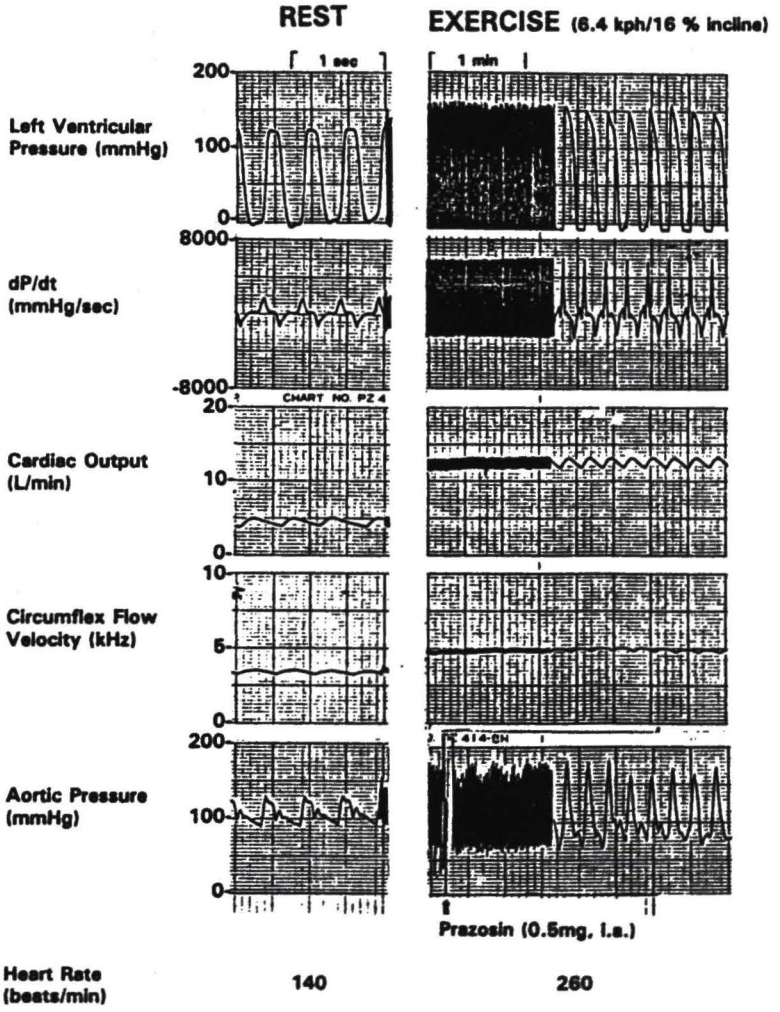

Figure 4. Tracing from a representative experiment showing the response to submaximal exercise before and after systemic injection of prazosin (0.5 mg, i.a.). Prazosin injection is indicated by the arrow.

TRANSITION

Coronary blood flow is regulated primarily by local metabolic mechanisms according to myocardial oxygen demand. However, two external factors are superimposed on local control mechanisms which can substantially modulate coronary blood flow: sympathetic vasoconstriction and vascular endothelium. First, sympathetic α_1-adrenergic coronary constriction has been shown to oppose metabolic vasodilation and limit oxygen supply to the myocardium during exercise. In this regard, studies have shown that removing this α_1-constrictor tone leads to an increase in coronary blood flow and, as a result, regional contractile function. Thus, the first study was performed to examine whether an α_1-constrictor tone, which limits coronary functional hyperemia during exercise, imposes a significant limitation on global cardiac performance as determined by cardiac output. Results demonstrated that removing coronary α_1-constrictor tone with prazosin during submaximal exercise leads to an increase in myocardial oxygen supply and, as a result, stroke volume and cardiac output.

Recently, nitric oxide released from the vascular endothelium has been implicated in flow-induced relaxation of coronary arteries. Accordingly, the next study will examine whether nitric oxide released from endothelium in response to shear stress and neurohumornal stimulation during exercise increases coronary blood flow, and thereby, contributes to the coronary vasodilation during exercise. Thus, this study will provide fundamental information defining the role of endothelium-

dependent vasodilating mechanisms, in addition to sympathetic vasoconstriction, on coronary circulation during exercise.

CHAPTER III

EFFECT OF INTRACORONARY NITRIC OXIDE BLOCKADE ON CORONARY BLOOD FLOW AND dP/dt_{max} DURING EXERCISE

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Running Title: Coronary blood flow during exercise

Submitted to: American Journal of Physiology

ABSTRACT

Nitric oxide (NO) released from vascular endothelium has been implicated in flow-induced relaxation of coronary arteries. The objective of this study was to assess the role of NO on coronary blood flow regulation during exercise. Seven dogs were chronically instrumented to measure left ventricular pressure, dP/dt_{max}, heart rate (HR), mean aortic pressure (MAP), and circumflex blood flow (CBF) at rest and during exercise. NO synthase was blocked by intracoronary infusion of N^G-monomethyl-L-arginine (L-NMMA, 35 mg), which avoided systemic effects of the drug. Each animal was subjected to a standardized treadmill exercise regimen before (control) and after L-NMMA on separate days. At rest and at low exercise workloads, MAP, HR, dP/dt_{max} and CBF were not different between control and L-NMMA conditions. However, during moderate and heavy exercise after L-NMMA, CBF significantly reduced, while hemodynamic variables did not differ from values at respective control conditions. At the same workloads, this attenuated increase in CBF caused a subsequent lesser increase in dP/dt_{max} and myocardial oxygen consumption. These findings suggest that in the conscious resting state and during mild levels of exercise, NO does not contribute to coronary vasodilation, but during moderate to heavy exercise workload, NO-mediated vasodilation is necessary for increases in coronary blood flow. These data indicate that endothelium-dependent flow-induced dilation, in addition to the metabolic vasodilation, is probably an important stimulus

to maintain myocardial oxygen supply during exercise.

KEY WORDS: coronary blood flow, ventricular function, nitric oxide, myocardial oxygen consumption, exercise, endothelium.

INTRODUCTION

The functional importance of the endothelium has been unappreciated for a long time until Furchgott and Zawadzki (16) demonstrated that the vascular relaxation induced by acetylcholine was dependent on the presence of the endothelium. Since then, many *in vitro* studies have shown that the endothelium modulates vasomotor tone via the synthesis and metabolism of various vasodilatory and vasoconstricting agents (2, 6). Among the important vasoactive substances synthesized by endothelial cells is endothelium-derived relaxing factor or nitric oxide (NO), which is synthesized from the amino acid L-arginine and catalyzed by nitric oxide synthase (16, 33). NO then diffuses into the underlying vascular smooth muscle and causes relaxation.

Although the physiological stimuli for generation of NO are not yet fully understood, flow-induced shear stress at the interface between blood and the endothelial cell surface is probably the most important stimulus in the endothelium-dependent control of vascular caliber (25, 27). Other stimuli include acetylcholine, bradykinin, serotonin, and calcium inophore A23187 (33). Endothelium-dependent flow-induced dilation has been described in conduit arteries, small arteries and veins both in *in vitro* and *in vivo* preparations (15, 25). In isolated vascular preparations, NO release increases when flow is made pulsatile, while damage to the endothelium abolishes this response to shear stress (30). Moreover, the activity of endogenous NO appears to be greatest in large arterioles, where resistance and shear stress are also highest (18). Based on this information, it is likely that NO-dependent vasodilator

tone is locally regulated, and is probably one of the simplest and yet most fundamental mechanisms in the cardiovascular system. Therefore, such flow-dependent control seems to be particularly important in the coronary circulation because of the extremely pulsatile nature of flow patterns in this vascular bed.

During exercise, the work output of the normal heart increases several-fold by the stimulation of the sympathetic nerves to the heart. The increased work output of the heart causes increased myocardial oxygen demand. Consequently, the coronary circulation undergoes vasodilation due to primarily local metabolic mechanisms (14). The elevation in shear stress caused by increases in coronary blood flow may trigger release of NO from the endothelium because of the extremely pulsatile nature of the flow. NO may be also released during exercise in response to various neurohumoral mediators, such as circulating catecholamines and vasopressin (33). Previous studies have investigated whether NO plays a role in dilation of the coronary artery during resting and exercise conditions in conscious dogs using intravenous administrations of the selective NO synthase inhibitors, N^G-monometyl-L-arginine (L-NMMA) or N-nitro-L-arginine (NLA) (1, 10, 26, 29). However, systemic administration of L-NMMA or NLA always induced alterations in hemodynamic parameters, including aortic blood pressure, heart rate, and systemic vascular resistance. Alterations in these factors have been demonstrated by themselves to have profound effects on coronary hemodynamics and myocardial oxygen consumption, thus making it difficult to identify the direct effects of NO on the coronary circulation. Accordingly, the

present study evaluated the role of NO on regulation of the coronary circulation during exercise by using the selective intracoronary infusion of L-NMMA in chronically instrumented conscious dogs, which effectively avoids the hemodynamic influences associated with systemic administration of the NO inhibitor. This study examined the hypothesis: vascular endothelial release of NO contributes to coronary hyperemia during exercise.

MATERIAL AND METHODS

Surgical Preparation

Experiments were performed on seven (7) mongrel dogs (20-30 kg) of either sex, selected on the basis of being heartworm free, in good health prior to surgery, and willing to run on a motor-driven treadmill. All dogs were premedicated with acepromazine (0.03 mg/kg, s.c.), anesthetized with surital (0.2 mg/kg, i.v.) and intubated. A surgical level of anesthesia was maintained using gaseous anesthesia with isoflurane (1-3 %) and oxygen. The tidal volume and rate of ventilation were set at approximately 15 ml/kg and 15-20 breaths/min, respectively.

Using sterile technique, a left thoracotomy was performed in the fifth intercostal space, and the heart was instrumented as illustrated in Figure 1. The aorta was exposed and a fluid-filled Tygon catheter (1.27 mm, OD) was inserted just distal to the aortic arch to monitor aortic pressure. The left ventricular pressure was

measured by implanting a Konigsberg P 6.5 transducer and a fluid-filled Tygon catheter in the apex of the ventricle. The catheter was used to calibrate the Konigsberg transducer at the beginning of each experiment. The maximum rate of rise of left ventricular systolic pressure ($+dP/dt_{max}$) was obtained from left ventricular pressure using an electronic differentiator. The proximal circumflex artery was dissected free of surrounding tissue and a 10 mHz Doppler flow probe was implanted around the vessel for measurement of circumflex inflow. An inflatable cuff hydraulic occluder was placed distal to the flow probe to check the zero flow reference of the Doppler flow probe and to evaluate coronary flow reserve following a 15 sec circumflex artery occlusion. A silastic catheter was implanted distal to the flow probe and occluder (17). This catheter was used for selective injection of drugs into the circumflex coronary artery and for collection of arterial blood samples. Another silastic catheter was inserted into the coronary sinus for myocardial venous blood sampling.

After instrumentation was completed, a chest tube was placed in the thoracic cavity to evacuate the pneumothorax and any post-surgical intrathoracic exudate accumulation. All wires and catheters were tunneled subcutaneously to exit between the scapula. The indwelling catheters were flushed daily with heparinized saline to maintain patency. Post-operative analgesics, antibiotics, and antipyretics were given as specified by the institutional veterinarian. At least 10-14 days were allowed before beginning experimentation.

Experimental Protocol

The intracoronary dose of L-NMMA (35 mg) was selected according to Cappelli-Bigazzi *et al* (8). In four resting dogs, the effectiveness of L-NMMA in blocking NO synthase in resting dogs was evaluated using graded intracoronary infusions of acetylcholine (ACh) (5, 10, and 20 μg/min), an endothelium-dependent vasodilator, and sodium nitroprusside (SNP) (20, 40, and 80 μg/min), an endothelium-independent vasodilator. These dose-responses to ACh and SNP were performed before and after intracoronary infusion of L-NMMA (1, 8). The concentrations of these vasodilators were formulated so that an identical range of infusion rates could be used for each (0.25 - 1.0 ml/min). The low infusion rate was chosen to avoid a hemodilution effect as a factor in the observed increases in coronary blood flow. Higher concentrations of ACh ($>$20 μg/min) and SNP ($>$80 μg/min) were not used, thus avoiding hemodynamic instability due to spill-over into the systemic circulation. The order of the infusions of ACh and SNP was randomized. Following adequate time for recovery, L-NMMA at dose of 1.75 mg/min for 20 min (35 mg) was infused into the left circumflex artery, and the graded intracoronary infusions of ACh and SNP was repeated.

The role of NO on coronary blood flow regulation during exercise was examined in seven dogs using the intracoronary infusion of L-NMMA. Experiments consisted of data collection while the dog was resting quietly in a sling or while running on a Quinton model 18-65 motor-driven treadmill. Each animal was

subjected to the standardized submaximal exercise regimen described by Tipton *et al* (32). The submaximal exercise test lasted 18 min and was divided into 3-min time blocks beginning with a warm-up at 4.8 kph, 0 % incline. The speed was increased to 6.4 kph, and the incline of the treadmill was varied at 3-min intervals to encompass 0, 4, 8, 12, and 16 % inclines. During exercise, data were collected during the last 20-30 sec of each 3 min block. In four of the dogs, simultaneous aortic and coronary sinus blood samples were withdrawn anaerobically at rest and during exercise at selected workloads of 4.8/0, 6.4/8, and 6.4/16 % incline. These samples were used for determination of myocardial oxygen extraction and myocardial oxygen consumption (described in detail below). On the next day, to evaluate the role of NO on coronary blood flow, L-NMMA (35 mg) was infused into the left circumflex artery for 20 min before taking resting data according to previous described methods (8). Following intracoronary infusion of L-NMMA, the submaximal exercise test was repeated and data were collected.

Circumflex vasodilator reserve was evaluated by examining the reactive hyperemia response to 15 sec occlusion to the baseline blood flow. If the peak reactive hyperemic response was approximately less than an expected 4-fold increase in blood flow, then the dog would have been eliminated from this study because of a possible circumflex artery stenosis due to the chronic instrumentation. However, all seven dogs had a normal reactive hyperemic responses and were included in the statistical analyses.

Since L-NMMA is a competitive inhibitor of the NO synthase, intracoronary administration of L-arginine (250 mg) was used to evaluate whether the effect of L-NMMA was reversed by L-arginine, the substrate of NO (2, 10, 26). In four of dogs, after intracoronary infusion of L-NMMA (35 mg), the exercise test was repeated up to a workload of 6.4 kph/8 % incline. At steady-state conditions, a bolus of L-arginine was injected into circumflex artery and data were collected.

After completion of all experiments, each dog was anesthetized with sodium pentobarbital (30 mg/kg, i.v.). The circumference of the circumflex artery under the Doppler flow probe was measured and used to calculate its cross-sectional area for conversion of flow velocity to volume flow rate (see below).

Data Acquisition and Analysis

Arterial blood pressure were measured with an Isotec® pressure transducer, which was calibrated using a mercury manometer. During each experiment, hemodynamic variables were recorded continuously both on a 8-channel strip recorder (Coulbourn Instruments) and on magnetic tape (Hewlett-Packard 8-channel tape recorder). The following data were analyzed from the recorded variables: mean aortic pressure (MAP), left ventricular systolic pressure (LVSP) and end-diastolic pressure (LVEDP), $+dP/dt_{max}$, heart rate (HR), and circumflex flow velocity (CFV).

Measurements of oxygen consumption in circumflex-perfused myocardium

were obtained to determine whether the change in vascular tone by NO during exercise was caused by changes in myocardial oxygen consumption (MVO_2). Oxygen saturation and hemoglobin content of arterial and coronary sinus blood samples were determined using a Radiometer OSM 2 hemoximeter. Arterial and venous oxygen content were obtained by multiplying the respective oxygen saturation by hemoglobin content and by 1.36 gm/ml (the oxygen carrying capacity of hemoglobin). MVO_2 was calculated by the Fick equation: MVO_2 (ml O_2/ml/min) = CBF x (Cao_2-Cvo_2), where CBF is circumflex blood flow (ml/min), Cao_2 is arterial oxygen content (ml O_2/ 100 ml blood), and Cvo_2 is coronary venous oxygen content (ml O_2/ 100 ml blood). Oxygen extraction was obtained by dividing the arterial-venous oxygen content difference by the arterial oxygen content. CFV was converted to circumflex blood flow (CBF) using the cross-sectional area of the vessel within the flow probe as determined by postmortem measurement. CBF was then calculated as: CBF (ml/min) = $(\pi \times D^2 \times V)/4$, where D is vessel diameter (cm), V is flow velocity (cm/sec).

Data are reported as mean $\pm$ standard error of the mean (SEM). Differences among means were considered statistically significant if the probability of their occurring by chance was less than 5 % ($p < 0.05$). For all comparisons, data within and among the protocols were analyzed for statistical significance using two way analysis of variance (ANOVA). In this analysis, factors were: A, with or without L-NMMA; and B, exercise levels; rest, 4.8/0, 6.4/ 0, 4, 8, 12, and 16 % incline. If the ANOVA detected significant differences within factor means, then these

differences were identified with the Student paired t-test for Factor A and with the Student-Newman-Keul procedure for Factor B.

RESULTS

Figure 2 shows the changes in CBF caused by intracoronary ACh and SNP in the resting dogs. This figure also compares responses of CBF to ACh and SNP before and after intracoronary administration of L-NMMA (35 mg). L-NMMA did not affect the increase in CBF at any dose of SNP. In contrast, blockade of NO synthase caused a significant attenuation of the CBF response to all doses of ACh ($p < 0.05$). These data demonstrate that this intracoronary dose of L-NMMA was effective in partially inhibiting ACh-induced vasodilation (46 % at 20 μg/min). Figure 3 demonstrates that a 15 sec occlusion of circumflex artery caused significant increase in blood flow after release of the occlusion (399 $\pm$ 20 %). At the highest level of exercise (6.4 kph/0 % incline), CBF increased 204 $\pm$ 12 % above basal flow, equivalent to only half of coronary vasodilatory reserve. Thus, chronic instrumentation did not cause a coronary stenosis.

Table 1 demonstrates that selective intracoronary infusion of L-NMMA had no significant effects on any measured hemodynamic variable or on CBF in resting conditions. These data indicating that the selected dose of L-NMMA (35 mg, i.c.) did not significantly spill-over into systemic circulation to alter MAP, HR, left ventricular function or CBF.

Figure 4 depicts a representative tracing from one dog showing circumflex blood flow at rest and during exercise. In the resting state, intracoronary L-NMMA *per se* did not affect CBF (see also Table 1). In the absence of L-NMMA (control), CBF increased as the intensity of exercise increased. In contrast, the magnitude of increase in CBF was significantly attenuated by L-NMMA at workloads higher than 6.4 kph/0 % incline. Data from all dogs are summarized in Figure 5 and Table 2. At rest and at a mild exercise workload (4.8 kph/0 % incline), CBF was not different between control and L-NMMA conditions. However, after blockade with L-NMMA, CBF was significantly reduced at workloads greater than 4.8 kph/0 % incline compared to respective control values ($p < 0.05$).

Figure 6 summarizes the effect of L-NMMA on hemodynamic variables at rest and during graded exercise. For both control and L-NMMA conditions, graded exercise produced progressive increases in MAP, HR, LVSP and LVEDP, and $+dP/dt_{max}$. Note that at rest and during exercise, MAP, HR, LVSP, and LVEDP were not different between control and L-NMMA conditions at any respective level of exercise, indicating that intracoronary infusion of L-NMMA *per se* did not affect systemic hemodynamic parameters. However, intracoronary infusion of L-NMMA did attenuate the increase in $+dP/dt_{max}$ during moderate to strenuous levels of exercise (i.e., workloads greater than 4.8 kph/0 % incline; $p < 0.05$).

In order to demonstrate that the attenuation of coronary hyperemia during exercise was indeed due to blockade of NO synthase, L-arginine (250 mg, i.c.) was

infused into four dogs while running at a workload of 6.4 kph/8 % incline in the presence of L-NMMA. A typical tracing from one experiment is shown in Figure 7. In all 4 dogs, intracoronary L-arginine (250 mg) restored both CBF and dP/dt_{max} to values equivalent to these observed in the control experiment.

Table 2 summarizes MVO_2 and related variables at rest and during exercise without (control) and after intracoronary infusion of L-NMMA. At rest, MVO_2 and oxygen extraction were not affected by intracoronary administration of L-NMMA. However, as the intensity of exercise increased for both conditions, arterial-venous oxygen content difference, oxygen extraction, and MVO_2 were increased significantly ($p < 0.05$), while coronary sinus oxygen content was decreased ($p < 0.05$). After L-NMMA blockade at workloads higher than 6.4 kph/8 % incline, CBF and MVO_2 were significantly reduced compared to respective control values ($p < 0.05$), but oxygen extraction was not changed. Figure 8 shows the relationship between coronary blood flow and MVO_2. These data indicate that intracoronary infusion of L-NMMA did not affect this relationship ($r=0.95$).

DISCUSSION

The most significant findings of this study are: 1) in the conscious resting state and at mild levels of exercise, NO does not appear to contribute to coronary vasodilation; 2) NO-mediated vasodilation is necessary for increases in coronary blood flow during moderate to heavy exercise; and 3) L-NMMA induces an attenuation of

coronary blood flow during exercise, thereby reducing myocardial contractility and myocardial oxygen consumption. These data indicate that endothelium-dependent flow-induced dilation, in addition to the metabolic vasodilation, is an important stimulus to maintain myocardial oxygen supply during exercise.

Previous studies have investigated whether NO plays a role in dilation of the coronary artery during resting and exercise conditions in conscious dogs using intravenous administrations of NO synthase inhibitors (1, 10, 26, 29). However, systemic administration of these inhibitors always caused increases in systemic vascular resistance, and consequently, increases in aortic pressure and baroreflex mediated decreases in heart rate. The use of selective intracoronary administration of L-NMMA in this study allowed us to avoid any confounding systemic effects, which simplified interpretation of the findings. In present study, intracoronary L-NMMA did not cause any changes in systemic hemodynamic parameters, demonstrating that the intracoronary dose 35 mg of L-NMMA did not specifically spill-over into systemic circulation to induce any systemic effects. Thus, any changes in coronary hemodynamic variables should be due to the direct local effects of NO blockade on the coronary circulation.

It was important to demonstrate that dissection and instrumentation of the circumflex coronary artery did not damage the circumflex artery adventitia and surrounding nerves as shown in a previous investigation (12). Gwirtz *et al* (17) also demonstrated that dissection of the circumflex coronary artery, as was done in this

study, does not alter the response of the circumflex artery to exercise. In present study, a 15 sec occlusion to produce maximum vasodilation caused 4-fold increase in peak coronary blood flow. These data indicated that the instrumentation of circumflex artery did not significantly alter the vascular function.

In present study, L-NMMA was used as a NO synthase inhibitor since the arginine analogue N^G-nitro-L-arginine methyl ester (L-NAME) has been shown to be a muscarinic receptor antagonist and to be long acting (over 24 h after intravenous administration in chronically instrumented, conscious dogs) (7, 31). An important consideration for this study was whether the NO synthase inhibitor remained effective throughout experiment (approximately 20 min). Rees *et al* (26) found that intravenous administration of L-NMMA (5-120 mg/kg) caused a significant dose-dependent increase in mean aortic pressure. Furthermore, L-NMMA at a dose greater than 50 mg did not further increase basal epicardial coronary vasomotor tone. Since the portion of the cardiac output comprising circumflex blood flow is only small (approximately 3-5 %), the intracoronary dose of L-NMMA and L-arginine used in this study were extrapolated from the intravenous doses used by Rees *et al* and others (8, 10, 26). Adequacy and specificity of this dose of L-NMMA were confirmed by observing the ability of L-NMMA to cause a significant attenuation of the coronary vasodilation induced by acetylcholine (endothelial-dependent vasodilator), while it did not affect the response to sodium nitroprusside (endothelial-independent vasodilator). The intracoronary dose of L-arginine selected did not affect coronary blood flow

during resting conditions before intracoronary administration of L-NMMA.

Dynamic exercise results in activation of the sympathetic nervous system which increases both the inotropic and chronotropic states of the myocardium. As a result, the increased work output of the heart causes increased coronary blood flow in proportion to the increased metabolic demand (14). Although the mechanisms for generation of NO are not fully understood, it has been proposed that an increase in blood flow, i.e., flow-induced shear stress, is probably the most important stimulus in the endothelium-dependent control of vascular caliber (25, 27). Using an anesthetized dog preparation, Smiesko *et al* (30) demonstrated that the flow-dependent femoral artery dilation induced by opening an arteriovenous shunt to increase flow velocity (shear stress) depended upon an intact endothelium, while removing the endothelium in the femoral artery by a balloon technique failed to induce dilation. This flow-dependent coronary dilation has also been demonstrated in humans (13). Many studies have investigated whether NO plays a role in the dilation of the coronary artery in physiological conditions, for example, during exercise (1, 3, 29). However, results from these studies are conflicting. Altman *et al* (1) reported that NO is not of critical importance for the increase in coronary blood flow which occurs in response to the increased myocardial oxygen demands during exercise. In contrast, there is a strong evidence that the endothelium is essential for the mediation of coronary dilation during exercise (3, 29). This conflict is probably attributable to factors associated with the intravenous administrations of NO inhibitors and/or the use of different NO

inhibitors used in these studies.

In present study, selective intracoronary infusion of L-NMMA avoided the systemic hemodynamic effects commonly associated with previous studies. In the conscious resting state and at low levels of exercise workload, intracoronary L-NMMA did not affect coronary blood flow, indicating that the increases in coronary blood flow during mild exercise was more likely due to local metabolic factors and increases in perfusion pressure. It may be possible that shear stress and/or other neurohumoral stimuli are not strong enough to induce NO synthesis at rest and at the low level of exercise. However, at moderate and strenuous levels of exercise, coronary blood flow was significantly attenuated. These data indicate that NO-mediated vasodilation is involved in the further increase in coronary blood flow during moderate to heavy exercise. This interpretation of results is supported by several studies (23, 35). Yamabe *et al* (35) compared the effects of 8-phenyltheophylline (8-PT), a specific competitive adenosine antagonist, to L-NMMA on myocardial reactive hyperemia response to a coronary artery. They found that the myocardial reactive hyperemia is regulated not only by adenosine but also by the endothelium-derived NO. Maekawa *et al* (23) examined the role of endothelium derived-NO and adenosine in functional myocardial hyperemia induced by atrial pacing, isoproterenol, and constriction of the aorta in anesthetized dogs. NLA, another NO synthase blocker, significantly attenuated the increase in coronary conductance during the functional myocardial hyperemia induced by all three

conditions, indicating that NO, as well as adenosine, plays an important role in functional hyperemia. Thus, our data which indicates that endothelium-dependent flow-induced dilation, in addition to the metabolic vasodilation, is probably an important stimulus to maintain myocardial oxygen supply during exercise is consistent with previous findings by others (23, 35).

It has been suggested that the α-adrenergic vasoconstriction is modulated by endothelial-dependent vasodilation in coronary arteries (4, 10). Although the precise mechanism of this interaction is unknown, Borkenboom *et al* (4) demonstrated that inhibition of NO synthesis potentiates the constriction induced by norepinephrine administration to isolated coronary arteries from dogs and humans. Furthermore, constriction of coronary microvessels (diameters between 40 and 250 μm) caused by α-adrenergic receptor activation with norepinephrine is markedly potentiated after inhibition of NO synthase activity (21). Thus, another possible explanation exists for the reduced coronary blood flow observed during exercise after L-NMMA in the present study. It is conceivable that a reduction in NO synthesis led to an unmasking of the coronary α-adrenergic constrictor tone during the sympathetic mediated stress of exercise.

The coronary sinus is known to be a major venous drainage pathway of the myocardium in the left ventricle. Since a catheter positioned in a posterior regional vein (which drains the circumflex perfusion territory) is technically difficult to maintain patent in the chronically instrumented dog for several weeks, a catheter was

positioned in the coronary sinus to allow sampling of coronary venous blood for determination of myocardial oxygen extraction and MVO_2. A previous study has demonstrated that left anterior descending artery (LAD) and left circumflex artery inflow drains primarily through the coronary sinus into the right atrium and accounts for approximately 86% of the coronary sinus effluent (20). The left circumflex artery contributed 45% of total coronary sinus effluent, which is the same contribution from the LAD artery (19). Thus, if the coronary sinus oxygen content during exercise is significantly less than values measured during respective control conditions, then the posterior regional venous oxygen content can be estimated because it is in the linear portion of the oxyhemoglobin dissociation curve above PO_2 25 mmHg. However, in present study, coronary sinus oxygen content at the similar workloads were not different for both control and L-NMMA conditions, indicating contamination from regions other than the posterior region had a negligible influence on calculated values of oxygen extraction and MVO_2.

The results of the current study demonstrated that the increase in CBF and dP/dt_{max} during exercise were less after NO blockade and as a result, decreased MVO_2. There are two possible explanations regarding the reduction in dP/dt_{max} and MVO_2 during exercise after blockade of NO production by L-NMMA. First, the reduction in NO caused the reduced coronary blood flow which led to the reduction in myocardial contractile function and MVO_2 as a result of the Gregg phenomenon. A second possible explanation of these results is that L-NMMA itself and/or the

reduction in NO synthesis directly caused a decrease in myocardial contractility, which led to the decrease in MVO_2. As a result, coronary flow will be lower during exercise. However, there is little to no evidence in the literature to support the latter possibility.

The three major determinants of myocardial oxygen consumption are heart rate, myocardial contractility, and ventricular wall tension (5). Since heart rate and ventricular wall tension, which are related to changes in arterial pressure and ventricular geometry, did not differ between control and L-NMMA conditions at respective levels of exercise, the reduced myocardial contractility after L-NMMA during exercise most likely caused the decrease in MVO_2. It has been established that increases in MVO_2 during exercise are matched by comparable increases in coronary blood flow because coronary arteriovenous oxygen extraction is near maximal (14). In present study, MVO_2 increased with increasing exercise workload. As a result, there was a linear relationship between coronary blood flow and MVO_2. However, at workloads higher than 6.4 kph/8 % incline, after L-NMMA MVO_2 was less compared to values at respective workloads in the control condition. We believe that this reduction of MVO_2 was most likely caused by reduced coronary blood flow because myocardial oxygen extraction did not increase to maintain MVO_2 at given workload. This assumption is an extension of the well known Gregg phenomenon which states that changes in coronary perfusion alters the strength of myocardial contraction (14). Recent studies by Kitakaze and Marban (22) and Schouten *et al* (28) demonstrated

that coronary perfusion modulates myocardial intracellular calcium ion concentration, and thus, myocardial contractile force. Both studies demonstrated that small to moderate reductions in coronary perfusion reduced intracellular calcium ion concentration and a concomitant reduction in force generation without any indication of ischemia or changes in sarcomere length.

The second possibility to explain these results is that reduced in myocardial contractility by L-NMMA caused a reduction in myocardial oxygen demand, which led to decrease coronary blood flow. However, there is little, but conflicting, evidence supporting the possibility that L-NMMA or NO blockade directly alters myocardial contractility and oxygen consumption. Amrani *et al* (2) demonstrated in the isolated working rat heart that L-NMMA decreased dP/dt_{max}, an index of cardiac contractility. However, they attributed this negative inotropic effect to decreases in coronary flow (i.e., to 39 % of its basal values), since the effects of L-NMMA were reversed with L-arginine and because L-NMMA did not alter the contractile activity of isolated cardiac myocytes. More recently, Weyrich *et al* (34) demonstrated that physiologically relevant concentrations of NO did not induce significant negative inotropic effects acutely in rat and cat myocardium in preparations ranging from isolated cardiac myocytes to isolated papillary muscles. Chu *et al* (10) also reported no effect of L-NMMA on dP/dt_{max} after systemic administration in chronically instrumented dogs. Therefore, we propose that during moderate and high levels of exercise workloads, intracoronary L-NMMA reduced dP/dt_{max} due to the reduction in

coronary blood flow, since at rest and at low level of exercise, intracoronary L-NMMA *per se* did not decrease dP/dt_{max}, and because a bolus intracoronary L-arginine restored coronary blood flow and dP/dt_{max}.

In recent years, it has been recognized that the vascular endothelium plays an important role in the local control of vascular smooth muscle tone and serves as a primary target organ in cardiovascular diseases such as atherosclerosis and hypertension (9). In studies of coronary arteries from patients with atherosclerosis and hypertension, the endothelium-dependent relaxation to acetylcholine is reduced, while the relaxation to sodium nitroprusside, an endothelium independent vasodilator, is not affected. Panza *et al* (24) reported that this reduction of endothelium-dependent relaxations in response to acetylcholine was directly related to the level of systolic blood pressure. Therefore, endothelial damage in coronary arteries may serve as one explanation for cardiac insufficiency observed in patients with coronary artery diseases, especially when they are subjected to stressful conditions such as exercise.

In summary, our results indicate that in the conscious resting state and at low levels of exercise, the increase in coronary blood flow was more likely due to local metabolic factors and increases in perfusion pressure rather than NO release induced by shear stress. However, during moderate to heavy exercise, NO-mediated vasodilation, in addition to the metabolic mediated vasodilation, appears to be an important mediator to maintain myocardial oxygen supply during exercise. After NO blockade, the reduction in coronary blood flow during exercise decreases myocardial

contractility and myocardial oxygen consumption. This indicates that endothelium-dependent flow-induced dilation, in addition to the metabolic vasodilation, is probably an important stimulus to maintain myocardial oxygen supply during exercise.

ACKNOWLEDGEMENTS

This research was performed as partial fulfillment of the dissertation requirements for the Ph.D. degree for Song-Jung Kim. We thank Abraham Heymann and Linda Legorreta for their outstanding technical assistance. This research was support by HL-34172 and HL-29232.

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Table 1. Lack of effect of intracoronary infusion of L-NMMA (35 mg) on hemodynamic variables and coronary blood flow at rest.

	Pre L-NMMA	Post L-NMMA
MAP (mmHg)	94 ± 3	97 ± 3
LVSP (mmHg)	119 ± 5	123 ± 5
LVEDP (mmHg)	1 ± 1	1 ± 1
$+dP/dt_{max}$ (mmHg/sec)	3034 ± 196	3134 ± 180
HR (beats/min)	82 ± 4	83 ± 5
CBF (ml/min)	29.5 ± 2.6	30.2 ± 2.6

Values are mean ± SEM, n = 7. MAP, mean aortic pressure; LVSP, left ventricular systolic pressure; LVEDP, left ventricular end-diastolic pressure; $+dP/dt_{max}$, maximum rate of change of left ventricular pressure; HR, heart rate; CBF, coronary blood flow.

Table 2. Myocardial oxygen consumption and related variables at rest and during exercise without (Control) and after intracoronary L-NMMA (L-NMMA).

	REST		EXERCISE (kph/% incline)					
			4.8/0		6.4/8		6.4/16	
	Control	L-NMMA	Control	L-NMMA	Control	L-NMMA	Control	L-NMMA
CBF (ml/min)	36 ± 2	33 ± 2	51 ± 6*	50 ± 7*	62 ± 6*	55 ± 6*§	72 ± 8*	62 ± 8*§
CvO_2 (ml O_2 /ml)	6.1 ± 0.3	5.7 ± 0.3	5.7 ± 0.3*	5.2 ± 0.5*	4.6 ± 0.6*	4.9 ± 0.7*	4.7 ± 0.8*	4.4 ± 0.6*
(a-v)O_2 diff (ml O_2/ml)	10.1 ± 0.5	9.3 ± 0.4	11.2 ± 1.9*	11.5 ± 1.1*	13.0 ± 0.8*	13.1 ± 1.0*	14.6 ± 1.1*	14.4 ± 0.7*
O_2 EXT (%)	62 ± 1	62 ± 2	66 ± 2*	69 ± 4*	74 ± 3*	73 ± 4*	76 ± 4*	77 ± 3*
MVO_2 (ml O_2/ml/min)	3.6 ± 0.4	3.1 ± 0.5	5.9 ± 1.0*	5.8 ± 1.1*	8.1 ± 1.2*	7.2 ± 1.1*§	10.8 ± 2.0*	9.1 ± 1.4*§

Values are mean ± SEM, n = 4. CBF, coronary blood flow; CvO_2, coronary sinus oxygen content; (a-v) O_2 diff, arterial-venous oxygen content difference; O_2 EXT, myocardial oxygen extraction; MVO_2, myocardial oxygen consumption. * $p<0.05$ vs respective rest values. § $p<0.05$ vs respective control values.

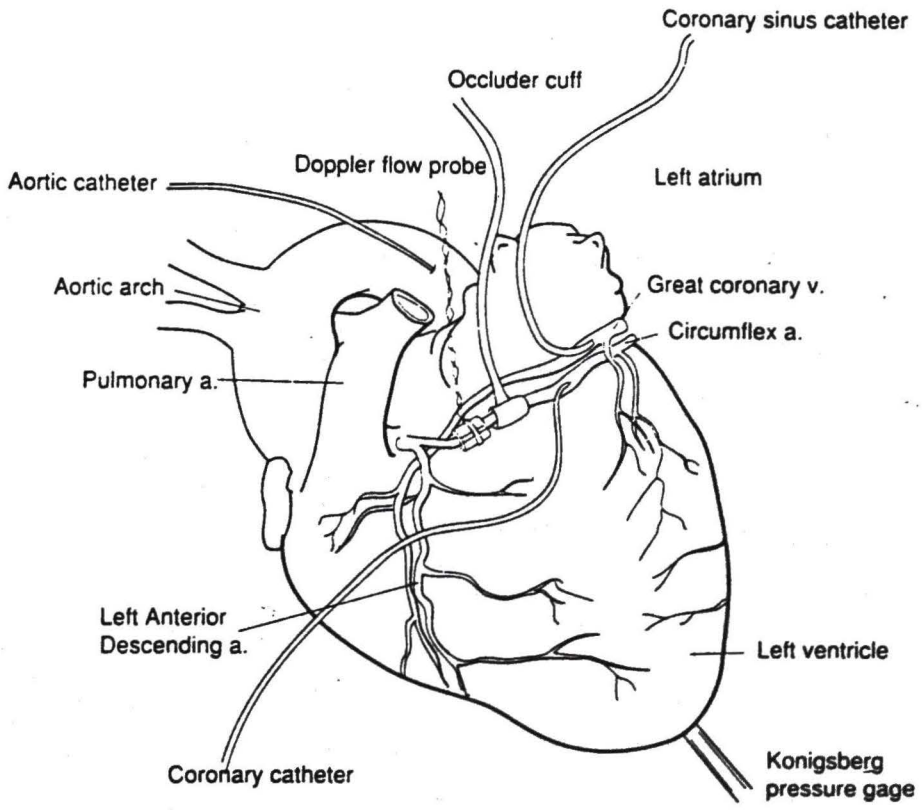

Figure 1. Illustration of the chronic surgical instrumentation used for measurement of cardiovascular parameters.

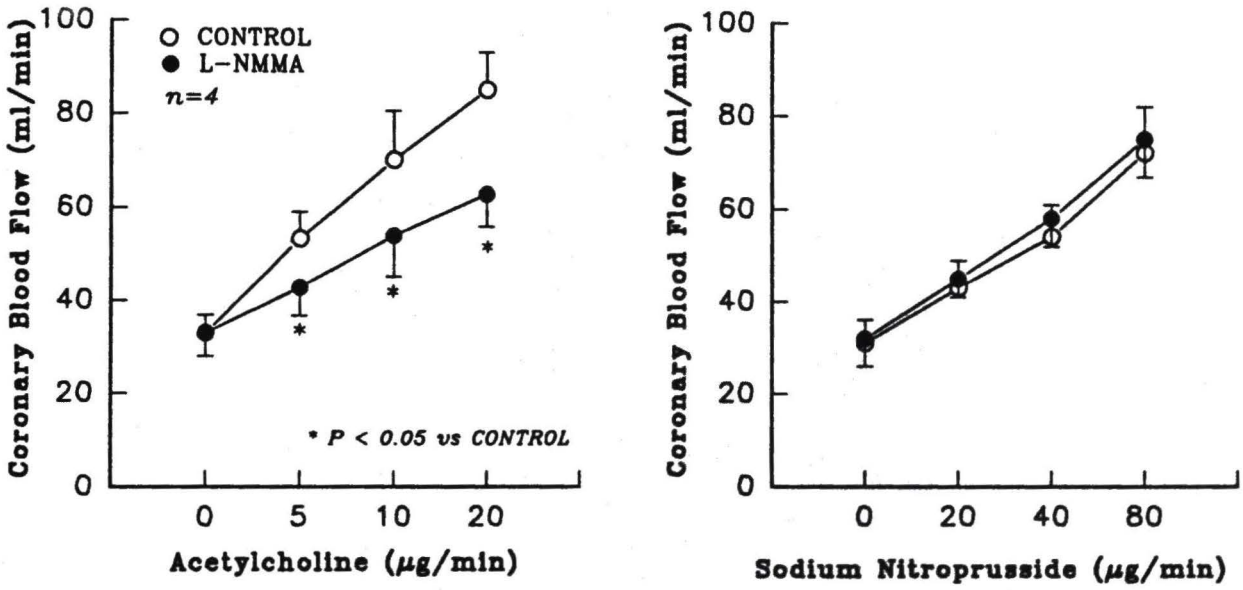

Figure 2. Changes in circumflex blood flow caused by graded intracoronary doses of acetylcholine and sodium nitroprusside before and after intracoronary administration of L-NMMA (35 mg).

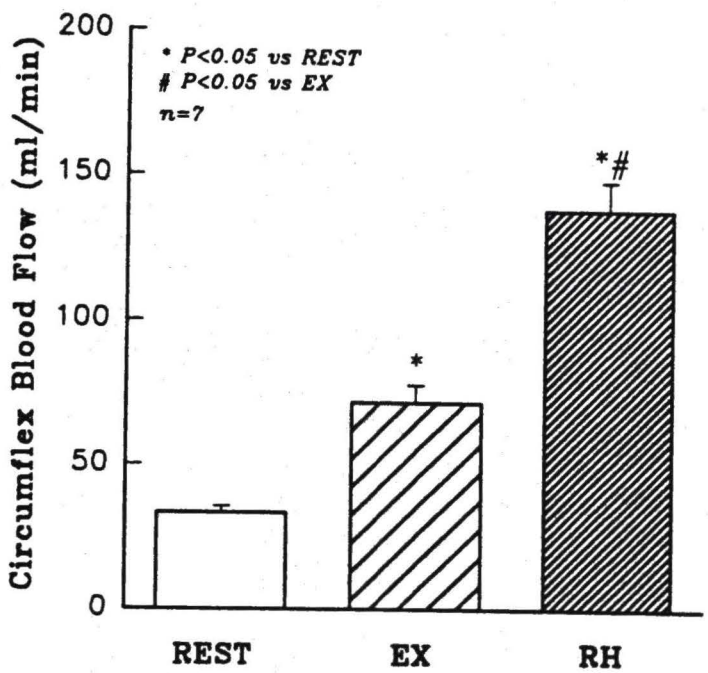

Figure 3. Increase in circumflex blood flow by exercise at 6.4 kph/16 % incline (EX) and during reactive hyperemia (RH) following a 15 sec circumflex artery occlusion.

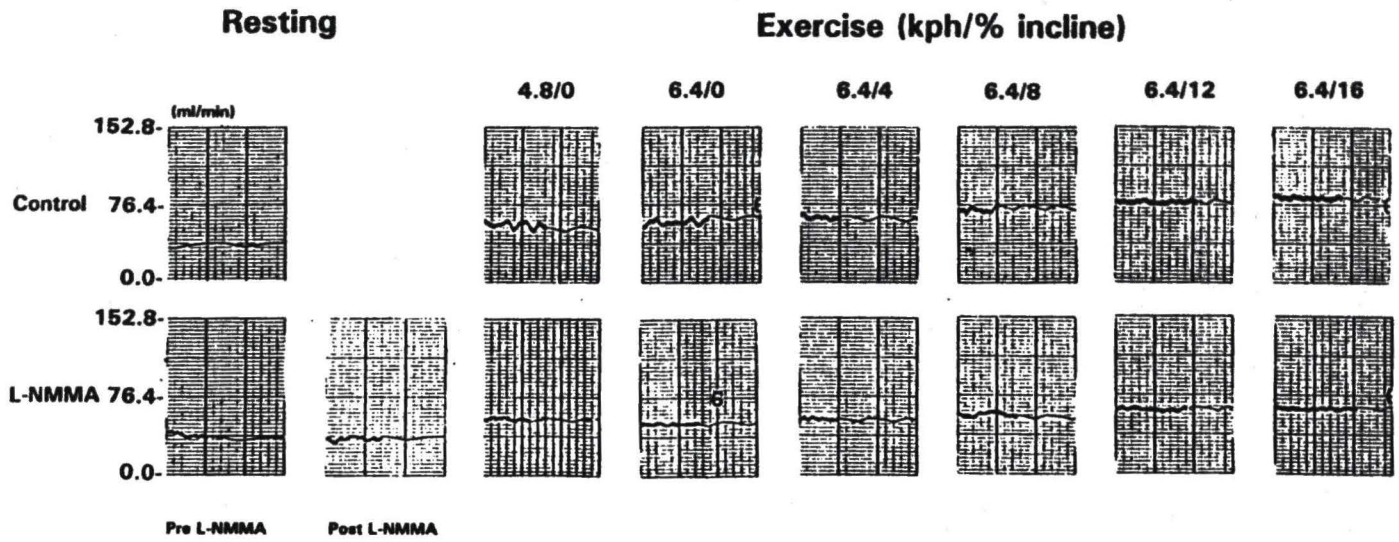

Figure 4. Original representative tracing showing circumflex blood flow at rest and during exercise.

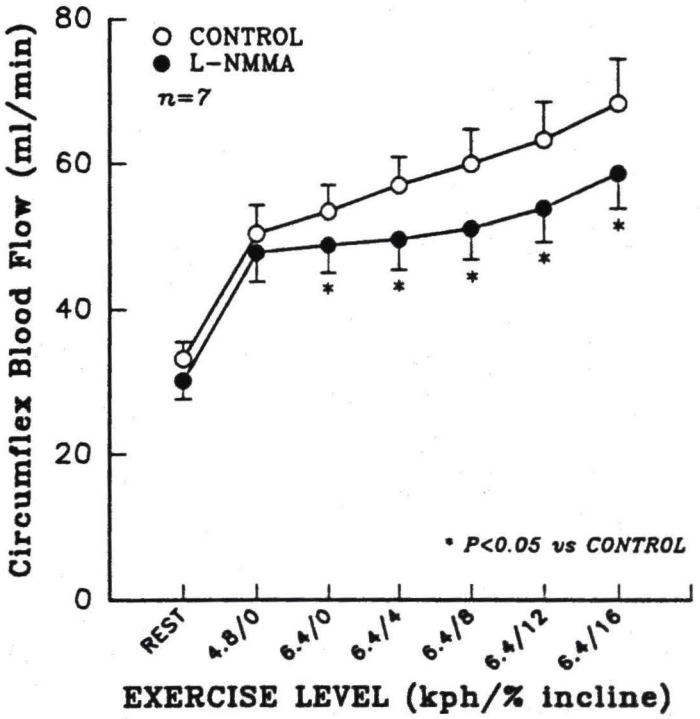

Figure 5. Effect of L-NMMA on circumflex blood flow at rest and during graded exercise.

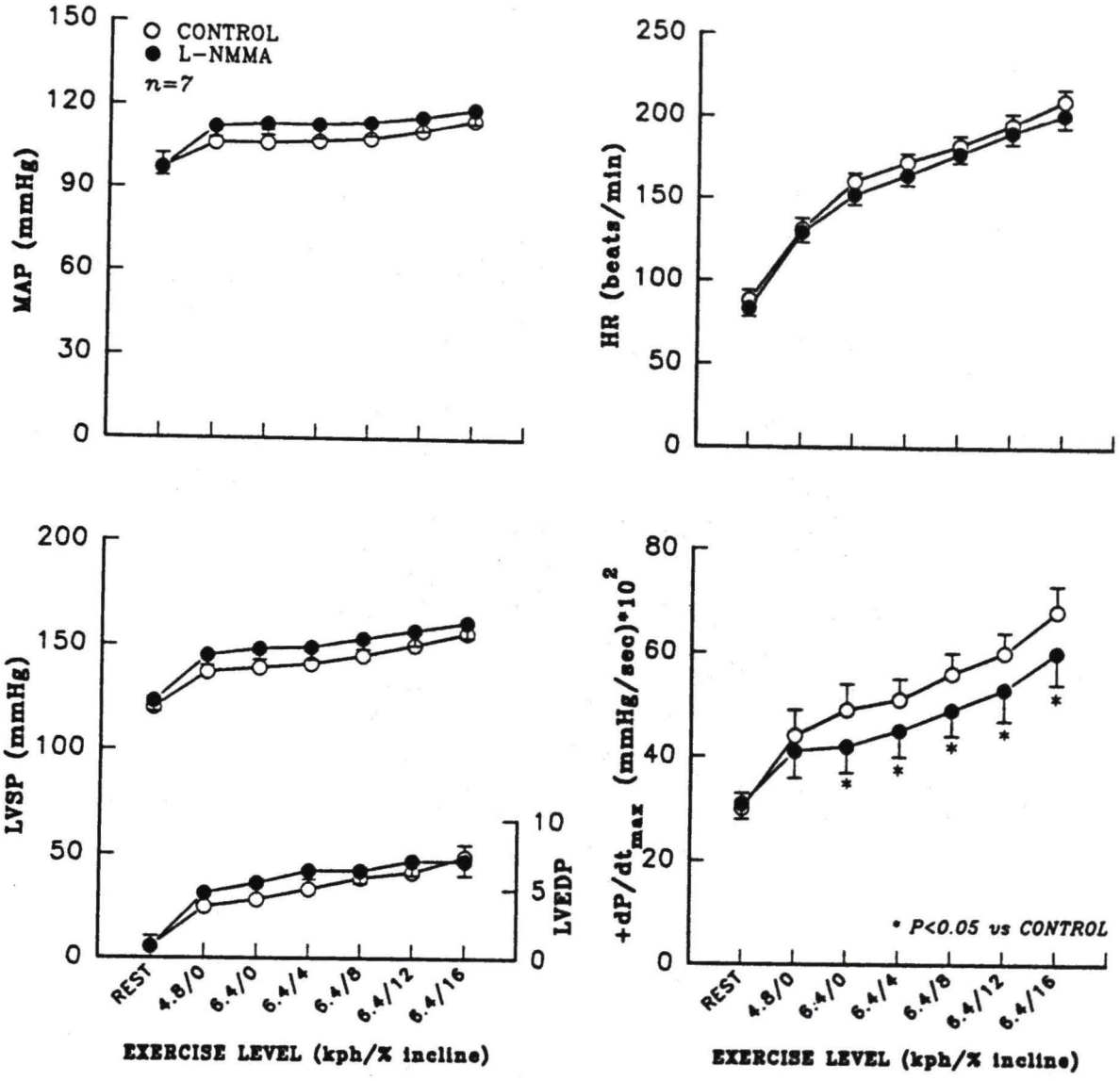

Figure 6. Effect of L-NMMA on hemodynamic variables at rest and during graded exercise. MAP, mean aortic pressure; HR, heart rate; LVSP, left ventricular systolic pressure; LVEDP, left ventricular end-diastolic pressure; $+dP/dt_{max}$, maximum rate of left ventricular pressure generation.

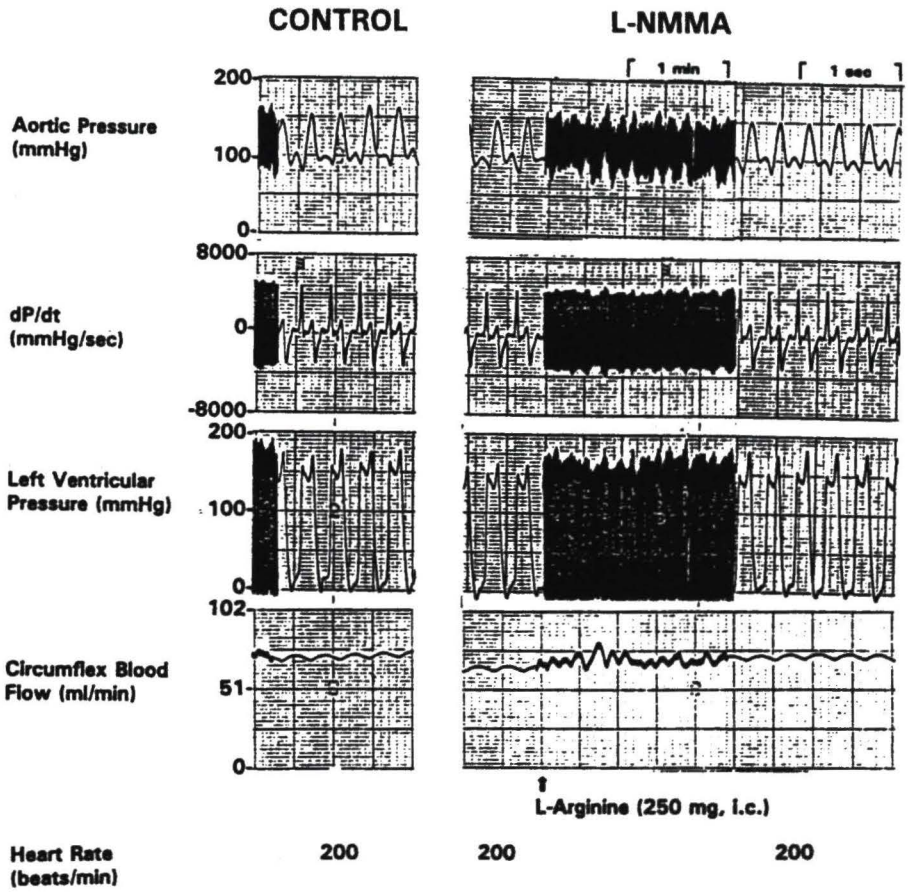

Figure 7. Original representative tracing showing response to exercise under control condition and during L-NMMA. Also shown is the reversal of effects of L-NMMA on circumflex blood flow and dP/dt_{max} by intracoronary L-arginine (250 mg) during exercise at a workload of 6.4 kph/8 % incline.

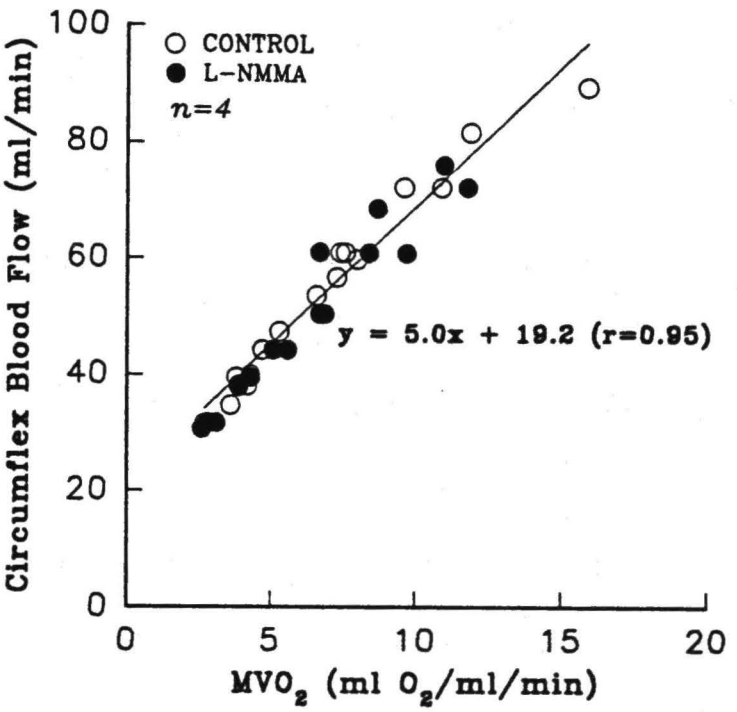

Figure 8. Relationship between circumflex blood flow and myocardial oxygen consumption (MVO_2). Individual values for all four dogs are shown.

CHAPTER IV

CONCLUSIONS

The first study was performed to examine whether an α_1-constrictor tone, which limits coronary functional hyperemia during exercise, imposes a significant limitation on global cardiac performance as determined by cardiac output. Results demonstrated that removing a coronary α_1-constrictor tone with prazosin during submaximal exercise leads to an increase in myocardial oxygen supply and, as a result, stroke volume and cardiac output. This limited flow by α_1-coronary constriction in coronary circulation might be beneficial for the delivery and distribution of cardiac output to the heart and working skeletal muscle during exercise.

The second study examined whether, in addition to the metabolic vasodilation, NO released by shear stress from the endothelium contributes to the coronary vasodilation during exercise. The significant findings of this study were 1) in the conscious resting state and at low level of exercise, NO does not contribute to coronary vasodilation, 2) during moderate to heavy exercise, NO-mediated vasodilation is necessary for increases in coronary blood flow, 3) this attenuation of coronary blood flow during exercise decreases myocardial contractility and

myocardial oxygen consumption. This indicates that endothelium-dependent flow-induced dilation, in addition to the metabolic vasodilation, is an important stimulus to maintain myocardial oxygen supply during exercise.

The proposed study provided fundamental information relating to the role of endothelium and sympathetic vasoconstriction on coronary circulation as well as cardiac output during exercise. It demonstrated how NO contributes to the intrinsic coronary blood flow control mechanisms during exercise in physiological conditions. However, it has brought other questions which could be clarified by future studies:

1. Is there an interaction between coronary α-adrenergic vasoconstriction and endothelial-dependent vasodilation mechanism which controls coronary blood flow during exercise?
2. Does endothelial-dependent vasodilation in pathophysiological conditions, such as hypertension, have a less functional role on the intrinsic coronary blood flow control mechanisms?