EXISTENCE OF AN ALPHA ONE-ADRENOCEPTOR-MEDIATED CORONARY VASOCONSTRICTOR REFLEX DURING ACUTE SYSTEMIC HYPOXIA, IN ANESTHETIZED, OPEN-CHEST DOGS

THESIS

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The presence of an alpha-adrenoceptor-mediated coronary vasoconstrictor reflex during acute systemic hypoxia was examined in thirteen chloralose-anesthetized dogs. Local vasodilator effects were avoided by perfusing the left common coronary artery (LCC) with normoxic blood, while the dogs were ventilated with 5% O_2-95% N_2. Left ventricular afterload was held constant and positive cardiac inotropic responses and beta two-adrenoceptor-mediated coronary vasodilation were blocked by propranolol. Parasympathetic-mediated bradycardia and coronary vasodilation were blocked with atropine. Systemic hypoxia decreased LCC flow to normoxic myocardium by 19.4$\pm$2.6 %. Although myocardial oxygen extraction increased 9.7$\pm$2.9 %, myocardial oxygen consumption decreased 16.5$\pm$2.6 %. Intracoronary prazosin prevented the reflex vasoconstriction during repeated hypoxia.

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CHAPTER I

INTRODUCTION

Hypoxia, a subnormal level of oxygen in blood or tissue, is considered to be the most potent of all physiological stimuli (53). In the neurologically intact conscious animal, systemic hypoxia increases ventilation, heart rate, ventricular contractility, cardiac output, blood pressure, and coronary blood flow (3,23,33,39,50,66, 71,76). During systemic hypoxia, increases in coronary blood flow result from vasodilatory substances released from hypoxic, hyperactive myocardium, chemoreceptor reflex-mediated coronary vasodilation (27,67), and possibly, a direct effect of hypoxia on coronary vessels (42,55).

Local Effect of Hypoxia on Blood Flow

Extensive investigation has been performed on the local effects of ischemia and hypoxia on tissue perfusion. The local effects of hypoxic blood perfusion are relatively consistent; local hypoxia causes vasodilation (21,29,55), although the degree of vasodilation varies with the organ being perfused. The coronary vascular system is one of the most sensitive and reactive vascular systems to hypoxia (53).

Mechanisms for hypoxia-induced vasodilation have been

proposed. Duling and Berne (29) studied the effects of hypoxia on hamster cheek pouch microvasculature. They demonstrated that the large surface area to volume ratio of arterioles combined with oxygen's high lipid solubility, allows for extremely rapid oxygen diffusion across vessels into surrounding tissue. When perfused with hypoxic blood, tissues readily remove oxygen from pre-capillary vessels reducing the availability of oxygen to vascular smooth muscle. The result is smooth muscle relaxation and vasodilation. In vitro helical strips of coronary artery have also been used to study the relationship between oxygen tension and vascular smooth muscle tone (42). Results indicate that during moderate myocardial hypoxia, vascular oxygen tension may have a more prominent role than adenosine in control of coronary vascular tone, yet the vasodilatory effects of adenosine may be more prominent during severe myocardial hypoxia. Studies by Downey et al. (25) have demonstrated the that coronary vasodilation during non-ischemic myocardial hypoxia occurs even though myocardial adenosine concentrations do not increase.

Physiology of the Autonomic Nervous System

Systemic hypoxia produces various changes in the cardiovascular system which are mediated by the autonomic nervous system (5,23,33,50,66,74,77,93,121). In order to appreciate and understand the significance of the autonomic

nervous system during systemic hypoxia-induced cardiovascular reflexes, one must first have a general working knowledge of this system.

The autonomic nervous system (ANS) is the division of the nervous system which controls the involuntary responses of various physiological systems. The ANS is divided into the sympathetic and parasympathetic nervous systems, which in general are antagonistic. The efferent pathways of both systems are characterized by pre- and post-ganglionic neurons, have post-synaptic cholinergic nicotinic-receptors on their post-ganglionic neurons, and utilize acetylcholine as a ganglionic synaptic neurotransmitter. Both systems also innervate and have prominent roles in the regulating the cardiovascular system.

Acetylcholine is the neurotransmitter of the parasympathetic nervous system at the neuro-effector sites, and exerts its effects on myocardium and coronary arteries via muscarinic-receptors. Cholinergic muscarinic-receptor stimulation decreases inotropic and chronotropic responses in the myocardium (47,82), and indirectly produces coronary vasodilation (41,115). Muscarinic-receptors are selectively blocked by atropine (119).

Cholinergic (muscarinic and nicotinic) receptors are also located on pre-junctional adrenergic neurons and modulate norepinephrine release. Stimulation of pre-junctional muscarinic-receptors inhibits norepinephrine

release, whereas stimulation of pre-junctional nicotinic-receptors promotes release of norepinephrine from adrenergic nerve terminals (4,111,115).

The catecholamines, norepinephrine and epinephrine, are the two principle neurotransmitters of the sympathetic nervous system. Norepinephrine is synthesized in and released from the sympathetic, post-ganglionic neurons, while both epinephrine and norepinephrine are synthesized in and released from the adrenal medulla (86). Norepinephrine and epinephrine interact with two types of adrenergic receptors (adrenoceptors), alpha and beta, located on effector or target cells. Alpha- and beta-adrenoceptors are further subdivided into alpha-one and alpha-two and beta-one and beta-two subtypes, respectively. Adrenoceptor subtype affinities for various agonists and antagonists are presented in Table I (64,109,110,120,121).

Alpha one-adrenoceptors are located post-synaptically on the systemic and coronary arterial smooth muscle cells, and stimulating these adrenoceptors causes vasoconstriction (42,64). These adrenoceptors are also found on myocytes, and experiments have demonstrated that stimulation of these myocardial adrenoceptors causes a positive inotropic effect in isolated ventricular myocytes of cats, guinea pigs, rats rabbits, dogs, chickens, and humans (13,34,109,110,111).

Alpha two-adrenoceptors are also found on the arterial smooth muscle cells; however, unlike alpha one-adrenoceptors

TABLE I

ADRENOCEPTOR AGONISTS AND ANTAGONISTS *

Adrenoceptor subtype	Agonists	Antagonists
Alpha-one	phenylephrine	prazosin
	norepinephrine	phenoxybenzamine
	epinephrine	phentolamine
	methoxamine	tolazoline
Alpha-two	clonidine	rauwolscine
	epinephrine	yohimbine
	norepinephrine	tolazoline
	methoxamine	phentolamine
Beta-one	isoproterenol	practolol
	epinephrine	atenolol
	norepinephrine	propranolol
Beta-two	salbutamol	propranolol
	isoproterenol	
	epinephrine	
	norepinephrine	

* Agonists and antagonists are listed in order of decreasing specificity for adrenoceptor subtypes.

they are preferentially distributed extra-synaptically (78). Stimulation of alpha two-adrenoceptors also causes smooth muscle cell contraction and vasoconstriction (78). Post-junctional alpha two-adrenoceptors are generally believed to primarily respond to stimulation by circulating (not neurally released) catecholamines (78), but evidence disagrees with this conclusion (60,61,62,105). Alpha two-adrenoceptors are also located on pre-junctional sympathetic neurons and are believed to regulate norepinephrine release through a negative feedback mechanism (4,63,64,77,87).

Beta one-adrenoceptors are located post-synaptically on atrial and ventricular cardiac myocytes. Stimulation of these adrenoceptors produces positive chronotropic and inotropic effects in both the atria and ventricles (11). Beta one-adrenoceptors have not been found in coronary arteries (85).

Beta two-adrenoceptors are situated on the arterial smooth muscle cells (85,110,121) as well as on atrial and ventricular cardiac myocytes of humans, dogs, cats and guinea pigs (11,80). Beta two-adrenoceptor stimulation mediates vasodilation (110,121) in arteries and may increase myocardial chronotropy and inotropy (11). Beta two-adrenoceptors have also been found on pre-junctional adrenergic neurons, and are believed to facilitate norepinephrine release when stimulated (4,111,116).

During physiological stress, such as exercise,

stimulation of beta-adrenoceptors increases heart rate and myocardial contractility thus increasing cardiac output, blood pressure, and tissue perfusion. Stimulation of alpha-adrenoceptors in the systemic vasculature increases peripheral resistance thus increasing systemic blood pressure, and redistributing blood flow to metabolically active organs. However, stimulation of coronary alpha-adrenoceptors can produce vasoconstriction which can limit coronary blood flow (88).

During physiological stress, restriction of coronary blood flow due to stimulation of coronary alpha-adrenoceptors could decrease myocardial oxygen delivery and myocardial oxygen consumption (44,96). This could be detrimental to myocardial metabolism. However, there may be beneficial effects of alpha-adrenoceptor induced coronary vasoconstriction during stress.

Coronary steal is the diversion of blood flow away from a one perfusion field to another with less resistance (17). Coronary steal can reduce coronary blood flow to ischemic myocardium causing this tissue to become more ischemic and possibly causing it to infarct. Constriction of vasculature in non-ischemic myocardium can improve collateral blood flow to ischemic myocardium by preventing coronary steal (17).

The steal phenomenon also occurs when blood flow distal to a coronary stenosis is distributed away from the subendocardium to lower resistance pathways in

subepicardium, i.e., transmural steal (36,94). Alpha-adrenoceptor mediated constriction in the subepicardial vessels may prevent flow from being diverted from subendocardium to subepicardium, and maintain oxygen delivery to the subendocardium (36,94). Even in the absence of coronary stenosis, alpha-adrenoceptor-mediated coronary vasoconstriction can prevent transmural steal in situations were the coronary vasculature was previously maximally dilated (68).

Alteration of Cardiovascular Hemodynamic Variables During Systemic Hypoxia

Selective severe myocardial hypoxia not only vasodilates coronary vessels, but also decreases cardiac function in situ (21,55). However, systemic hypoxia results in stimulation of aortic and carotid chemoreceptors and the central nervous system and initiates reflexes which modify cardiac function and systemic vascular tone. Specifically, myocardial contractility increases (23) and peripheral vasculature constricts (20,23,50) during systemic hypoxia.

Carotid chemoreceptor stimulation with hypoxic blood or other noxious substances decreases heart rate and cardiac contractility, but increases peripheral vascular resistance (19,27,49,74,97). Hypoxia-induced carotid chemoreflex bradycardia can be blocked with atropine and/or bilateral vagotomy (15,112). Selective carotid body stimulation with nicotine, cyanide, or hypoxic blood activates a vagal-

mediated, cholinergic coronary vasodilator reflex which has been detected only in the presence of atrial pacing and beta-adrenoceptor blockade (30,49,67). Thus, carotid body stimulation activates reflexes which increase sympathetic discharge to the peripheral vasculature while simultaneously increasing parasympathetic discharge to the heart.

This reflex may account for observed peripheral vasoconstriction and increased systemic blood pressure during systemic hypoxia, but it does not explain the increases in heart rate and ventricular performance observed during systemic hypoxia.

Some experimenters have observed both vasodilatory and vasoconstrictory coronary reflexes during carotid body activation. Hashimoto et al. (52), using a canine Langendorf preparation, noted an increase in coronary blood flow when carotid chemoreceptors were stimulated with nicotine, lobeline, 5-hydroxytryptamine, or acetylcholine. When the dog was given atropine, the reflex vasodilation was eliminated and vasoconstriction was unmasked.

Biphasic coronary responses after carotid body stimulation with nicotine have been observed in the right and left coronary artery of conscious, artificially ventilated dogs (91). The initial vasodilation was abolished by atropine. The subsequent vasoconstriction was abolished by the alpha-adrenoceptor blockade and attenuated by bilateral adrenalectomy. These results suggest the

presence of an alpha-adrenoceptor mediated coronary vasoconstriction during stimulation of carotid chemoreceptors which has both neural and adrenal origins. Experimenters have not observed a coronary vasoconstrictor reflex when carotid chemoreceptors are stimulated with hypoxic blood.

Carotid chemoreflexes in conscious dogs can be potentially modified by alterations in ventilation (104,117,118). Carotid body stimulation with nicotine produces spontaneous hyperventilation followed by a decrease in coronary resistance and an increase in coronary flow (118). After beta-adrenoceptor and muscarinic-receptor blockade, intracarotid nicotine causes a coronary vasodilation, but only if accompanied by hyperventilation. During controlled ventilation with beta-adrenoceptor and muscarinic-receptor blockade, intracarotid nicotine produces no change in coronary resistance or blood flow (117). Stimulation of carotid chemoreceptors apparently elicits coronary vasodilation through a minor vagal component directly involving chemo-reflexes and a major component involving withdrawal of sympathetic tone via the lung inflation reflex.

Aortic chemoreceptor stimulation with hypoxic blood or other noxious substances (72,113) increases heart rate and myocardial contractility. Aortic pressure and peripheral resistance also increase (15). The increase in cardiac

contractility during aortic chemoreceptor stimulation is unaffected by muscarinic-receptor blockade, but is significantly reduced by beta-adrenoceptor blockade (112). Aortic body stimulation also produces a vagal-mediated reflex coronary vasodilation which can be blocked by bilateral vagotomy or muscarinic-receptor blockade with atropine (48). The lack of coronary vasoconstriction during muscarinic-receptor and beta-adrenoceptor blockade suggests the absence of a coronary sympathetic vasoconstrictor mechanism during aortic chemoreceptor activation.

Reduction in central nervous system (CNS) oxygen tension increases systemic sympathetic tone (1,2,5,22,26,46,106). Isolated perfusion of the CNS with hypoxic blood produces increases in heart rate, peripheral resistance, systemic blood pressure, and ventricular contractility (1,2,5,22,26). These are the same cardiovascular responses seen during cerebral ischemia (46,106) and during systemic hypoxia. Complete aortic and carotid denervation does not significantly alter the cardiovascular responses to systemic hypoxia (9,74,76,98). Although studies on peripheral vasculature have been performed, no studies have specifically examined changes in coronary tone and coronary blood flow during selective CNS hypoxia or cerebral ischemia.

Rationale for this Investigation

Systemic hypoxia is characterized by increases in ventilation, heart rate, peripheral vasoconstriction, systemic blood pressure, and cardiac output. Evidence strongly suggests that cardiovascular responses to hypoxia are primarily mediated through a cerebral hypoxic reflex and may be modified by afferent carotid and aortic chemoreceptor input. Isolated carotid chemoreceptor stimulation with hypoxic blood has been shown to cause a parasympathetic-mediated bradycardia in intact hearts and vasodilation of coronary vessels. Since systemic hypoxia evokes sympathetic discharge which constricts numerous peripheral vascular beds, then the cardiac component of this discharge might be expected to produce coronary vasoconstriction. In light of parasympathetic-mediated coronary vasodilation that occurs during hypoxia and especially the potent local metabolic vasodilatory mechanisms that match coronary blood flow with changes in cardiac function, it is not surprising that coronary vasoconstriction is not manifested during hypoxia-induced increases in heart rate, afterload, and myocardial contractility. Therefore, during systemic hypoxia, if parasympathetic-mediated coronary vasodilation was blocked, and changes in myocardial oxygen requirements could be avoided, then a sympathetic-mediated coronary vasoconstriction might be unmasked.

CHAPTER II

METHODS

Experimental Animals

All experiments were performed on mongrel dogs of either sex that weighed 15-25 kg. The dogs (Canis familiaris), which were in good health, were obtained from either the Dallas Animal Shelter or the Burleson Animal Shelter, and were housed in the Texas College of Osteopathic Medicine (T.C.O.M.) Vivarium. All dogs were used in experiments within two weeks upon arrival at T.C.O.M.

Pharmacological Tools (Drugs)

The following pharmacological tools (drugs) were used in this experiment: Alpha-chloralose (Nutritional Biochemical, Cleveland, OH), concentration-130 mg/ml; Atropine (Elkins-Sinn Inc., Cherry Hill, NJ), concentration-0.4 mg/ml; Heparin (Elkins-Sinn Inc., Cherry Hill, NJ), concentration-5000 U/ml; Isoproterenol (Elkin-Sinn Inc., Cherry Hill, NJ), concentration-0.01 mg/ml; Phenylephrine (Sigma Chemical Company, St. Louis, MO), concentration-200 ug/ml; Prazosin hydrochloride (a gift from Pfizer Pharmacuetical Company, Groten, CN), concentration-0.5 mg/ml; Propranolol (Sigma Chemical Company, St. Louis, MO), concentration-5.0 mg/ml; Sodium bicarbonate (Fisher Chemical

Company, Fair Lawn, NJ), concentration-75mg/ml; Surital (thiamylal sodium) (Park-Davis, Morris Plains, NJ) concentration-25 mg/ml.

All drugs, except alpha-chloralose and Surital, were dissolved in normal saline. Alpha-chloralose and Surital were dissolved in distilled water.

General Preparation

Thirteen dogs were sedated with Surital (5 mg/kg, iv), and then anesthetized with alpha-chloralose (130 mg/kg, iv). Alpha-chloralose was used due to its non-vagolytic actions and its reduced capability to impair neural reflexes (51). Supplemental doses of alpha-chloralose (10-20 mg/kg, iv) were administered at one hour intervals in order to maintain the dogs in an surgical state of anesthesia, as indicated by an attenuated eye reflex. The trachea was intubated and the lungs were ventilated (15-20 cc/kg) at positive pressure with room air, supplemented with 100% O_2, using a Harvard model 607 respirator. A water trap was connected to the respirator expiration-vent to prevent atelectasis by maintaining a positive end-expiratory pressure of 3-5 mmHg. The dogs were kept warm by a circulating-water heating pad. Left thoracotomy was performed in the fourth intercostal space, and the third and fourth ribs were removed. The upper and middle lobes of the left lung were retracted in order to expose the heart and thoracic vessels. The left

ventral ansa subclavia was dissected free from the distal left subclavian artery and surrounding tissue, and the left subclavian artery was isolated near its origin. The proximal descending aorta was prepared for cannulation by isolating, ligating and cutting the first three or four pairs of vertebral arteries. Care was taken to prevent damage to visible nerves during the subclavian artery and aortic isolations. After these surgical procedure were performed, heparin (500 U/kg, iv) was administered to the dog to prevent blood coagulation. Additional heparin (2000 U, iv) was administered every hour.

The heart was exposed through pericardotomy and suspended in a pericardial cradle. For measurement of left ventricular pressure, a Millar PC-380 transducer tipped catheter was inserted into the left atrial appendage, and advanced into the left ventricle. The left ventricular pressure signal was differentiated by a Beckman 9879 coupler and recorded as left ventricular dP/dt. For measurement of left atrial pressure, a polyethylene catheter, attached to a Statham P23 Db pressure transducer, was inserted into the left atrial appendage for measurement of left atrial pressure. Heart rate was measured using a Beckman 9857 tachometer coupler which was driven by signal output from the ventricular dP/dt coupler. Aortic pressure was measured with a Statham P23 Db transducer connected to a polyethylene catheter advanced through the left femoral artery into the

descending aorta. The experimental hemodynamic variables, left atrial pressure, aortic pressure, LCC perfusion pressure, left ventricular pressure, left ventricular dP/dt_{max}, LCC blood flow and heart rate, were recorded by an eight channel Beckman R611 recorder.

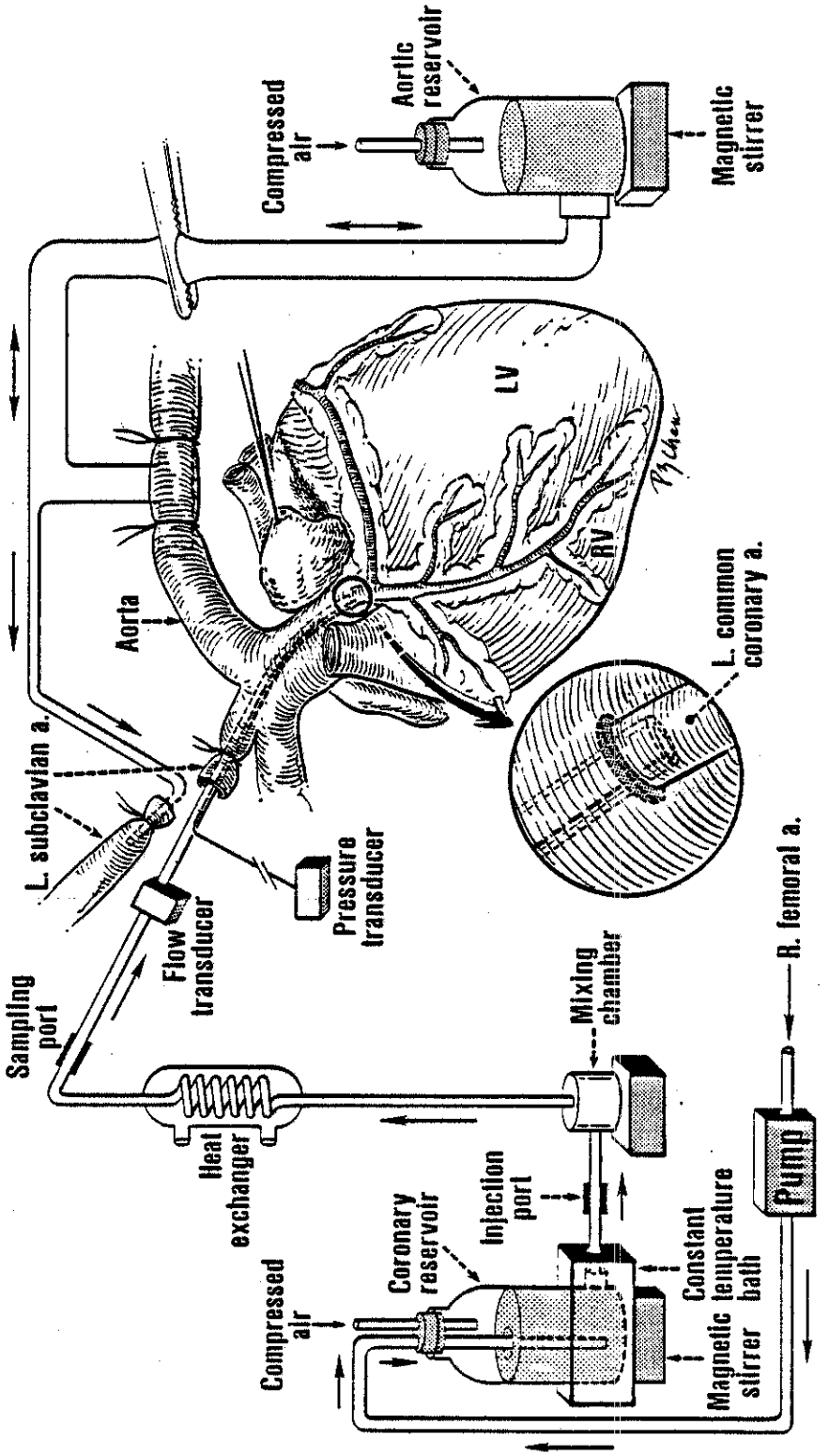
Aortic Blood Pressure Stabilization

A diagram of the aortic blood pressure stabilization apparatus is presented in Figure 1. Aortic blood pressure during systemic hypoxia was maintained constant by an external pressurized blood reservoir connected to the proximal descending aorta. The reservoir was a one liter glass bottle pressurized with compressed air. The proximal descending aorta was cannulated with a polyvinyl T-tube (0.8 mm I.D., 1.1 mm O.D.) which was connected to the reservoir via thick-walled Tygon tubing (0.8 mm I.D., 1.5 mm O.D.). Stagnation and separation of blood in the reservoir was prevented by a magnetic stirrer. Aortic pressure was controlled by increasing or decreasing the pressure within the reservoir, thus, infusing or removing blood from the animal.

Perfusion of the Left Common Coronary Artery

A schematic diagram of the perfusion system used in these experiments is shown in Figure 1. The left common coronary artery (LCC) was perfused at a constant pressure with blood from a pressurized blood reservoir connected to a

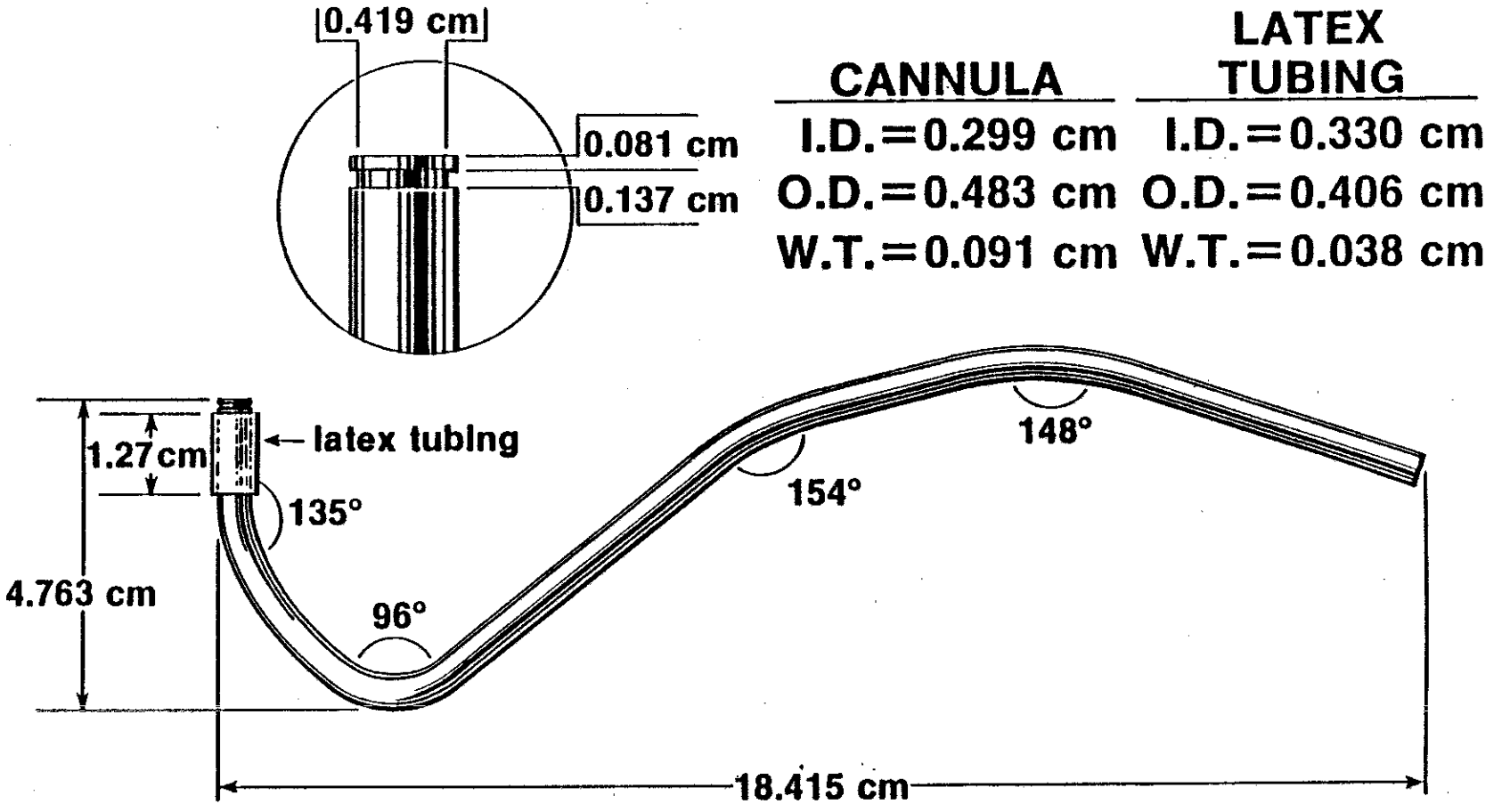
Figure 1. A schematic diagram of the aortic blood pressure stabilization system and the coronary artery perfusion system. LV = left ventricle; RV = right ventricle.


Compressed air
Aortic reservoir
Magnetic stirrer
LV
RV
Aorta
L. subclavian a.
L. common coronary a.
Pressure transducer
Flow transducer
Sampling port
Mixing chamber
R. femoral a.
Heat exchanger
Injection port
Coronary reservoir
Pump
Compressed air
Constant temperature bath
Magnetic stirrer

modified stainless steel Gregg cannula. A technical drawing of the Gregg cannula is presented in Figure 2. The cannula, which had a latex rubber sleeve near its distal orifice, was inserted into the proximal left subclavian artery, advanced across the ascending aorta, and securely wedged into the left coronary ostium where it was held in place by variable, opposite tension applied at high and low points along its stem. The coronary reservoir was filled with normoxic blood withdrawn from the right femoral artery and stagnation and separation of blood in the reservoir was prevented by a magnetic stirrer. LCC blood flow was measured with a Carolina Medical Inc. EP 612 electromagnetic in-line flow-transducer which was inserted into the perfusion line between the LCC cannula and reservoir. Perfusion pressure was measured with a Statham P23 Db transducer connected to a polyethylene catheter which has been inserted into the perfusion line. Blood perfusing the LCC was maintained at 39^{o} C by a heat exchange coil which was warmed with circulating water.

Proper position of the cannula in the LCC was verified by: 1) a drop in coronary perfusion pressure to 20-30 mm Hg at zero blood flow through the LCC cannula and 2) a reduction in LCC blood flow upon external compression of the circumflex and/or left anterior descending arteries. At the end of each experiment, India ink was injected into the LCC

Figure 2. A technical drawing of the modified stainless steel Gregg cannula used to perfuse the left common coronary artery.



	CANNULA	LATEX TUBING
	I.D. = 0.299 cm	I.D. = 0.330 cm
	O.D. = 0.483 cm	O.D. = 0.406 cm
	W.T. = 0.091 cm	W.T. = 0.038 cm

perfusion line to stain the perfused area of the heart. Uniform staining of the left ventricle and septum also confirmed proper position of the LCC cannula. The hearts were excised, rinsed with running tap water, and blotted dry. The atria were removed and discarded and the perfused region was then separated from the non-perfused regions and weighed. The weight of the LCC perfusion territories ranged from 59.2 g to 134.2 g and averaged 87.9 $\pm$ 5.7 g.

Blood Gas Tension, Oxygen Content and pH Measurements

During all experiments, systemic arterial, coronary arterial and coronary venous sinus blood samples were withdrawn and analyzed for PO_2, PCO_2, pH and oxygen content. Systemic arterial blood samples were drawn anaerobically through a polyethylene catheter inserted into the right femoral artery. Coronary arterial blood samples were drawn anaerobically through the coronary LCC perfusion line. Coronary venous sinus blood samples were drawn anaerobically through a polyethylene catheter inserted into the right jugular vein and advanced through the superior vena cava and into the coronary venous sinus. The PO_2, PCO_2, and pH of blood samples were measured by a Corning 175 automatic blood pH/gas analyzer and blood O_2 content was measured by an Instrumental Laboratory 282 CO-oximeter.

Determination of Myocardial Oxygen Consumption

Oxygen consumption for the LCC perfused region was

computed by the Fick equation (47):

$$MVO_2 = CBFG \times (CaO_2 - CvO_2),$$

where MVO_2 is regional myocardial oxygen consumption (ml O_2/min/100g); CBFG is LCC blood flow per one-hundred grams of myocardium (ml/min/100g); $(CaO_2 - CvO_2)$ is the coronary arterio-venous oxygen difference for the LCC region (ml O_2/100 ml of blood).

Method for Determining Contamination of Coronary Venous Sinus Blood with Systemic Hypoxic Blood

It was necessary to determine that changes in coronary venous sinus oxygen content resulted from changes in left ventricular myocardial oxygen consumption, but not by the contamination of the LCC circulation or coronary venous sinus with systemic hypoxic blood. Systemic hypoxic blood could enter into the LCC circulation via leakage around the LCC cannula, while hypoxic blood could enter into the coronary sinus via venous drainage from the right ventricle. Properly positioning the coronary sinus cannula assured that blood drawn through this cannula was not contaminated by blood from right atrium (75). Therefore, the presence systemic blood in the LCC circulation and coronary venous sinus was tested at the end of three experiments by measuring the presence of systemically administered Evans blue dye in coronary venous sinus blood while the LCC was perfused with blood absent of Evans blue dye.

These animals were instrumented as previously

described. The LCC perfusion reservoir was filled with normoxic arterial blood, free of Evans blue dye, from the experimental dog. At that point, the pump used to fill the coronary perfusion reservoir was shut off, and 3 ml of venous sinus and systemic venous blood were drawn. Evans blue dye (4 mg/kg) was then administered systemically via a femoral vein. Over the next two minutes, 3 ml samples of coronary sinus and systemic venous blood were drawn every thirty seconds.

All samples were then centrifuged in a Beckman TJ-6 centrifuge, and the plasma was removed. The plasma samples were analyzed for Evans blue dye in a Beckman spectrophotometer with the absorbance wavelength of 610 nm. The plasma from the control coronary sinus samples was used as the zero reference, and the presence of Evans blue was tested in the other coronary sinus and systemic samples.

Systemic Blood Flow Analysis by the Microsphere Technique

The general microsphere procedures described by Heyman et al. (103) and Domenech et al. (24) were followed. Fifteen micron (15 u) microspheres (New England Nuclear) were used to measure regional systemic blood flows normoxia and hypoxia. Microspheres were labeled with one of the following radionucleides: ^{57}Co, ^{46}Sc, or ^{113}Sn. One million of each type of microsphere was administered to ensure an adequate number of microspheres in each tissue sample (14).

Prior to injection, microsphere aggregations were dispersed by ultrasonication for two minutes and then by vortex stirring for one minute. Microspheres were introduced into the left atrium through a polyethylene catheter. Reference blood samples were withdrawn by a Is/matec 7624-02 micropump through two polyethylene catheters, inserted into the right femoral artery, and advanced into the descending aorta. The duplicate reference samples were used as internal checks. Each simultaneously drawn reference should have approximately the same radioactivity.

Blood flow was measured in the following tissues: 1) renal cortex; 2) renal medulla; 3) duodenum; 4) adrenal gland; 5) skin; 6) skeletal muscle (scapular region); 7) liver; 8) pancreas; 9) pons; 10) medulla oblongata; 11) cerebral frontal lobe; 12) cerebral parietal lobe; 13) cerebral occipital lobe; 14) cervical spinal cord. Each tissue sample weighed 3-6 g. Radioactivity levels of organ and blood samples were measured by a Packard Auto-Gamma three channel gamma counter and recorded by a Commadore 64 Computer interfaced to the gamma counter. Radioisotope separation and blood flow calculation were done on a Commadore 64 Computer by a program written by H. F. Downey, Ph.D.. In general, this program calculates blood flow according to the following equation (84):

$$BF = FR \times Mr/Rr,$$

where BF is tissue blood flow (ml/min); FR is the reference

blood sampling rate (ml/min); Mr is the radioactivity in a tissue sample (counts/min); Rr is the radioactivity in the reference blood sample. Blood flow per unit weight was obtained by dividing the calculated blood flow by the weight of the specific tissue sample.

Experimental Protocol

The experimental protocol was divided into five separate phases, each having a specific function vital to the success of the experiment.

In Phase I, the integrity of sympathetic and parasympathetic nervous systems' innervation of the myocardium and coronary arteries was physiologically and pharmacologically tested. Increases in cardiac inotropy and chronotropy, following left ventral ansa subclavia stimulation (30 sec, 10 V, 10 Hz), verified the intact function of the cardiosympathetic nerves (6,113). Vasoconstriction, noted as a decrease in coronary blood flow, following an intracoronary bolus injection of phenylephrine (20-40 ug), verified intact alpha one-adrenoceptor function. Bradycardia following right vagus nerve stimulation (30 sec, 10 V, 10 Hz) verified the intact function of the cardio-parasympathetic nerves (6,113).

In Phase II, muscarinic-receptors were blocked with atropine (0.5 mg/kg, iv) to prevent hypoxia-induced reflex bradycardia and coronary vasodilation (67). Adequacy of

muscarinic-receptor blockade was verified by the absence of bradycardia during right vagus stimulation. Beta-adrenoceptors were then blocked with propranolol (2 mg/kg, iv) to prevent hypoxia-induced reflex increases in chronotropy and inotropy. The adequacy of beta-adrenoceptor blockade was verified by the absence of increases in left ventricular dP/dt_{max} and heart rate during left ventral ansa subclavia stimulation and also by a bolus injection of isoproterenol (2 ug, ic). Chronotropic and inotropic responses to parasympathetic and sympathetic stimulation were not quantitated, but the absence of changes in these variables during pharmacological and physiological challenges were indicators of adequate receptor blockade.

In Phase III, physiological measurements before and during hypoxia were performed on each dog, which acted as its own control. The coronary artery reservoir was filled with 500-700 ml of normoxic blood while supplemental blood was infused into the dogs. Control measurements of LCC flow were made, and LCC arterial and venous sinus blood samples were taken. Hypoxia was induced by ventilating the dog with a 5% O_2-95% N_2 mixture for three minutes. Ventilation rates were maintained at twelve breaths/minute in order to prevent hyperventilation induced reflex coronary vasodilation (117). During hypoxia, coronary arterial, coronary sinus, and systemic arterial blood samples were taken at one, two, and three minutes. Hypoxic ventilation was stopped at three

minutes, and the coronary perfusion reservoir was refilled. Recovery of the dog's systemic blood pressure, gas tensions and pH were aided by supplemental oxygen and sodium bicarbonate.

In Phase IV, Phase II procedures were again performed in order to test the integrity of cardiac and coronary sympathetic innervation and pharmacological blockades.

In Phase V, when coronary vasoconstriction was observed during ventilation with hypoxic gas, Phases I-IV were repeated during alpha one-adrenoceptor blockade with prazosin (1.0 mg,ic). Adequacy of coronary alpha one-adrenoceptor blockade was always assessed immediately prior to and after induction of systemic hypoxia. If coronary vasoconstriction was observed during left ventral ansa subclavia stimulation or administration of intracoronary phenylephrine, then an additional bolus of prazosin (1.0 mg, ic) was administered.

In Condition 1 (no intracoronary alpha one-adrenoceptor blockade, n=13), Phases I, II, III, and IV were performed. In five dogs, regional blood flows were measured with microspheres during Phase III: 1) just prior to the onset of hypoxic ventilation; 2) three minutes after the induction of hypoxia; 3) after hypoxia when systemic blood gas and pH values approximately equaled pre-hypoxia control values.

In Condition 2 (intracoronary alpha one-adrenoceptor blockade, n=8), Phases I, II, III, IV and V were performed,

however, regional blood flows were not measured with microspheres.

Myocardial Oxygen Metabolism During Restricted Coronary Blood Flow

An approximate twenty per cent reduction in coronary blood flow was observed during systemic hypoxia. To determine whether comparable reductions in coronary blood flow would produce equivalent alterations in myocardial metabolism, a "simulated hypoxic flow reduction" was performed a total of eleven times in three dogs. All dogs were instrumented, as previously described, and subjected to Phases I though IV of the Experimental Protocol, except, in Phase III, LCC blood flow was restricted by twenty per cent instead of ventilating the dogs with hypoxic gas. LCC flow was slowly decreased by occluding the LCC perfusion line over a two minute period. During the second minute of flow restriction, coronary arterial and coronary venous sinus oxygen contents were measured.

Statistical Analyses

Data are reported as mean $\pm$ standard error of the mean (SEM), and differences are considered to be statistically significant if the probability of their occurring, due to chance, was less than five per cent ($p < 0.05$). Systemic arterial and coronary perfusate blood gas and pH values, cardiovascular hemodynamic variables values, and coronary

blood flow and myocardial oxygen metabolism values are reported separately for Condition 1 and Condition 2.

In minute by minute analysis, all hypoxia values in Condition 1 and Condition 2 were compared to respective pre-hypoxia control values by randomized block analysis of variance (ANOVA) and a Student-Newman-Kuels multiple range test. Minute by minute values during Condition 1 were compared to corresponding minute by minute values during Condition 2 by analysis of variance (ANOVA).

In maximal effect analysis, all hypoxia values in Condition 1 and 2 were compared to respective pre-hypoxia control values using a paired Student's t-test. Significant changes in systemic arterial and coronary perfusate blood gas values, cardiovascular hemodynamic values, and LCC blood flow and myocardial oxygen metabolism values during hypoxia between Condition 1 and 2 were identified by analysis of co-variance (ANCOVA).

Significant changes in regional blood flows during control, hypoxic, and recovery periods were identified by randomized block analysis of variance (ANOVA) and a Student-Newman-Keuls multiple range test.

All statistical analysis techniques utilized have been described by Zar (122), and were performed with Statistical Analysis System (SAS) (107,108) using an IBM NAS 8083 Dual Processor.

CHAPTER III

RESULTS

Determination of Possible Contamination of Coronary Venous Sinus Blood with Systemic Blood

The possible contamination of coronary sinus blood with right ventricular venous drainage or aortic blood from leakage around the LCC cannula was studied in three dogs over a two minute period using systemically administered Evans blue dye (4 mg/kg). Spectrophotometric analysis of coronary sinus and systemic venous blood demonstrated that Evans blue dye was present in systemic venous blood. However, it was not detectable in coronary sinus blood samples which were drawn simultaneously with the systemic samples.

Evans blue dye was spectrophotometrically detectable at a minimum concentration of 1 ug/ml of plasma. Each ml of plasma from systemic venous blood contained approximately 30 ug of Evans blue dye. Coronary venous sinus blood contained less than 1 ug/ml of Evans blue dye. This suggests that during systemic hypoxia, coronary sinus blood was not contaminated by blood from right ventricular venous drainage or leakage of aortic blood into the LCC circulation.

Analyses of Experimental Variables

Experimental variables were analyzed and reported in two manners: 1) minute by minute values versus pre-hypoxia control values and 2) the maximum response values versus pre-hypoxia control values.

In four of the thirteen Condition 1 experiments, coronary arterial and venous sinus blood samples were not drawn during the first and second minutes of hypoxia. Therefore, minute by minute analysis was performed on only nine Condition 1 experiments. A typical experimental recording is presented in Figure 3.

Minute by Minute Analyses

Effects of Systemic Hypoxia on Systemic Arterial Blood Gases and pH

Condition 1.--The effects of systemic hypoxia on systemic blood gases and pH in the absence of intracoronary alpha one-adrenoceptor blockade are presented in Table II. Ventilating these nine dogs with 5% O_2-95% N_2 mixture significantly decreased both systemic arterial oxygen content and PO_2 after only one minute of hypoxia. These variables decreased maximally by 58.3 $\pm$ 4.6 per cent and 72.8 $\pm$ 4.6 per cent respectively at the third minute. PCO_2 significantly increased 11.5, 8.4, and 10.3 per cent during minutes one, two and three, respectively. pH was not altered significantly from pre-hypoxia control values at any time during three minutes of hypoxia.

Figure 3. A representative tracing of cardiovascular variables in response to two minutes of systemic hypoxia, in the presence and absence of intracoronary prazosin. Note the change of scale for LCC blood flow during pre-hypoxia control and hypoxia, in the presence of intracoronary alpha one-adrenoceptor blockade. LCC = left common coronary artery.

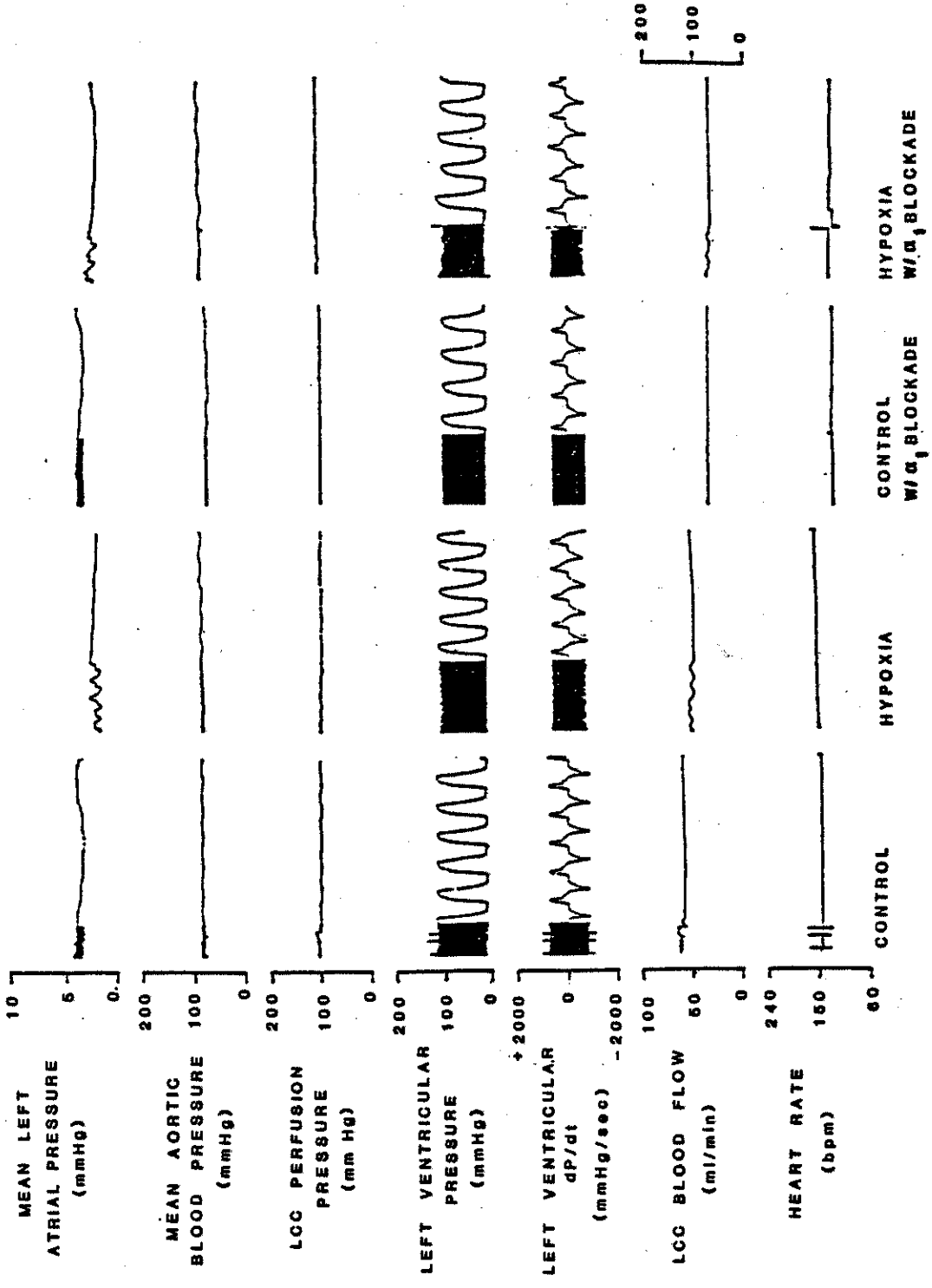

MEAN LEFT ATRIAL PRESSURE (mmHg)
10
5
0
MEAN AORTIC BLOOD PRESSURE (mmHg)
200
100
0
LCC PERFUSION PRESSURE (mm Hg)
200
100
0
LEFT VENTRICULAR PRESSURE (mmHg)
200
100
0
LEFT VENTRICULAR dP/dt (mmHg/sec)
+2000
0
-2000
LCC BLOOD FLOW (ml/min)
100
50
0
HEART RATE (bpm)
240
150
60
200
100
0
CONTROL
HYPOXIA
CONTROL
W/ α_1 BLOCKADE
HYPOXIA
W/ α_1 BLOCKADE

TABLE II

MINUTE BY MINUTE ANALYSIS OF SYSTEMIC ARTERIAL BLOOD GAS AND pH VALUES DURING SYSTEMIC HYPOXIA, IN THE PRESENCE OF SYSTEMIC MUSCARINIC-RECEPTOR AND BETA-ADRENOCEPTOR BLOCKADE

Variable	Duration of Hypoxia (minutes)			
	Control	1	2	3
N	9	7	7	9
O_2 content (ml O_2/dl blood)	17.8 ± 0.9	9.4 ± 1.2*	7.8 ± 1.0*	6.5 ± 0.7*
PO_2 (mmHg)	108.5 ± 15.1	32.5 ± 2.4*	28.2 ± 1.0*	26.5 ± 1.3*
PCO_2 (mmHg)	41.7 ± 2.0	46.5 ± 2.6*	45.2 ± 1.7*	46.0 ± 1.3*
pH	7.39 ± 0.01	7.37 ± 0.01	7.37 ± 0.01	7.37 ± 0.02

Values are means ± standard errors of the mean (SEM).

* Significantly different at $p < 0.05$ from respective control values (analysis of variance).

Condition 2.--The effects of systemic hypoxia on systemic arterial blood gases and pH in the presence of intracoronary alpha one-adrenoceptor blockade are presented in Table III. Ventilating these eight dogs with a 5% O_2-95% N_2 mixture significantly decreased both systemic arterial oxygen content and PO_2 by 44.2 $\pm$ 5.6 and 66.5 $\pm$ 6.2 per cent, respectively after only one minute of hypoxia. Both variables decreased maximally at the third minute by 59.6 $\pm$ 1.7 per cent and 71.3 $\pm$ 5.6, respectively. PCO_2 significantly increased 6.4, 8.1, and 8.6 per cent during minutes one, two and three, respectively. pH was not altered significantly at any time during the hypoxia.

Comparison of Condition 1 and Condition 2.--Student t-test indicated that there were no significant differences between Condition 1 and Condition 2 for systemic arterial oxygen content, PO_2, PCO_2, or pH during hypoxia.

Effects of Systemic Hypoxia on Cardiovascular Hemodynamic Variables

Condition 1.--The effects of systemic hypoxia on cardiovascular hemodynamic variables in the absence of alpha one-adrenoceptor blockade are presented in Table IV. Mean left atrial pressure, mean aortic pressure, left ventricular pressure, left ventricular positive dP/dt_{max}, and heart rate did not change significantly during any of the three minutes of hypoxia.

TABLE III

MINUTE BY MINUTE ANALYSIS OF SYSTEMIC ARTERIAL BLOOD GAS AND pH VALUES DURING SYSTEMIC HYPOXIA, IN THE PRESENCE OF SYSTEMIC MUSCARINIC-RECEPTOR AND BETA ADRRENOCEPTOR BLOCKADE, WITH INTRACORONARY ALPHA ONE-ADRENOCEPTOR BLOCKADE

Variable	Duration of Hypoxia (minutes)			
	Control	1	2	3
N	8	7	7	8
O_2 content (ml O_2/dl blood)	16.1 ± 0.6	8.3 ± 1.0*	7.6 ± 0.8*	6.0 ± 0.5*
PO_2 (mmHg)	102.7 ± 13.8	31.8 ± 1.9*	30.4 ± 2.4*	26.3 ± 1.2*
PCO_2 (mmHg)	40.9 ± 2.5	43.5 ± 1.5	43.8 ± 1.6	44.2 ± 1.3
pH	7.39 ± 0.03	7.37 ± 0.02	7.37 ± 0.02	7.37 ± 0.02

Values are means ± standard errors of the mean (SEM).

* Significantly different at $p < 0.05$ from respective control values (analysis of variance).

TABLE IV

MINUTE BY MINUTE ANALYSIS OF CARDIOVASCULAR HEMODYNAMIC VARIABLES DURING SYSTEMIC HYPOXIA, IN THE PRESENCE OF SYSTEMIC MUSCARINIC-RECEPTOR AND BETA-ADRENOCEPTOR BLOCKADE

Variable	Duration of Hypoxia (minutes)			
	Control	1	2	3
N	9	9	9	9
Mean Aortic Pressure (mmHg)	84 ± 3	85 ± 3	84 ± 3	85 ± 4
Left Ventricular Systolic Pressure (mmHg)	103 ± 6	103 ± 6	102 ± 5	103 ± 5
Mean Left Atrial Pressure (mmHg)	5 ± 1	5 ± 1	4 ± 1	4 ± 1
Heart Rate (beats/min)	130 ± 8	131 ± 8	132 ± 9	136 ± 9
Left Ventricular dP/dt (mmHg/sec)	1310 ± 100	1300 ± 95	1280 ± 70	1245 ± 65

Values are means ± standard errors of the mean (SEM).

Hypoxia values did not differ significantly from pre-hypoxia control values.

Condition 2.--The effects of systemic hypoxia on cardiovascular hemodynamic variables in the presence of alpha one-adrenoceptor blockade are presented in Table V. Mean left atrial pressure, mean aortic pressure, left ventricular pressure, left ventricular dP/dt_{max}, and heart rate did not change significantly during any of the three minutes of systemic hypoxia.

Comparison of Condition 1 and Condition 2.--Analysis with Student t-test indicated that there were no significant differences in the minute by minute values between Condition 1 and Condition 2. Overall, all hemodynamic variables, in the presence or absence of alpha one-adrenoceptor blockade, did not change significantly from pre-hypoxia control values during systemic hypoxia.

Effects of Systemic Hypoxia on LCC Blood Flow and Myocardial Oxygen Metabolism

Condition 1.--The effects of systemic hypoxia on LCC blood flow and myocardial oxygen metabolism responses in the absence of intracoronary alpha-one adrenoceptor blockade are presented in Table VI. Coronary blood flow significantly decreased 9.7 $\pm$ 3.4 per cent, 11.9 $\pm$ 2.0 per cent, 10.2 $\pm$ 2.4 per cent during the first, second, and third minutes of hypoxia, respectively. Decreases in oxygen delivery accompanied the decreases in coronary blood flow. Oxygen delivery significantly decreased 11.9 $\pm$ 1.0 per cent, 15.9 $\pm$ 2.7 per cent, and 14.2 $\pm$ 3.2 per cent during the first,

TABLE V

MINUTE BY MINUTE ANALYSIS OF CARDIOVASCULAR HEMODYNAMIC VARIABLES DURING SYSTEMIC HYPOXIA, IN THE PRESENCE OF SYSTEMIC MUSCARINIC-RECEPTOR AND BETA-ADRENOCEPTOR BLOCKADE, WITH INTRACORONARY ALPHA ONE-ADRENOCEPTOR BLOCKADE

Variable	Duration of Hypoxia (minutes)			
	Control	1	2	3
N	8	8	8	8
Mean Aortic Pressure (mmHg)	71 ± 5	70 ± 5	72 ± 5	71 ± 5
Left Ventricular Systolic Pressure (mmHg)	91 ± 7	92 ± 7	92 ± 7	93 ± 7
Mean Left Atrial Pressure (mmHg)	5 ± 1	5 ± 1	5 ± 1	5 ± 1
Heart Rate (beats/min)	121 ± 6	123 ± 6	122 ± 6	123 ± 7
Left Ventricluar dP/dt (mmHg/sec)	1140 ± 80	1150 ± 80	1175 ± 70	1165 ± 60

Values are means ± standard errors of the mean (SEM).

Hypoxia values did not differ significantly from pre-hypoxia control values.

TABLE VI

MINUTE BY MINUTE ANALYSIS OF LCC BLOOD FLOW AND LEFT VENTRICULAR MYOCARDIAL OXYGEN METABOLISM DURING SYSTEMIC HYPOXIA, IN THE PRESENCE OF SYSTEMIC MUSCARINIC-RECEPTOR AND BETA-ADRENOCEPTOR BLOCKADE

Variable	Duration of Hypoxia (minutes)			
	Control	1	2	3
N	9	9	9	9
LCC Perfusion Pressure (mmHg)	100 ± 1	100 ± 1	100 ± 1	99 ± 1
LCC Blood Flow (ml/min/100 g)	82.1 ± 6.7	74.4 ± 6.6*	72.5 ± 6.6*	74.4 ± 7.0*
Arterial-Venous Oxygen Difference (ml O_2/dl blood)	11.4 ± 0.9	11.8 ± 0.8	12.0 ± 0.9	12.0 ± 0.8
Oxygen Delivery (ml/min/100 g)	13.9 ± 1.2	12.5 ± 1.2*	11.9 ± 1.0*	12.1 ± 1.0*
Myocardial Oxygen Extraction (%)	66.4 ± 4.1	69.9 ± 4.4*	72.0 ± 4.6*	72.1 ± 4.1*
Myocardial Oxygen Consumption (ml O_2/min/100 g)	9.1 ± 0.9	8.6 ± 0.8	8.4 ± 0.7	8.6 ± 0.7

Values are means ± standard errors of the mean (SEM).

* Significantly different at $p < 0.05$ from respective control values (analysis of variance).

LCC = left common coronary artery.

second and third minutes of hypoxia, respectively. Myocardial oxygen extraction increased significantly 4.9 ± 2.0 per cent, 7.6 ± 2.3 per cent, and 8.1 ± 2.7 per cent during the first, second, and third minutes of hypoxia. Myocardial oxygen consumption did not change significantly during the three minutes of hypoxia.

Oxygen content, PO_2, PCO_2, and pH values of the coronary perfusate are presented in Table VII. These values were in the physiological range, and did not change significantly during hypoxia.

Condition 2.--The effects of systemic hypoxia on coronary blood flow and myocardial oxygen metabolism in the presence of alpha one-adrenoceptor blockade are presented in Table VIII. After intracoronary administration of prazosin, systemic hypoxia no longer produced significant decreases in LCC blood flow. During all three minutes of hypoxia, values for arterial-venous oxygen difference and oxygen delivery did not change significantly from pre-hypoxia control values. Myocardial oxygen consumption did not change significantly from pre-hypoxia control during the first minute of hypoxia. However, during the second and third minutes of hypoxia, myocardial oxygen consumption increased significantly 14.7 ± 0.8 per cent and 8.8 ± 0.7 per cent from pre-hypoxia values.

Oxygen content, PO_2, PCO_2, and pH values of the coronary perfusate are presented in Table IX. These values

TABLE VII

MINUTE BY MINUTE ANALYSIS OF LCC PERFUSATE BLOOD GAS AND pH VALUES DURING SYSTEMIC HYPOXIA, IN THE PRESENCE OF SYSTEMIC MUSCARINIC-RECEPTOR AND BETA-ADRENOCEPTOR BLOCKADE

Variable	Duration of Hypoxia (minutes)			
	Control	1	2	3
N	9	9	9	9
O_2 content (ml O_2/dl blood)	17.1 ± 0.8	16.1 ± 0.9	16.8 ± 1.0	16.2 ± 0.9
PO_2 (mmHg)	119.1 ± 10.3	117.6 ± 9.8	117.4 ± 8.0	109.7 ± 9.1
PCO_2 (mmHg)	42.4 ± 1.3	43.3 ± 1.1	42.3 ± 1.5	43.8 ± 0.9
pH	7.37 ± 0.01	7.36 ± 0.01	7.37 ± 0.01	7.36 ± 0.01

Values are means ± standard errors of the mean (SEM).

Hypoxia values did not differ significantly from pre-hypoxia control values.

LCC = left common coronary artery.

TABLE VIII

MINUTE BY MINUTE ANALYSIS OF LCC BLOOD FLOW AND LEFT VENTRICULAR MYOCARDIAL OXYGEN METABOLISM DURING SYSTEMIC HYPOXIA, IN THE PRESCENCE OF SYSTEMIC MUSCARINIC-RECEPTOR AND BETA-ADRENOCEPTOR BLOCKADE, WITH INTRACORONARY ALPHA ONE-ADRENOCEPTOR BLOCKADE

Variable	Duration of Hypoxia (minutes)			
	Control	1	2	3
N	8	8	8	8
LCC Perfusion Pressure (mmHg)	101 ± 1	101 ± 1	100 ± 1	100 ± 1
LCC Blood Flow (ml/min/100 g)	65.3 ± 4.8	68.0 ± 4.0	69.8 ± 4.3	66.4 ± 4.5
Arterial-Venous Oxygen Difference (ml O_2/dl blood)	10.7 ± 0.7	10.7 ± 0.5	11.0 ± 0.5	11.2 ± 0.6
Oxygen Delivery (ml O_2/min/100 g)	10.2 ± 0.8	10.4 ± 0.8	10.7 ± 0.8	10.2 ± 0.7
Myocardial Oxygen Extraction (%)	67.8 ± 0.4	69.8 ± 0.4	71.8 ± 0.4	72.9 ± 0.5*
Myocardial Oxygen Consumption (ml O_2/min/100 g)	6.8 ± 4.4	7.2 ± 5.5	7.8 ± 4.4*	7.4 ± 5.9*

Values are means ± standard errors of the mean (SEM).

* Significantly different at $p < 0.05$ from respective control values (analysis of variance).

LCC = left common coronary artery.

TABLE IX

MINUTE BY MINUTE ANALYSIS OF LCC PERFUSATE BLOOD GAS AND pH VALUES DURING SYSTEMIC HYPOXIA, IN THE PRESENCE OF SYSTEMIC MUSCARINIC RECEPTOR AND BETA ADRENOCEPTOR BLOCKADE, WITH INTRACORONARY ALPHA ONE-ADRENOCEPTOR BLOCKADE

Variable	Duration of Hypoxia (minutes)			
	Control	1	2	3
N	8	8	8	8
O_2 content (ml O_2/dl blood)	15.9 ± 1.0	15.5 ± 0.9	15.5 ± 0.9	15.6 ± 0.9
PO_2 (mmHg)	115.7 ± 11.3	111.4 ± 10.6	111.4 ± 11.0	112.4 ± 10.3
PCO_2 (mmHg)	41.1 ± 1.3	40.6 ± 1.5	41.3 ± 1.5	40.7 ± 1.4
pH	7.38 ± 0.02	7.38 ± 0.02	7.38 ± 0.02	7.38 ± 0.02

Values are means ± standard errors of the mean (SEM).

Hypoxia values did not differ significantly from pre-hypoxia control values.

LCC = left common coronary artery.

were within the physiological range and did not change significantly during hypoxia.

Comparison of Condition 1 and Condition 2.--Student t-test was utilized to compare the response to hypoxia for corresponding minute variables in the absence and presence of coronary alpha one-adrenoceptor blockade. Changes in myocardial blood flow, myocardial oxygen delivery, and myocardial oxygen consumption in Condition 1 differed significantly from corresponding changes in Condition 2 during all three minutes of hypoxia. Arterial-venous oxygen difference and myocardial oxygen extraction did not differ significantly between Condition 1 and Condition 2.

Maximal Effects Analyses

Effects of Systemic Hypoxia on Systemic Arterial Blood Gases and pH

Condition 1.--The effects of systemic hypoxia on blood gases and pH are presented in Table X. Ventilating thirteen dogs with a 5% O_2-95% N_2 gas mixture produced a 55.4 $\pm$ 6.2 per cent reduction in systemic arterial oxygen content and a 70.7 $\pm$ 6.6 per cent reduction in PO_2 from respective pre-hypoxia control values. Systemic PCO_2 increased significantly by 21.2 $\pm$ 2.3 per cent, yet the systemic pH did not decrease significantly.

Condition 2.--The effects of hypoxia on systemic arterial blood gases and pH in the presence of alpha one-adrenoceptor blockade are presented in Table X. Ventilating

TABLE X

SYSTEMIC ARTERIAL BLOOD GAS AND pH VALUES DURING SYSTEMIC HYPOXIA, IN THE PRESENCE OF SYSTEMIC MUSCARINIC-RECEPTOR AND BETA-ADRENOCEPTOR BLOCKADE, WITH AND WITHOUT INTRACORONARY ALPHA ONE-ADRENOCEPTOR BLOCKADE

Variable	Condition	Without Alpha-block N		With Alpha-block N	
O_2 Content (ml O_2/dl blood)	Control	13	17.1 ± 0.8	8	15.0 ± 0.9
	Hypoxia	13	7.3 ± 1.0*	8	6.6 ± 0.8*
PO_2 (mmHg)	Control	13	113.9 ± 10.8	8	101.8 ± 10.1
	Hypoxia	12	28.9 ± 1.1*	8	28.8 ± 1.3*
PCO_2 (mmHg)	Control	13	38.9 ± 1.7	8	41.4 ± 1.9
	Hypoxia	12	47.1 ± 1.5*	8	44.2 ± 1.2
pH	Control	13	7.37 ± 0.01	8	7.38 ± 0.02
	Hypoxia	12	7.33 ± 0.02	8	7.37 ± 0.02

Values are means ± standard error of the mean (SEM).

* Significantly different at $p < 0.05$ from respective control values (Student t-test).

eight dogs with a 5% O_2-95% N_2 gas mixture reduced systemic oxygen tension by 47.6 ± 4.1 per cent and systemic PO_2 by 68.6 ± 4.8 per cent from respective pre-hypoxia control values. Systemic PCO_2 and pH did not increase significantly during systemic hypoxia.

Comparison of Condition 1 and Condition 2.--Analysis by Student t-test revealed no significant differences in systemic arterial oxygen content, PO_2, PCO_2, or pH in response to hypoxia between Condition 1 and Condition 2.

Effects of Systemic Hypoxia on Cardiovascular Hemodynamic Variables

Condition 1.--The effects of systemic hypoxia on systemic cardiovascular hemodynamic variables are presented in Table XI. Mean left atrial pressure, mean aortic pressure, left ventricular pressure, left ventricular positive dP/dt_{max} and, heart rate values did not differ significantly from pre-hypoxia control values.

Condition 2.--The effects of systemic hypoxia on systemic cardiovascular hemodynamic variables are presented in Table XI. Mean left atrial pressure, mean aortic pressure, left ventricular pressure, left ventricular dP/dt_{max}, and heart rate values did not differ significantly from respective pre-hypoxia control values.

Comparison of Condition 1 and Condition 2.--Overall, there were no significant changes in hemodynamic variables during systemic hypoxia in either group. Analysis of

TABLE XI

CARDIOVASCULAR HEMODYNAMIC VARIABLES DURING SYSTEMIC HYPOXIA, IN THE PRESENCE OF SYSTEMIC MUSCARINIC-RECEPTOR AND BETA-ADRENOCEPTOR BLOCKADE, WITH AND WITHOUT INTRACORONARY ALPHA ONE-ADRENOCEPTOR BLOCKADE

Variable	Condition	Without Alpha-block N=13	With Alpha-block N=8
Mean Aortic Pressure (mmHg)	Control	81 ± 3	71 ± 5
	Hypoxia	88 ± 3	71 ± 5
Left Ventricular Systolic Pressure (mmHg)	Control	95 ± 6	91 ± 7
	Hypoxia	100 ± 1	93 ± 7
Mean Left Atrial Pressure (mmHg)	Control	4 ± 1	5 ± 1
	Hypoxia	4 ± 1	5 ± 1
Heart Rate (beats/min)	Control	134 ± 8	121 ± 6
	Hypoxia	134 ± 9	121 ± 6
Left Ventricular dP/dt (mmHg/sec)	Control	1270 ± 100	1140 ± 75
	Hypoxia	1285 ± 90	1175 ± 65

Values are means ± standard error the mean (SEM).

Hypoxia values did not differ significantly from pre-hypoxia control values.

co-variance demonstrated that there were no significant differences in hemodynamic variables in response to hypoxia between Condition 1 and Condition 2.

Effects of Systemic Hypoxia on LCC Blood Flow and Myocardial Oxygen Metabolism

Condition 1.--The effects of systemic hypoxia on coronary blood flow and myocardial oxygen metabolism in the absence of intracoronary alpha one-adrenoceptor are presented in Table XII. LCC blood flow maximally decreased from 90 ml/min/100 g to 73 ml/min/100 g. This significant 19.4 $\pm$ 2.6 per cent reduction in blood flow produced a significant 23.6 $\pm$ 2.3 per cent decrease in myocardial oxygen delivery. Despite a 9.7 $\pm$ 2.9 per cent increase in myocardial oxygen extraction, myocardial oxygen consumption decreased significantly by 16.5 $\pm$ 2.6 per cent.

Oxygen content, PO_2, PCO_2, and pH values for the coronary perfusate are presented in Table XIII. These values were within the physiological range and did not change significantly during hypoxia.

Condition 2.--The effects of hypoxia on coronary blood flow and myocardial oxygen metabolism in the presence of intracoronary alpha one-adrenoceptor blockade are presented in Table XII. During systemic hypoxia, values for LCC blood flow, arterial-venous oxygen difference, oxygen delivery, and myocardial oxygen extraction did not change significantly from pre-hypoxia control values. However,

TABLE XII

LCC BLOOD FLOW AND LEFT VENTRICULAR MYOCARDIAL OXYGEN METABOLISM DURING SYSTEMIC HYPOXIA, IN THE PRESENCE OF SYSTEMIC MUSCARINIC-RECEPTOR AND BETA-ADRENOCEPTOR BLOCKADE, WITH AND WITHOUT INTRACORONARY ALPHA ONE-ADRENOCEPTOR BLOCKADE

Variable	Condition	Without Alpha-block N=13	With Alpha-block N=8
LCC Perfusion Pressure (mmHg)	Control	100 ± 1	101 ± 1
	Hypoxia	100 ± 1	102 ± 2
LCC Blood Flow (ml/min/100 g)	Control	90.1 ± 6.3	65.3 ± 6.6
	Hypoxia	73.2 ± 5.6*	70.2 ± 7.0**
Coronary Arterial-Venous Oxygen Difference (ml O_2/dl blood)	Control	10.7 ± 0.7	10.7 ± 0.7
	Hypoxia	11.1 ± 0.7	10.8 ± 0.5
Myocardial Oxygen Extraction (%)	Control	62.6 ± 3.4	67.8 ± 3.7
	Hypoxia	68.5 ± 3.7*	70.9 ± 4.0
Oxygen Delivery (ml O_2/min/100 g)	Control	15.2 ± 1.1	10.2 ± 0.8
	Hypoxia	11.5 ± 0.8*	10.7 ± 0.9**
Myocardial Oxygen Consumption (ml O_2/min/100 g)	Control	9.3 ± 0.7	6.8 ± 0.4
	Hypoxia	7.6 ± 0.5*	7.5 ± 0.5*,**

Values are means ± standard errors of the mean (SEM).

* Significantly different at $p < 0.05$ from respective control values (Student t-test).

** Significantly different at $p < 0.05$ from response to hypoxia without alpha one-adrenoceptor blockade (analysis of co-variance).

LCC = left common coronary artery.

TABLE XIII

LCC PERFUSATE BLOOD GAS AND pH VALUES DURING SYSTEMIC HYPOXIA, IN THE PRESENCE OF SYSTEMIC MUSCARINIC-RECEPTOR AND BETA-ADRENOCEPTOR BLOCKADE, WITH AND WITHOUT INTRACORONARY ALPHA ONE-ADRENOCEPTOR BLOCKADE

Variable	Condition	Without Alpha-block N=13	With Alpha-block N=8
O_2 Content ($ml\ O_2/dl$ blood)	Control	17.1 ± 3.0	16.0 ± 2.9
	Hypoxia	16.3 ± 3.1	15.5 ± 2.6
PO_2 (mmHg)	Control	119.1 ± 11.3	115.7 ± 11.3
	Hypoxia	111.3 ± 10.8	119.4 ± 9.3
PCO_2 (mmHg)	Control	42.4 ± 1.3	41.1 ± 1.3
	Hypoxia	42.8 ± 1.1	41.1 ± 1.3
pH	Control	7.37 ± 0.01	7.36 ± 0.01
	Hypoxia	7.38 ± 0.02	7.38 ± 0.02

Values are means ± standard errors of the mean (SEM).

Hypoxia values did not differ significantly from pre-hypoxia control values.

myocardial oxygen consumption increased significantly by 9.4 ± 3.3 per cent.

Oxygen content, PO_2, PCO_2, and pH values for the coronary perfusate are presented in Table XIII. These values did not change significantly from pre-hypoxia control values.

Comparison of Condition 1 and Condition 2.--Coronary arterial-venous oxygen differences did not differ from pre-hypoxia control values in either group. Analysis of co-variance indicated significant differences in LCC blood flow, oxygen delivery, and myocardial oxygen consumption in response to hypoxia between Condition 1 and Condition 2. Also, analysis of co-variance revealed no significant differences in coronary perfusate oxygen content, PO_2, PCO_2, and pH values in response to hypoxia between Condition 1 and Condition 2.

Myocardial Oxygen Metabolism During Restricted Coronary Perfusion

The effects of restricted coronary perfusion on myocardial oxygen consumption are presented in Table XIV. Systemic hypoxia produced a 19.4 ± 2.6 per cent decrease in LCC blood flow accompanied by left ventricular myocardial oxygen metabolism alterations. Hypoxia-induced alterations in myocardial oxygen metabolism were compared to alterations produced during manually-reduced LCC blood flow. During restricted LCC blood flow, myocardial oxygen metabolism was

TABLE XIV

COMPARISON OF PER CENT CHANGES IN LEFT VENTRICULAR MYOCARDIAL OXYGEN METABOLISM BETWEEN HYPOXIA-INDUCED CORONARY VASOCONSTRICTION AND MANUALLY RESTRICTED CORONARY BLOOD FLOW

Variable	Per cent Changes from Control	
	Hypoxia N=13	Manual N=11*
LCC Blood Flow (ml/min/g)	-19.4 ± 2.6	-19.8 ± 0.2
Coronary Arterial-Venous Oxygen Difference (ml O_2/dl blood)	4.3 ± 3.2	9.7 ± 2.0
Myocardial Oxygen Extraction (%)	9.7 ± 2.9	11.0 ± 1.7
Oxygen Delivery (ml O_2/min/100 g)	-23.6 ± 2.3	-20.8 ± 0.7
Myocardial Oxygen Consumption (ml O_2/min/100g)	-16.5 ± 2.6	-12.0 ± 1.7

Values are means ± standard error of the mean (SEM).

* Performed in three dogs.

LCC = left common coronary artery.

measured eleven times in three muscarinic-receptor and beta-adrenoceptor blocked dogs. Reducing LCC blood flow by 19.8 ± 0.2 per cent produced a 20.8 ± 0.7 per cent decrease in oxygen delivery and a 12.0 ± 1.7 per cent decrease in myocardial oxygen consumption despite an 11.0 ± 1.7 per cent increase in myocardial oxygen extraction.

Systemic Regional Blood Flows During Systemic Hypoxia

The effects of systemic hypoxia on regional blood flow, studied in five dogs, are given in Table XV. Parametric analysis of variance (ANOVA) and a Student-Newman-Keuls multiple range test demonstrated that during hypoxia, blood flow values increased significantly from pre-hypoxia control values in the cerebellum, cerebral frontal lobe, cerebral parietal lobe, cerebral occipital lobe, medulla oblongata, pons, and spinal cord. During hypoxia, blood flow values decreased significantly from pre-hypoxia control values in the kidney cortex, kidney medulla, pancreas, and spleen. Recovery blood flow values did not differ significantly from pre-hypoxia control blood flow values.

TABLE XV

REGIONAL BLOOD FLOWS IN SPECIFIC ORGANS DURING CONTROL, SYSTEMIC HYPOXIA, AND RECOVERY

Organ	Flow (ml/min/g)		
	Control N=5	Hypoxia N=5	Recovery N=5
	% Change		
Kidney cortex	2.04 ± 0.40	0.23 ± 0.06*	2.29 ± 0.60
	-87.4 ± 4.6		
Kidney medulla	0.39 ± 0.10	0.03 ± 0.01*	0.30 ± 0.10
	-89.5 ± 5.6		
Spleen	0.24 ± 0.10	0.03 ± 0.01*	0.12 ± 0.10
	-80.1 ± 10.7		
Pancreas	0.13 ± 0.02	0.05 ± 0.01*	0.08 ± 0.02
	-50.9 ± 16.2		

TABLE XV--Continued

Liver	0.38 ± 0.20	0.09 ± 0.03	0.27 ± 0.10
		-28.3 ± 31.9	
Duodenun	0.31 ± 0.05	0.20 ± 0.03*	0.24 ± 0.04
		-34.8 ± 8.4	
Adrenal gland	2.08 ± 0.40	1.28 ± 0.30*	1.93 ± 0.40
		-33.3 ± 14.2	
Frontal lobe	0.31 ± 0.03	0.92 ± 0.20*	0.31 ± 0.02
		194.1 ± 25.2	
Parietal lobe	0.31 ± 0.03	0.91 ± 0.10*	0.30 ± 0.03
		192.0 ± 20.1	
Occipital lobe	0.35 ± 0.04	0.99 ± 0.20*	0.33 ± 0.03
		181.9 ± 19.3	
Pons	0.34 ± 0.10	1.01 ± 0.10*	0.33 ± 0.03
		211.2 ± 27.3	

TABLE XV--Continued

Cerebellum	0.31 ± 0.04	0.97 ± 0.20*	0.30 ± 0.03
		201.1 ± 19.1	
Medulla	0.25 ± 0.03	0.80 ± 0.10*	0.25 ± 0.05
		217.9 ± 27.2	
Spinal cord	0.09 ± 0.01	0.42 ± 0.10*	0.10 ± 0.01
		377.7 ± 85.3	
Skeletal muscle	0.03 ± 0.01	0.02 ± 0.01	0.03 ± 0.01
		-43.4 ± 25.6	
Skin	0.03 ± 0.01	0.04 ± 0.04	0.04 ± 0.03
		12.4 ± 46.1	

All values are means ± standard errors of the mean (SEM).

* Significantly different at $p < 0.05$ from respective control values (analysis of variance).

CHAPTER IV

DISCUSSION

The role of the autonomic nervous system during systemic hypoxia has been extensively examined in a variety of models including human (58,54,83). Acute systemic hypoxia effects the cardiovascular system by increasing systemic blood pressure, heart rate, myocardial contractility, and cardiac output (3,23,33,50,66,71,76,122), with all of these sympathetic-mediated reflexes originating from various chemosensitive areas.

Systemic hypoxia-induced increases in heart rate and ventricular positive dP/dt have been attributed to stimulation of myocardial beta-adrenoceptors (23,33), while increases in systemic blood pressure have been attributed to stimulation of peripheral vascular alpha-adrenoceptors (23).

The increase in cardiac function and arterial blood pressure results in a marked increase in coronary blood flow during systemic hypoxia. Erickson and Stone (33) observed the effects of coronary blood flow during systemic hypoxia in the conscious dog. When these dogs were subjected to a hypoxic atmosphere, heart rate increased by 25 per cent, dP/dt increased 39 per cent, and coronary blood flow increased by 163 per cent. When the same dogs were

subjected to a hypoxic atmosphere during beta-adrenoceptor blockade, heart rate increased 13 per cent, dP/dt increased 24 per cent, and coronary blood flow increased 120 per cent. When heart rates were kept constant by pacing, coronary blood flows during hypoxia and beta-adrenoceptor blockade did not differ significantly from coronary blood flows during hypoxia without beta-adrenoceptor blockade. Their results suggest that reduced increases in coronary blood flow during hypoxia in the presence of beta-adrenoceptor blockade result from an attenuated cardiac metabolic vasodilation normally produced by increases in heart rate and myocardial contractility. They did not examine the role of alpha-adrenoceptor regulation of coronary blood flow during systemic hypoxia.

The role of alpha-adrenoceptor mediated regulation of coronary blood flow in the intact dog during systemic hypoxia was later examined by Doherty and Liang (23). They found that alpha-adrenoceptor blockade, with or without beta-adrenoceptor blockade, did not enhance coronary blood flow during systemic hypoxia. It is likely that hypoxia-induced coronary vasoconstriction was masked by vasodilation resulting from direct hypoxia-induced relaxation of coronary vessels (42,55), and carotid chemoreceptor reflexes (27,67).

Active hyperemia during systemic hypoxia also results from increased sympathetic cardiac stimulation (23,50) and increased ventricular afterload (10) due to increased

peripheral resistance. However, under normoxic conditions alpha-adrenoceptors modulate coronary vasomotor tone, which can compete with metabolic regulation of coronary blood flow (88). Since systemic hypoxia increases cardiac sympathetic stimulation, we hypothesized that coronary blood flow could be reduced via an alpha-adrenoceptor mediated increase in coronary vasomotor tone if hemodynamic variables were held constant and the coronary arteries were perfused with normoxic blood.

The present experiments examined the role of an alpha-adrenoceptor mediated coronary constrictor reflex in the open-chest anesthetized dog during acute, severe systemic hypoxia. This model permitted: 1) Normoxic perfusion of the left common coronary artery (LCC) during hypoxic perfusion of the remaining cardiovascular system; 2) Control of systemic hemodynamic variables; and 3) Selective blockade of coronary alpha one-adrenoceptors. Results of these studies indicate that acute systemic hypoxia produces a reflex coronary vasoconstriction that is modulated by alpha one-adrenoceptors.

Systemic Arterial Blood Gas and pH Values

Ventilating dogs with a 5% O_2-95% N_2 mixture caused a significant, abrupt reduction in systemic arterial oxygen content and PO_2. Arterial PCO_2 increased significantly to approximately 47 mmHg in both maximum response analysis and

minute by minute response analysis. However, these variables were still within the physiological range. Also, arterial pH did not decrease significantly despite the increase in PCO_2. Thus, the observed systemic and coronary arterial reflex responses most likely resulted from hypoxic and not from hypercapnic or acidic stimulation of the various peripheral or central chemoreceptors or the central nervous system (22,26,27).

Systemic Hemodynamic Variables

Coronary vascular resistance is affected by extravascular compression of the coronary vasculature (8,36) which varies with heart rate, myocardial contractility, and systolic pressure. In the intact animal, systemic hypoxia alters these variables, which can alter coronary resistance and coronary blood flow (36). Although the left coronary circulation was perfused with normoxic blood in this experimental preparation, it was necessary to block systemic hypoxia-induced reflexes that would alter heart rate and myocardial contractility. Thus, muscarinic-receptor and beta-adrenoceptor blockade was successfully utilized to prevent significant neurally mediated changes in heart rate and myocardial contractility during systemic hypoxia in both Conditions 1 and 2.

During Condition 1, aortic and left ventricular systolic pressures, reflecting left ventricular afterload,

did not increase significantly during systemic hypoxia, although some individual animals had notable increases. These increases in aortic and left ventricular pressure occurred in the initial five experiments in which microspheres were used to measure regional systemic blood flows. In order to prevent the flow of microspheres into the reservoir used to maintain aortic pressure constant, the line to the reservoir was clamped for one minute. During this period aortic and left ventricular pressures increased. When these five experiments are omitted, mean aortic and left ventricular pressures during systemic hypoxia, in the absence of alpha one-adrenoceptor blockade, increased by less than three per cent. In any case, coronary vasoconstriction occurred even in the animals with elevated left ventricular afterload, a condition which would normally increase myocardial oxygen demand and thus, produce metabolic coronary vasodilation.

During Condition 2, microspheres were not used to measure systemic blood flows during systemic hypoxia, so the line to the aortic reservoir was never clamped during systemic hypoxia. As a result, neither aortic nor left ventricular pressures could increase during systemic hypoxia.

Prazosin, a selective alpha one-adrenoceptor antagonist, can prevent catecholamine induced vasoconstriction. After prazosin was administered, coronary

vasoconstriction did not occur during systemic hypoxia. Vasoconstriction occurred in the eight experiments in which aortic and left ventricular pressures did not increase, and was subsequently eliminated by intracoronary administered prazosin. Therefore, it is unlikely that increased extravascular compression produced the reduction in LCC blood flow during systemic hypoxia.

Coronary Blood Flow and Myocardial Oxygen Metabolism

The lack of significant changes in oxygen content, PO_2, PCO_2, and pH of the arterial perfusate suggests that observed decreases in coronary blood flow were not produced by alteration in these variables (16). Coronary perfusion pressure remained constant during systemic hypoxia, indicating that changes in this variable also did not contribute to the reduction in LCC flow. This was important since changes in LCC perfusion pressure could have produce changes in LCC blood flow which might have been incorrectly interpreted as vasodilation or vasoconstriction.

During Condition 1, LCC blood flow decreased by a maximum of 19.4 $\pm$ 2.6 per cent, while coronary arterial oxygen extraction increased by 9.7 $\pm$ 2.9 per cent during systemic hypoxia. As flow decreased, oxygen extraction increased, apparently in attempt to maintain normal myocardial oxygen consumption. Despite increases in oxygen extraction, myocardial oxygen consumption significantly

decreased. The increased coronary arterial-venous oxygen difference supports the concept that decreased myocardial oxygen consumption was the result of rather than the cause of decreased coronary blood flow.

The dependence of myocardial oxygen consumption on blood flow was further exemplified when LCC blood flow was manually restricted. Alterations in myocardial oxygen consumption during hypoxia-induced vasoconstriction were compared to those produced by manually restricting LCC blood flow to determine whether or not independent reductions in LCC blood flow would produce changes in myocardial oxygen consumption similar to those observed during hypoxia. LCC blood flow was restricted by manually constricting the coronary perfusion tubing which connected the LCC cannula to the coronary perfusion reservoir over a two minute period. Manually reducing LCC blood flow by approximately twenty per cent reduced myocardial oxygen consumption 12.0 $\pm$ 1.7 per cent, while myocardial oxygen extraction increased 11.0 $\pm$ 1.7 per cent. During hypoxia, an equivalent reduction in LCC blood flow reduced myocardial oxygen consumption 16.5 $\pm$ 2.6 per cent, while myocardial oxygen extraction increased 9.7 $\pm$ 2.9 per cent. Manually-produced decreases in blood flow were similar to hypoxia-induced decreases in blood flow and this suggests that changes in myocardial oxygen metabolism observed during hypoxia were coronary blood flow dependent.

Minute by minute analysis of coronary blood flow during Condition 1, revealed an interesting trend. Coronary blood flow during all three minutes of systemic hypoxia was reduced significantly from control values. However, the maximal flow reduction was not maintained during the entire duration of systemic hypoxia. Flow was reduced at one minute, reached a maximal reduction at two minutes, and showed a slightly less reduction by the third minute. Similar transient decreases and increases in coronary blood flow were noted by Ek and Ablad (31) when they simultaneous stimulated right and left stellate ganglia for two minutes in the presence of beta-adrenoceptor blockade. The transient decrease and increase in coronary blood flow during systemic hypoxia can be explained by: 1) a local metabolic regulation (8,36) or 2) inhibition of norepinephrine release involving pre-junctional and ganglionic receptors (4,111,116).

The most likely explanation the waning of the coronary vasoconstriction during systemic hypoxia is metabolic counterbalance. When flow initially decreased, myocardial oxygen requirements were apparently unfulfilled. At the same time active hyperemia was prevented by beta-adrenoceptor blockade. Under these conditions, myocardial release of adenosine, potassium, or other vasodilatory substances would be expected (8,36) and would have attenuated the systemic hypoxia-induced, reflex-mediated

vasoconstriction.

Pre-junctional inhibition of norepinephrine release could have also accounted for the increasing trend in coronary flow during the third minute of systemic hypoxia. Activation of pre-junctional alpha two-adrenoceptors has been shown to inhibit norepinephrine release from adrenergic nerve terminals in anesthetized and conscious dogs (4,63,87,111,116). If pre-junctional alpha two-adrenoceptors could be selectively blocked, then the increase in coronary blood flow observed during the third minute could possibly be eliminated.

Stimulation of pre-junctional muscarinic-receptors has been demonstrated to inhibit norepinephrine release from adrenergic nerve terminals (4,111,116). Efferent parasympathetic regulation of sympathetic neural discharge must also be disregarded as a cause for the increasing trend in coronary flow during the third minute of systemic hypoxia in the current investigation, since muscarinic-receptors were blocked with systemically administered atropine. Thus, parasympathetic inhibition of norepinephrine release was prevented.

Nature of the Hypoxia-Induced Coronary Vasoconstriction

The nature of sympathetic-induced coronary vasoconstriction has been a debated issue. In this study, intracoronary alpha one-adrenoceptor blockade alleviated the

systemic hypoxia-induced coronary vasoconstriction. This demonstrated a role the for alpha one-adrenoceptors in sympathetic reflex mediated coronary vasoconstriction. Previous works by Heusch and Deusen (60) and Saeed et al. (105) disagree with the concept of neurally mediated alpha one-adrenoceptor vasoconstriction, and suggests that neural coronary vasoconstriction is predominantly mediated by alpha two-adrenoceptors. Their results showed that alpha one-adrenoceptor blockade attenuated but did not prevent sympathetic-induced vasoconstriction in response to cardiac nerve stimulation. In other studies, Heusch et al. (61,62) suggested that alpha one-adrenoceptors mediate constriction in larger epicardial coronary arteries, whereas alpha two-adrenoceptors are predominantly responsible for constriction in smaller coronary arteries and arterioles.

Intracoronary prazosin eliminated the systemic hypoxia-induced LCC vasoconstriction as well as the LCC vasoconstriction produced by left ventral ansa stimulation. This demonstrated the vasoconstriction was alpha one-adrenoceptor mediated. However, one must also consider the possibility that prazosin produced a direct coronary vasodilation which masked the vasoconstriction response elicited during systemic hypoxia.

Prazosin was initially expected to produce vasodilation by inhibiting vascular smooth muscle phosphodiesterase (59). However, Gwirtz et al. (42) and Heyndrickx et al. (63) have

demonstrated that prazosin does not cause dilation independent of its action an alpha one-adrenoceptor antagonist. Even if prazosin caused direct coronary vasodilation in this preparation, it is unlikely that sympathetically induced coronary vasoconstriction could be masked since Johanssen et al. (68) observed such vasoconstriction in left circumflex arteries which were previously dilated by adenosine.

Even though alpha two-adrenoceptors antagonists were not used in this experiment, our results indicate the insignificance of alpha two-adrenoceptors in systemic hypoxia-induced reflex coronary vasoconstriction. Alpha two-adrenoceptors may have a greater role in regulating coronary tone and reactivity in response to changing concentration of circulating catecholamines, especially epinephrine, which is a slightly more potent alpha two-agonist than alpha one-agonist (64,78,87). Indeed, Holtz et al. (65) demonstrated the importance of alpha two-adrenoceptors, and the insignificance of alpha one-adrenoceptors, in causing coronary vasoconstriction during infusion of norepinephrine. Indirectly, these previous studies support the findings of the present study. Alpha two-adrenoceptors are predominately located in the media-intima border of arteries while alpha one-adrenoceptors are found in the media-adventitia border of arteries (78). The location of alpha two-adrenoceptors may make them more

susceptible to blood-borne catecholamine stimulation, while "protecting" them from neurally released norepinephrine. Likewise, the location of alpha one-adrenoceptors shields them from humoral stimulation. Experimenters have demonstrated that circulating catecholamine concentrations increase during systemic hypoxia (7,114). In the present study during systemic hypoxia, the LCC was perfused with normoxic blood from a reservoir which was not contaminated with systemic hypoxic blood. Even though catecholamine concentrations in the LCC perfusate were not measured, it is safe to say that these concentrations did not change during systemic hypoxia.

The dominance of an alpha one-adrenoceptor mediated coronary vasoconstrictor influence has been demonstrated during other physiological perturbations: exercise (44,96) and coronary hypoperfusion (70,81). Responses observed in exercising dogs are more applicable to the present experiment. Exercise evokes cardiovascular responses which are similar to those noted during systemic hypoxia. Both exercise and hypoxia increases heart rate, cardiac output, cardiac contractility, and systemic blood pressure. The importance of cardio-sympathetic nerve discharge producing increases in heart rate and cardiac contractility has been demonstrated in both exercise (43) and hypoxia (71,93,121).

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Origins of the Reflex Coronary Vasoconstriction

The present studies clearly demonstrate the existence of a sympathetically mediated coronary vasoconstriction during systemic hypoxia. However, the origin of this reflex is yet to be defined. Hypoxic stimulation of carotid and aortic chemoreceptors and the central nervous system produces similar and redundant peripheral vascular responses; however, cardiac responses differ depending upon which hypoxia-sensitive area is stimulated.

It has been well demonstrated that carotid chemoreceptor stimulation with hypoxic blood, or other

noxious substances, produces parasympathetic-mediated bradycardia, negative cardiac inotropy, (15,26,49,97,112), and coronary vasodilation (48,67). However, two other studies have reported coronary vasoconstriction during carotid chemoreceptor stimulation.

Hashimoto et al. (52) demonstrated the presence of both coronary vasodilation and vasoconstriction during carotid body stimulation with acetylcholine, nicotine, lobeline, or 5-hydroxytryptamine. When the dogs were given atropine, vasodilation was eliminated, and coronary vasoconstriction was unmasked. This constriction reduced coronary flow by approximately seven per cent. The receptor mechanism responsible for the vasoconstriction was not determined. It is difficult to explain their results, since chemical stimulation was not specifically limited to the carotid chemoreceptors. The carotid sinuses were not isolated from blood vessels distal to the sinuses. The chemicals used to stimulate the carotid chemoreceptors were able to travel into the cerebral circulation where, they could stimulate cerebral chemoreceptors or the medullary cardio-accelerator center (79). Hashimoto investigated the possibility that the coronary vasoconstrictor reflex originated from cerebral areas by injecting the previously mentioned chemicals directly into the cerebral circulation. However, once in cerebral circulation, these chemicals did not produce a reflex coronary vasoconstriction.

Murray et al. (91) reported a late onset coronary vasoconstriction in artifically ventilated, conscious dogs during carotid chemoreceptor stimulation with nicotine. The vasoconstriction, mediated by alpha-adrenoceptors, was shown to originate from both circulating and neurally released catecholamines. It is unlikely that the response noted by Murray et al. (91) had the same origin as that observed in the present investigation. Circulating catecholamines, well as other vasoactive substances, were unable to enter the LCC circulation in our preparation, so the subsequent LCC vasoconstriction could only have had neural origins. In the study by Murray et al. (91), the neurally produced vasoconstriction could possibly have resulted from cerebral chemoreceptor stimulation or nicotine-induced ganglionic stimulation, and not specifically carotid body stimulation. Nicotine was introduced into the carotid sinus, and immediately induced a coronary vasodilatory reflex. Since the carotid sinus was not isolated from other blood vessels, blood containing nicotine could then travel into the cerebral circulation and stimulate cerebral chemoreceptors or the medullary cardio-accelerator center. Stimulation of cerebral chemoreceptors with nicotine has been shown to elicit sympathetic-induced pressor reflexes, while stimulation of the medullary cardio-excitatory center increases sympathetic neural discharge to the heart (79). As the nicotine continued to circulate through the body, it

also could have stimulated sympathetic ganglia, further increasing sympathetic drive to the heart, and thus producing coronary vasoconstriction.

The majority of previous experiments overwhelmingly demonstrate that carotid chemoreceptor stimulation with hypoxic blood, or other noxious substance, produces a pronounced bradycardia, coronary vasodilation, and systemic vasoconstriction. Carotid chemoreceptor-reflex-induced coronary vasoconstriction has only been demonstrated during chemical but not hypoxic stimulation of carotid chemoreceptors. It is our belief that the sympathetic reflex induced coronary vasoconstriction during systemic hypoxia did not result from carotid body stimulation.

Any hypoxia-induced reflex which increases sympathetic discharge to the heart could be involved in coronary vasoconstriction. Since stimulation of carotid chemoreceptors probably does not produce reflexes that increase sympathetic discharge to the heart, increases in sympathetic stimulation to the heart during systemic hypoxia must originate from other sources. Stimulation of aortic chemoreceptors with hypoxic blood and/or the cerebral hypoxic response could have triggered the coronary vasoconstrictor reflex observed in the present experiment.

Aortic chemoreceptors stimulation has been shown to produce reflex mediated increases in heart rate, ventricular pressure, and dP/dt_{max} (72,112). If these sympathetically

mediated increases in myocardial function were suppressed, then any accompanying sympathetic discharge to the coronary vessels would no longer be masked by metabolic vasodilation.

Such experiments were performed by Hackett et al. (48), but when they stimulated aortic chemoreceptors with nicotine or cyanide, the left circumflex coronary circulation dilated. Coronary vasodilation was eliminated by atropine and bilateral vagotomy indicating that this was a parasympathetic reflex. These results, which are inconsistent with the anticipated outcome, might be traced back to inadequacies in the experimental design. Inadequate localization of the stimuli to aortic chemoreceptors could have allowed for stimulation of the adrenal glands, parasympathetic and sympathetic ganglia, cardiac chemoreceptors and carotid chemoreceptors. Inadequate control of cardiovascular hemodynamics could also have produced erroneous results. Arterial pressure and left ventricular dP/dt were allowed increase by twenty-three per cent and fourteen per cent, respectively. Increases in these variables are associated with increases in myocardial oxygen demand and coronary active hyperemia, which produces coronary vasodilation and increased coronary blood flow (10). In the present studies, cardiovascular hemodynamics (aortic pressure, left ventricular pressure and dP/dt) were controlled.

The CNS hypoxic response must also be considered as a

possible mechanism producing the coronary vasoconstriction. Previous experiments have demonstrated that CNS hypoxia provokes reflexes which promote positive chronotropic and inotropic effects in normoxically perfused hearts (1,2,22,26,28). This evidence strongly suggests that CNS reflexes may have the predominant role in increasing sympathetic cardiac tone during systemic hypoxia (1,2,22,26,28,46,106). Experiments have shown that ventilating conscious sino-aortic-denervated dogs with a hypoxic gas mixture produced cardiac responses which differed very little from those of intact dogs (77,98). Similar results were also observed in sino-aortic denervated cats that were exposed to a hypoxic gas mixtures (1,2). However, the reflex effects of cerebral systemic hypoxia on coronary blood flow have not been previously studied. Even though the CNS was not selectively perfused with hypoxic blood in the present experiment, we suggest that the observed reflex alpha-adrenergic coronary vasoconstriction originated primarily from CNS stimulation, and secondarily from aortic chemoreceptor stimulation.

Comparison of Hypoxia-Induced Coronary Vasoconstriction to Other Types of Sympathetic-Induced Coronary Vasoconstriction

Evidence strongly indicates that the resulting coronary vasoconstriction was mediated by alpha-adrenoceptors. However, how do these results compare to other adrenergic-

mediated forms of coronary vasoconstriction? An alpha-mediated coronary vasoconstriction has been observed in a number of circumstances such as during direct sympathetic nerve stimulation (31,95,102), baroreceptor reflex (32,37,93,100), exercise (43,44,45,96), and intracoronary catecholamine infusion (57,88). An alpha-adrenoceptor mediated vasoconstrictor tone has also been found to exist in hypoperfused coronary vessels (38,69,70,81). Results from selected previous experiments are presented in Table XVI.

One can readily note the similarities in per cent flow reduction observed in previous studies with those observed in the present study of systemic hypoxia. Decreases in left coronary blood flow observed during direct sympathetic nerve stimulation, exercise, and coronary hypoperfusion closely approximate hypoxic reflex vasoconstriction as well as each other. On the other hand, coronary vasoconstriction elicited by baroreceptor stimulation does not appear to be as powerful as that evoked by systemic hypoxia. Reductions in right coronary blood flow resulting from direct neural stimulation appear to be much greater than decreases in left coronary blood flow noted in other experiments (95) and the present experiment. Intracoronary infusion of catecholamines appears to produce greater increases in coronary vascular resistance than other forms of sympathetic adrenoceptor-mediated stimulation. Increased coronary

TABLE XVI

EFFECTS OF ADRENERGIC STIMULATION ON CORONARY VASCULAR TONE

Reference	Measured variable	Condition	Response
		Electrical Stimulation of Cardiac Sympathetic Nerves	
Ek and Ablad (29)	LCxBF	Beta-blockade	13% decrease
Feigl (33)	LCxBF	Beta-blockade	23% decrease
Naylor and Carson (93)	RCBF	Beta-blockade	40% decrease
Ross and Mulder (101)	LCxR	Beta-blockade	75% increase
	LADR		19% increase
Heusch et al. (61)	LCxR	During coronary stenosis	10% decrease
		Baroreceptors Reflex	
Powell and Feigl (99)	LCCBF		5% decrease
	LCCR		21% increase
Ely et al. (30)	RCBF		13% decrease
	RCR		14% increase
Murray and Vatner (91)	RCBF		4% decrease
	RCR		32% increase

TABEL XVI--Continued

Exercise		
Gwirtz et al. (42)	LCxBF	21% increase after alpha-blockade
Gwirtz and Stone (43)	LCxBF	21% increase after alpha-blockade
Intrcoronary Infusion of Catacholamines		
Morhman and Feigl (87)	LCCBF	30% increase after alpha-blockade
Intrinsic Sympathetic Tone During Coronary Hypoperfusion		
Jones et al. (68)	LCCBF	22% increase after alpha-blockade
Liang and Jones (80)	LCCBF	22% increase after aalpha-blockade

LCC - Left common coronary artery
LCx - Left circumflex coronary artery
LAD - Left anterior descending coronary artery
RC - Right coronary
BF - Blood flow
R - Coronary resistance

resistance during catecholamine infusion is likely due to stimulation both alpha one- and alpha two-adrenoceptors, as described earlier.

Changes in systemic blood flows were also measured during systemic hypoxia in this study, so these alterations in flow can be related to alterations in LCC blood flow. Trends in splanchnic and renal blood flow during systemic hypoxia observed in this experiment agree with those observed in hypoxic dog by Crystal et al. (20). Blood flow reductions in the pancreas, spleen, duodenum, renal cortex and renal medulla were very drastic, each decreased over thirty per cent during systemic hypoxia. These reductions in blood flow are considerably greater than those observed in the LCC. Unfortunately, the reductions in LCC and systemic blood flows can not be directly compared to each other since they were not measured under the same conditions. However, they do demonstrate the predominance of a sympathetic vasoconstrictor influence during systemic hypoxia.

Cerebral blood flows, in contrast to splanchnic and renal blood flows, increased during 5% O_2-95% N_2 breathing. Increases in cerebral flows during systemic hypoxia were most likely produced by local metabolic mechanisms rather than neural mechanisms (56,57). The absence of vasoconstriction in cerebral vessels agrees with evidence which suggests that cerebral blood flow is independent of

chemoreceptor-induced reflexes that normally increases sympathetic tone in other vascular bed (40,57).

Human Applications

Results of the present experiment clearly demonstrate the existence of a sympathetic coronary vasoconstrictor reflex during systemic hypoxia. The physiological applicability of this finding should be considered. Unlike other types of stimuli (baroreflex, exercise) which can elicit reflex coronary vasoconstriction, systemic hypoxia in intact animals elicits a variety of direct and indirect coronary responses which enhance and oppose one another. Sympathetic-induced coronary vasoconstriction has been shown to compete with metabolic vasodilatory responses in both dogs (88) and humans (89), and also with hypoxic vasodilatory responses in dogs (55). Even though the conditions established in this experiment could never be reproduced in a normal physiological situation, a sympathetic alteration in coronary tone could possibly affect coronary blood flow during systemic hypoxia. Sympathetic stimulation has been regarded as contributing factor in coronary vasospasm (85), producing myocardial ischemia, infarction, or ventricular fibrillation. Although coronary flow is not normally reduced during systemic hypoxia in the intact animal (3,50,80), increased alpha-adrenoceptor stimulation might trigger coronary spasms.

Humans with coronary artery disease could possibly experience the previously mentioned myocardial problems, since reflex coronary vasoconstriction has been shown to frequently occur in these individuals (12,73,90).

Beta-adrenoceptor blockers have proven to be an effective pharmaceutical aid in controlling various forms of cardiovascular disease including angina pectoris, hypertension, cardiac arrythmias and pheochromocytoma (99,120). However, beta-adrenoceptor blockade has been shown to unmask sympathetic mediated coronary vasoconstriction not only in experimental animals, but also in humans (90). Therefore, the use of beta-blockers, common in the treatment of cardiovascular disease, may also compound the tendency for coronary artery constriction or vasospasm during systemic hypoxia.

Future Experimentation

This experiment has answered the proposed question, "Does a sympathetic-induced coronary vasoconstrictor reflex exist during acute systemic hypoxia?" However, it has raised other questions which could be answered by future experimentation:

1. What are the effects of alpha two-adrenoceptor blockade and non-specific alpha-adrenoceptor blockade on hypoxia-induced reflex coronary vasoconstriction?
2. Is coronary blood flow altered when the isolated

cerebral circulation is perfused with hypoxic blood?

3. Is coronary blood flow altered when isolated aortic chemoreceptors are stimulated with hypoxic blood?
4. Will hypoxia-induced coronary vasoconstriction alter transmural distribution of coronary blood flow?
5. Do hypoxia-induced reflexes effect the right and left coronary circulations in a similar manner?

CHAPTER V

SUMMARY AND CONCLUSION

1. When the left common coronary artery (LCC) was perfused with normoxic blood, and cardiovascular hemodynamics were maintained constant during acute systemic hypoxia, a reduction in LCC blood flow was observed.

2. Minute by minute analysis of myocardial oxygen metabolism indicates that hypoxia-induced reductions in LCC blood flow were accompanied by increases in myocardial oxygen extraction which were sufficient to prevent decreases in myocardial oxygen consumption. Maximum effects analysis of myocardial oxygen metabolism indicated that hypoxia-induced reductions is LCC blood flow were accompanied by increases in myocardial oxygen extraction which were in sufficient to prevent decreases in myocardial oxygen consumption.

3. Intracoronary administration of the prazosin, a selective alpha one-adrenoceptor antagonist, abolished the hypoxia-induced reduction in LCC blood flow. This indicated that reduction LCC blood flow in the unblocked condition was due to a sympathetic-mediated coronary vasoconstrictor reflex which was modulated by alpha one-

adrenoceptors.

4. Measurement of regional blood flow with the microsphere technique indicated that during hypoxia, renal and splanchnic vascular beds constrict and cerebral vascular beds dilate.

In conclusion, the results of this investigation indicate that if hypoxia-induced coronary vasodilation is prevented during systemic hypoxia, a alpha one-adrenoceptor-mediated coronary vasoconstriction reflex is unmasked.

"What does not destroy me, makes me stronger."

Nietzsche

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