379
N81
NO. 6/31

THE ADENOSINE ANTAGONIST AMINOPHYLLINE ATTENUATES
PACING-INDUCED CORONARY FUNCTIONAL HYPEREMIA

THESIS

Presented to the Graduate Council of the
North Texas State University in Partial
Fulfillment of the Requirements

For the Degree of

MASTER OF SCIENCE

By

John Robert Randall

Denton, Texas

December, 1984

Randall, John R., The Adenosine Antagonist Aminophylline Attenuates Pacing-induced Coronary Functional Hyperemia. Master of Science (Basic Health Sciences), December, 1984, 72 pp., 3 tables, 11 illustrations, bibliography, 65 titles.

Left coronary blood flow (LCBF), left ventricular oxygen extraction $[(a-v)O_2]$, and myocardial oxygen consumption ($M\dot{V}O_2$) were monitored in 10 dogs. HR was paced at 120 bpm and then increased to 180 bpm to elicit a hyperemic response ($\Delta LCBF$). During the hyperemia, the vasodilatory response to exogenous adenosine (F_{AD}) was tested. Twenty min. after injection of aminophylline (100 mg/i.v.), HR was again increased. F_{AD} was again tested. The pacing-induced increase in $M\dot{V}O_2$ ($\Delta M\dot{V}O_2$) was not affected by aminophylline ($P > 0.05$). However, the slope $\Delta LCBF / \Delta M\dot{V}O_2$ was decreased, and the slope $(a-v)O_2 / \Delta M\dot{V}O_2$ was increased. F_{AD} was also decreased and the magnitude of the reduction was correlated with the decrease in $\Delta LCBF / \Delta M\dot{V}O_2$ ($r = 0.82$). These results suggest that adenosine may play an role in coronary functional hyperemia induced by increases in heart rate.

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CHAPTER I

INTRODUCTION

Coronary artery disease is the leading cause of death in America, and in the last few decades it has shown an increasing occurrence. The importance of understanding the pathophysiology of the coronary vasculature is clear, yet this knowlegde has not been easily acquired. Although centuries of investigation have explored the heart and its circulation, the relationship between coronary blood flow and disease states such as angina pectoris and myocardial infarction was not uncovered until early this century.

Investigation of the circulatory system began possibly as early as 3000 B.C. in Egypt. Evidence of this investigation was discovered in 1872 by the Egyptologist George Ebers, when Ebers translated a papyrus scroll which had been preserved in mummy-cloths (11). The scroll contained a historical collection of medical lore, which described the surgical, pharmaceutical, biological knowledge and procedures of that period. Included in these records was the observation that the pulse of the circulation was related to the beating of the heart. Most of these early observations were focused on describing the anatomy of the heart and the systemic vessels. Hippocrates (460 B.C.) described the

heart as a "firm, thick mass so richly supplied with fluid that it does not suffer pain" (22). In 130 B.C. Galen (20) described the anatomy of the vessels supplying the heart as a wreath or crown of vessels and is credited with coining the term "coronary" although the term had been used by others during this period. An interesting index of the progress of research at this time is that it was then believed that the heart was nourished by venous blood from the liver rather than arterial blood. Before Galen, most observations were concerned mostly with the gross anatomical features of the heart and systemic vessels and how they were affected by disease. The coronary vasculature itself began receiving more attention during the Renaissance as is evidenced by Leonardo da Vinci's excellent sketches of the coronary vessels.

The seventeenth and eighteenth centuries showed progress being made both in the recognition of cardiovascular disease, its clinical manifestations, and its relationship to the coronary circulation. In 1768 Heberden coined the term angina pectoris in "Some Account of a Disorder of the Breast" (23), but there was still no direct relationship to this syndrome and the heart or coronary arteries. This important connection was proposed by Jenner in 1799 in Parry's An Inquiry into the Symptoms and Causes of the Syncope Anginosa, Commonly called Angina Pectoris, Illustrated by Dissections (46) in which Parry wrote:

It was suggested by Dr. Jenner, that angina pectoris arose from some morbid change in the structure of the heart, which change was probably ossification, or some similar disease, of the coronary arteries.

Further developments followed. Burns (1809) proposed that angina pectoris was due to ischemia of the heart muscle (21). Work was done examining the effects of coronary ligation on cardiac action by Ericksen, and later the proposal that ossification of the coronary arteries and thromboses lead to angina pectoris was supported (22). After centuries of examination it was finally apparent by the nineteenth century that the syndrome of chest pain and sudden death known to the ancient Egyptians was related to a pathological decrease in coronary blood flow. Since this was learned, many techniques for clinical quantification of cardiovascular function and pharmacological interventions for the treatment of coronary heart disease have been developed. It is important to realize that almost every improvement in our methods of treating coronary heart disease have been based on furthering our knowledge of the physiology of the heart and coronary arteries.

Once it was recognized that much of cardiac disease was actually coronary artery disease; interest in the regulation of coronary blood flow increased. It was learned that coronary blood flow was under neural and local regulation. Local regulation is an intrinsic regulation of coronary

blood flow by the heart, and three different manifestations of local regulation can be demonstrated in the heart. These manifestations are autoregulation, reactive hyperemia, and functional hyperemia. Autoregulation is defined as "the intrinsic tendency of an organ to maintain constant blood flow despite changes in arterial perfusion pressure" (26). Reactive hyperemia is defined as the increase in blood flow in response to a decrease in oxygen supply following a brief arterial occlusion or a decrease in the oxygen content of the blood (e.g. anemia). Functional hyperemia, on the other hand, is an increase in blood flow in response to a greater need for oxygen by the tissue (e.g. exercise). Each of these responses is believed by many to be mediated primarily by one regulatory mechanism, although some controversy exists regarding the nature of the mechanism.

The most popular theory stated that the intrinsic regulation of coronary blood flow was mediated by a myocardial metabolite which was released in response to an imbalance between the myocardial oxygen demand and the coronary supply of oxygen. This metabolite was believed to act on the coronary vasculature to increase coronary blood flow. The subject of the present study is a potent vasodilator metabolite which has been shown to be released from the myocardium in response to several stimuli, and many believe it to be the mediator of intrinsic coronary blood flow

regulation. This metabolite is the purine nucleoside adenosine.

CHAPTER II

REVIEW OF THE LITERATURE

Vasoactivity of Adenosine and the Origin of the Adenosine Hypothesis.--The adenine containing compounds have been recognized as having vasodilator activity since 1929 when Drury and Szent-Gyorgi (17) demonstrated the vasodilator effect of these compounds in the mammalian heart. The idea that the release of one of these purine nucleotide derivatives may have a role in adjusting coronary blood flow to meet the changing oxygen requirements of the myocardium was suggested in 1932 by Rigler (50). Twenty-three years later, Wolfe and Berne (65) quantitated the coronary vasodilatory responses to the purine nucleosides and found ATP and ADP to be about four times more potent than AMP or adenosine as vasodilators. Yet, the observation that adenosine was the only one of these compounds able to easily cross the myocardial cell membrane (25) made adenosine seem to be the most likely candidate as a primary mediator of coronary vasodilation. In 1963, Berne (6) proposed that adenosine may be the vasoactive substance that is released from the myocardium during hypoxia. This proposal was based on the results obtained from two sets of experiments. One set of experiments utilized a Langendorff isolated cat heart

preparation, and the other set of experiments used open-chest anesthetized dogs. The isolated cat hearts were perfused initially with oxygenated Tyrode's solution for 15 min after which the perfusate was collected and analyzed for nucleotide derivative content. The hearts were then made hypoxic by infusion of deoxygenated Tyrode's solution for 15 min and again the perfusate was collected for analysis. For the in vivo dog heart study, the condition of hypoxia was created by partial asphyxiation. Arterial and coronary sinus blood samples were taken before and during the hypoxic state. The blood samples were then analyzed for purine nucleotide derivative content. The results of both sets of experiments failed to show adenine nucleotides or adenosine in either the arterial or venous perfusates collected before or during hypoxia. However, both studies demonstrated the appearance in the venous perfusates of inosine and hypoxanthine which had previously been shown to be degradation products of adenosine (24). In addition, it was observed that the inosine and hypoxanthine concentrations decreased upon reoxygenation of the myocardium. From these data, and data gathered on coronary adenosine infusions, Berne proposed that hypoxia stimulated the production and release of adenosine from the myocardium. He further hypothesized that this released adenosine could serve as the primary mediator of coronary blood flow regulation. Thus, it is a potent vasodilator which is rapidly deactivated by adenosine

deaminase in blood and tissue. Also, a large potential source of adenosine exist in the intracellular adenine nucleotide pool.

Role of Adenosine in Reactive Hyperemia.--Berne's proposal of the adenosine hypothesis was followed by many studies which further examined its role in the reactive hyperemic response (1,8,14,32,42,48,53). The reactive hyperemic response was chosen as the model used to test the adenosine hypothesis because it requires a simple preparation, and it consists of a large increase in flow which made measurement of metabolite concentrations in venous effluents easier. Reactive hyperemia following a brief occlusion of a coronary artery was also shown to be a response which could be obtained repeatedly without deleterious effects on the myocardium (13).

In some of the initial studies adenosine levels were estimated by the recovery of adenosine degradation products from arterial and coronary sinus blood (6). This was done because sampling techniques which prevented the rapid degradation of adenosine, and measurements sensitive enough to detect the low concentrations of adenosine had yet to be developed. Methods for measuring adenosine from hypoxic hearts in the presence of adenosine deaminase inhibitors were used in later studies (32,49), but still required a severe hypoxic stimulus to acquire the quantity of adenosine

which could be detected. Katori and Berne (32) used an adenosine deaminase inhibitor to measure adenosine in the perfusates of isolated cat and guinea pig hearts subjected to anoxia or severe hypoxia. The sum of adenosine, inosine, and hypoxanthine was found to increase in proportion to the increase in coronary flow caused by graded hypoxia. This relationship was also found to be inversely related to the O_2 tension of the coronary perfusate. These results further supported the hypothesis that hypoxia results in the breakdown of myocardial adenine nucleotides and the release of adenosine into the interstitium. Improvements in sampling and measuring techniques made quantification of much smaller amounts of adenosine in blood possible. Rubio et al. (53) measured arterial and coronary sinus blood for adenosine content prior to and immediately following coronary occlusions of 30-60s in open-chest anesthetized dogs. Adenosine was found in coronary sinus blood, but not in arterial blood. The results of this study suggest that adenosine was released from the myocardium into the interstitium during periods of ischemia and that the amount released during the occlusion was great enough to elicit a maximal dilatation of the coronary bed (30-60s).

Olsson (42) demonstrated that myocardial adenosine levels increase during coronary occlusions from 5-15 s in duration, and he also noted that a linear relationship exist

between myocardial adenosine level and the number of heart beats that occurred during the occlusion. Since heart rate is one of the primary determinants of myocardial oxygen consumption (9), this relationship suggests that adenosine production is tightly coupled to myocardial metabolism. This study introduced myocardial adenosine concentration as an alternate index of interstitial adenosine concentration. The measurements which Olsson performed in this study were made possible by improvements in spectrophotometric sensitivity and modifications in the enzymatic assay for adenosine. The assay for adenosine involved degradation of adenosine to uric acid which could be quantitated with greater sensitivity.

A third index of interstitial adenosine concentration was developed following a study by Rubio and Berne (52) in which they demonstrated that adenosine was released by the epicardial surface of the myocardium in the normal, oxygenated heart. In these experiments, using open-chest anesthetized dogs, the pericardial space was perfused by Tyrode's or Krebs-Henseleit solutions for 1 to 3 hrs, and then analyzed for adenosine content. Adenosine was found in the perfusate of the normal, oxygenated heart, and was shown to increase during hypoxia. This observation suggested that hypoxia was not a necessary stimulus for the production and release of adenosine, furthermore, that the pericardial fluid adenosine was representative of the interstitial

adenosine content. Later, a pericardial perfusate technique (41) was developed in which pericardial fluid perfusate samples (40 ml) were left in contact with the myocardium for 0.5 - 18 min and then measured for adenosine content. The results of the study showed that turnover of adenosine in the perfusate was rapid and that the adenosine level did not reach equilibrium with the tissue adenosine levels. However, the pericardial perfusate adenosine level did correlate significantly with increases in coronary blood flow brought about by stellate ganglia stimulation, which supported its use as an index of interstitial adenosine.

Although three measurements have been used as indices of interstitial adenosine concentration, it is still very controversial as to which is the best. Techniques, other than those mentioned above, have also been employed as interstitial adenosine indices. The advantages and disadvantages of each method have been discussed in many publications (4,5,35,45). Until a satisfactory method is developed, we shall have to rely on the best indices available. It is important to point out that although we may not have accurate quantitative measurements of interstitial adenosine, most researchers believe that the primary indices being used are reflective of the directional changes of interstitial adenosine concentration.

A major challenge to the adenosine hypothesis comes

from the studies which have examined the effect of the methylxanthines, theophylline and aminophylline, on reactive hyperemia. These compounds have been shown to effectively attenuate the coronary vasodilatory response to exogenous adenosine administration in the intact heart (1,2,3,8,12,30). Results of studies examining the effect of methylxanthines on reactive hyperemia have been controversial. Several studies have reported that the methylxanthines have little or no significant effect on the peak flow (2,8,30), nor the duration (8,39,59) of the reactive hyperemic response. In the study performed by Bittar and Pauly (8) reactive hyperemia following occlusions of 30, 60, and 120 s remained unchanged after administration of aminophylline, however, the response to exogenous adenosine was attenuated. These studies suggest that adenosine is unnecessary for expression of reactive hyperemia. Yet, other studies (14,31,55) have demonstrated an attenuation of reactive hyperemia in the presence of the methylxanthines. The reasons for the differences between the two groups of observations is unclear at this time, but several possible explanations have been offered, such as differences in the time of occlusion, or the accumulation of other vasodilatory substances. Merrill et al. (40) investigated the influence of perfusate pH on the coronary vasodilatory response to exogenous adenosine and the inhibition of this response by theophylline. This study was performed in isolated, per-

fused guinea pig hearts. The pH of the perfusate was decreased by increasing P_{CO_2} . The results showed that at the lower pH tested, the concentration of adenosine required to elicit a vasodilatory response was less, likewise, the response to a given amount of adenosine infused was greater. According to Merrill, the inhibition of the adenosine vasodilatation was not pH sensitive at the range of pH tested in this study, but it is proposed that the inability of the methylxanthines to "regularly attenuate reactive hyperemia might be related to enhanced adenosine dilation caused by increased tissue hydrogen activity."

Summarizing the results from the reactive hyperemia studies, one finds much evidence supporting the adenosine hypothesis. From this data it appears that adenosine may play a significant role in the regulation of the reactive hyperemic response. Decreasing the oxygen supply to the myocardium creates an imbalance between the oxygen supply and demand, resulting in an increase in the production of adenosine. Adenosine then is proposed to act on the coronary smooth muscle cells and elicit a vasodilatory response which would increase coronary blood flow. Thus, the oxygen delivery to the myocardium would be increased and the balance between oxygen supply and demand would be re-established.

Role of Adenosine in Functional Hyperemia.--The adenosine hypothesis was developed using severe ischemia or hypoxia as the stimulus for the hyperemic response. It later became warranted to examine the role of adenosine in coronary flow regulation with a more physiological stimulus, such as that seen in functional hyperemia. Functional hyperemia is the increase in flow which automatically accompanies an increase in cardiac activity and myocardial oxygen consumption. If adenosine does indeed play a major role in the regulation of coronary blood flow, it would be expected to be involved in the functional hyperemic response. Many studies have recently examined this possibility.

Katori and Berne (32) observed in two experiments that epinephrine increased adenosine release from the perfused cat heart in the presence of the adenosine deaminase inhibitor, 8-azaguanine. This study was followed by that of Wiedmeier and Spell (64) who tested the effects of the positive inotropic agents; histamine, epinephrine and nor-epinephrine, and the vasodilator nitroglycerin, on adenosine release in the perfused guinea pig heart. The positive inotropic agents increased the oxygen consumption of the myocardium, but nitroglycerin does not have an inotropic action and thus, did not increase oxygen consumption. Therefore, if adenosine is tightly coupled to both cardiac metabolism and coronary blood flow, adenosine levels in the

perfusates should increase proportionately with the increase in oxygen consumption induced by the inotropic agents. Likewise, if adenosine is intimately involved, one would expect adenosine levels to remain the same or decrease in the presence of a pure vasodilator, such as nitroglycerin. The results using histamine and the catecholamines did show an increase in coronary flow, a positive inotropic effect and a decrease in coronary sinus oxygen levels. The decrease in coronary sinus oxygen was accompanied by increased levels of adenosine in the perfusates. Nitroglycerin also produced an increase in coronary flow, however, there was no increase in contractile force and coronary sinus oxygen content increased while the coronary sinus adenosine level was unchanged. These results suggest that adenosine release is indeed involved in coronary dilation associated with a decrease in coronary sinus oxygen levels. It was also demonstrated that a linear relationship existed between myocardial oxygen consumption and the perfusate adenosine level. Other laboratories tested the hypothesis by increasing oxygen demand by other methods. Foley et al. (19) examined the formation of adenosine induced by aortic constriction in rat myocardium. Aortic pressure is the load against which the left ventricle must pump and thus, is a direct determinant of left ventricular oxygen demand (10,56). This study showed that myocardial adenosine levels

increased with increases in aortic pressure due to acute aortic constriction. Two studies have examined pericardial perfusate adenosine concentrations during graded and supra-maximal bilateral stellate ganglion stimulation (41,63). In one of these studies (41) pericardial adenosine levels were measured before and during graded stellate stimulation. Significant correlations between pericardial fluid adenosine, coronary blood flow and oxygen consumption were found. In addition to these findings, experiments were performed in which both tissue and pericardial levels of adenosine and lactate were measured. Neither the tissue nor the pericardial lactate levels increased during stellate stimulation indicating that the observed correlations were not effected by an ischemic myocardium. The researchers also felt that the parallel increases in tissue and pericardial perfusate adenosine content during stellate ganglion stimulation supported the use of pericardial perfusate content as an index of tissue adenosine production. The second study (63) evaluated the changes in pericardial perfusate adenosine during supramaximal stellate ganglion stimulation in anesthetized dogs, and during treadmill exercise in unanesthetized dogs. Both groups of experiments demonstrated an increase in the adenosine level when cardiac activity was increased by stellate stimulation or exercise. Furthermore, data from the bilateral stellate stimulation experiment correlated pericardial adenosine content with the pressure-

rate product (HR x MAP), and left ventricular dP/dt which are indices of cardiac performance. In the exercising dogs pericardial perfusate adenosine correlated significantly with heart rate.

The correlations between myocardial oxygen consumption, indices of interstitial adenosine concentration and coronary blood flow have been continually reinforced by studies using a variety of stimuli. These or similar correlations have been found to be consistent in anesthetized dogs with aortic constriction (38,54), isoproterenol (38,54), norepinephrine (33), atrial and paired pacing (33,54), vagal stimulation (33), and calcium chloride (33). In conscious dogs, experiments using treadmill exercise (18,38,54), or excitement induced by loud noises or feeding (4) have also yielded similar results. The study by Knabb et al. (33) perhaps summarizes the overall results best. Using six of the experimental stimuli mentioned above (two to five interventions per dog), measurements of coronary blood flow, myocardial oxygen consumption, and pericardial perfusate adenosine were made. The results showed significant linear relationships for coronary blood flow vs. myocardial oxygen consumption, pericardial perfusate adenosine vs. myocardial oxygen consumption and pericardial perfusate adenosine vs. coronary blood flow. In addition, it was demonstrated that for aortic constriction and calcium chloride infusion that

these linear relationships remained consistent with and without beta-blockade by propranolol. Knabb wrote that "for a given change in $\dot{MVO}_2$, the amount of adenosine entering the [pericardial infusate] is the same, regardless of what stimulus initiated the change in $\dot{MVO}_2$." These results support the hypothesis that adenosine couples changes in myocardial oxygen demand to coronary blood flow.

It can be seen that much evidence has been supportive of a role for adenosine in functional hyperemia. However, correlations offer no direct proof of a cause-effect relationship. Therefore, in a previous study (28) we more directly examined the role of adenosine in coronary functional hyperemia by using the methylxanthine aminophylline to block the vascular effect of adenosine. This study used the experimental preparation shown in Figure 1. Cardiac activity was increased by infusing the positive inotropic agent norepinephrine directly into left coronary artery. The results of one experiment is shown in Figure 2. The tracings on the left show the results under control conditions. Infusion of norepinephrine elicited an increase in coronary blood flow ($\Delta LCBF$), oxygen extraction $[(a-v)O_2]$ and calculated myocardial oxygen consumption ($\Delta \dot{MVO}_2$). Consequently, the ratio of the change in LCBF to the change in $\dot{MVO}_2$, or the flow response slope, was 5.2. Also note the increase in LCBF due to exogenous adenosine infusion during the functional hyperemia. The tracings on the

Figure 1. Experimental preparation for norepinephrine-induced functional hyperemia study. Diagram of in situ dog heart preparation used to monitor left coronary blood flow (LCBF), left ventricular oxygen extraction $[(a-v)O_2]$, coronary perfusion pressure at the tip of the coronary cannula (CPP), and mean arterial pressure (MAP). The pulsatile arterial pressure signal was used to monitor heart rate. Left ventricular oxygen consumption was calculated from $(a-v)O_2$ and LCBF (normalized to 100g left ventricle). The rate of the constant pressure pump was regulated by CPP, and CPP was maintained at 92 mmHg. Norepinephrine and adenosine were infused through a rubber bulb into the coronary circuit using a syringe pump.

Figure 2. Results of one norepinephrine-induced functional hyperemia experiment. Recordings in one dog before (control) and after administration of aminophylline. The functional hyperemic response during norepinephrine infusion was not altered by aminophylline as shown by comparison of both the increases in coronary flow (LCBF) and the flow response slopes ($\Delta\text{LCBF}/\Delta\dot{\text{MVO}}_2$). However, the coronary response to infusion of adenosine during the functional hyperemic response was completely blocked by aminophylline.

right show the results obtained approximately 15 min after administration of aminophylline. Under these conditions, infusion of norepinephrine still elicited a substantial increase in LCBF and $\dot{MVO}_2$, resulting in a flow response slope (5.0) nearly identical to the control flow response slope. Also note that the infusion of exogenous adenosine during functional hyperemia after aminophylline caused no further increase in LCBF indicating successful blockade of the adenosine receptor. Thus, there was no change in the functional hyperemic response before and after administration of aminophylline suggesting that adenosine was not necessary for the full expression of functional hyperemia induced by an inotropic stimulus. The mean results of these experiments is shown in Table I.

One other possible interpretation of these results still existed. If adenosine was being released at a greater rate in the presence of aminophylline, it would be possible for adenosine to replace aminophylline at the receptor by competitive inhibition. This could happen, according to the adenosine hypothesis, if the imbalance between oxygen supply and demand continued to increase because aminophylline was preventing an increase in oxygen supply which would reestablish the oxygen balance. To examine this possibility it is first important to note that the blockade of the vasodilatory response to exogenous adenosine by aminophylline was tested during the actual conditions of functional

TABLE I. Results of norepinephrine-induced functional hyperemia study.

	CONTROL	POST-AMINOPHYLLINE
<u>PRE-NOREPINEPHRINE</u>		
<u>INFUSION DATA:</u>		
MAP (mmHg)	105 $\pm$ 4	96 $\pm$ 4 *
HR (bpm)	159 $\pm$ 7	168 $\pm$ 8
LCBF (ml/100g/min)	86 $\pm$ 6	92 $\pm$ 6
(a-v)O ₂ (ml/100ml)	14.5 $\pm$ 1.6	14.4 $\pm$ 1.5
MVO ₂ (ml/100g/min)	11.7 $\pm$ 3.5	12.8 $\pm$ 4.2
<u>CHANGES DURING</u>		
<u>NOREPINEPHRINE INFUSION:</u>		
Δ LCBF (ml/100g/min)	29 $\pm$ 4	33 $\pm$ 3
Δ (a-v)O ₂ (ml/100ml)	1.0 $\pm$ 0.8	1.6 $\pm$ 1.0
Δ MVO ₂ (ml/100g/min)	5.7 $\pm$ 1.6	6.6 $\pm$ 1.4
Δ LCBF/ Δ MVO ₂	5.2 $\pm$ 0.9	5.0 $\pm$ 0.9
Δ F _{AD} (ml/100g/min)	16 $\pm$ 3	2 $\pm$ 1 *

Values are mean $\pm$ S.E. * indicates $P < 0.05$ compared to control value.

Abbreviations: MAP = mean arterial pressure; HR = heart rate; LCBF = left coronary blood flow; (a-v)O₂ = left ventricular oxygen extraction; MVO₂ = left ventricular oxygen consumption; F_{AD} = increase in LCBF caused by infusion of adenosine.

hyperemia. One of the major criticisms of the reactive hyperemia studies using methylxanthines was that it could not be demonstrated whether or not the methylxanthine was still blocking the adenosine receptor in the presence of elevated endogenous adenosine during hyperemia because the blockade could be tested only before and after reactive hyperemia and not during the reponse. Our results showed that aminophylline almost completely abolished the vasodilatory response to the exogenous adenosine, which is presumed to be additive to the endogenous adenosine. Therefore, suggesting that the aminophylline was not being displaced from the receptor by the concentrations of adenosine present during the functional hyperemic response.

To further test the possibility that adenosine concentration may increase to higher levels in the presence of aminophylline, a second set of experiments was performed to measure myocardial adenosine concentrations before and during norepinephrine-induced hyperemia with and without the presence of aminophylline in 4 groups of dogs. The results of these experiments are shown in Figures 3 and 4. These figures show that the concentration of myocardial adenosine in the presence of aminophylline did not increased to levels greater those than in the control groups before or during hyperemia. The failure of aminophylline to attenuate the inotropically-induced functional hyperemia was therefore not attributable to greater concentrations of adenosine

Figure 3. Relationship between $\dot{MVO}_2$ and myocardial adenosine. Each symbol represents one experiment. The linear regression was statistically significant ($P < 0.05$). Circles represent measurements taken before functional hyperemia and squares are those taken during functional hyperemia. The open symbols represent the absence of aminophylline and the closed symbols represent the presence of aminophylline.

Figure 4. Relationship between myocardial adenosine and LCBF. Each symbol represents one experiment (same groups as in figure 3). The linear regression was statistically significant ($P < 0.05$).

displacing aminophylline at the coronary vascular receptor. In addition, the linear relationships between myocardial adenosine concentration and MVO_2 , and myocardial adenosine concentration and LCBF were no different from those before aminophylline. In this regard, it is interesting to note that these linear relationships observed in our previous experiments are compatible with the results by others (4,18,19,33,38,41,54,63) and indicate the potential for adenosine involvement in the functional hyperemia accompanying an increase in inotropic state, although our results further indicated that adenosine was not necessary for the response.

Statement of Specific Aims.--The results of our previous study (28) indicated that the potential for adenosine involvement exists in inotropically-induced functional hyperemia, but that adenosine was not necessary for full expression of that hyperemia. It is conceivable that functional hyperemia elicited by different stimuli may have different underlying mechanisms. This possibility has been pursued in the present study with the following aim:

To determine if the adenosine antagonist aminophylline attenuates coronary functional hyperemia secondary to an increase in heart rate.

The results of this study were intended to provide insight into the role of adenosine in mediating hyperemia accompanying an increase in rate. Furthermore, by comparing these results with those previously obtained with inotropically induced hyperemia, insight can be gained regarding whether functional hyperemia elicited by different cardiac stimuli may have different mechanisms.

CHAPTER III

METHODS

Ten mongrel dogs of either sex weighing between 15-25 kg were used. Each dog was anesthetized by intravenous injection of sodium pentobarbital (30 mg/kg).

Experimental Preparation.--The experimental preparation is shown diagrammatically in Figure 5. A tracheotomy was performed, the animal was intubated and respired with room air using a Harvard Apparatus Model 613 respirator pump. A femoral vein and artery were then isolated and catheterized with polyethylene tubing filled with heparinized saline. The arterial catheter was connected to a Statham P23ID pressure transducer and was used to monitor mean arterial pressure (MAP) throughout the experiment. Succinylcholine chloride (20 mg) was administered intravenously to facilitate opening of the chest by electrocautery. The chest was opened through the left fourth intercostal space, and the third, fourth, and fifth ribs were removed. The left lung was loosely retracted with a wet gauze sponge. The left subclavian artery was isolated, the pericardium was opened, and the left common coronary artery was also isolated. After hemostasis, sodium heparin (1,000 U/kg) was infused through the femoral vein catheter. A double lumen cannula

Figure 5. Experimental preparation for pacing-induced functional hyperemia study. Diagram of in situ dog heart preparation used in pacing functional hyperemia study. Note the similarity to the preparation used a previous study (Figure 1) with the difference being the stimulus used to increase cardiac activity. In this preparation the heart rate was set by pacing electrodes.

was inserted into the left subclavian artery. The cannula is shown in Figure 6 and was constructed of two brass tubes welded together and polished. One lumen was inserted and secured in the main left coronary artery, while the other lumen was positioned in the ascending aorta. This cannula was connected to a Tygon tubing loop filled with heparinized saline. Before placing the cannula in the coronary ostium, the Tygon loop was filled with blood from the aorta, and the system was inspected for the presence of air bubbles. The cannula was then tied securely in the subclavian artery, and the flanged tip was tied in the left coronary artery. Once the cannula was positioned, blood flowed from the aorta through the external Tygon loop and perfused the left coronary bed. Intracoronary administration of solutions could be performed through a Latex rubber bulb in the loop. Coronary perfusion pressure (CPP) at the coronary cannula tip was measured with an 18.5 gauge heparinized saline-filled catheter passed through a side arm of the external loop. The catheter was connected simultaneously to a Harvard model 377 pressure transducer and a Statham P23ID pressure transducer. The signal from the Harvard transducer was input to a Harvard model 1200 servo-controlled peristaltic pump. Pump rate was determined by the CPP signal. Throughout each experiment; CPP was maintained at a level of 87-93 mmHg. The signal from the Statham transducer was used for recording CPP. Left coronary blood flow (LCBF) was

Figure 6. Diagram of the brass cannula used to cannulate the left coronary artery. (A) Top view. (B) Side view.

A. SIDE VIEW

B. TOP VIEW

monitored with an electromagnetic flow probe (Carolina Medical Electronics, model EP 612) in the external loop. The probe was connected to a Carolina Medical Electronics model 501 square wave electromagnetic flowmeter. This method of flow measurement is described by Spencer and Denison (60). The baseline output of the flowmeter was tested by performing a 15 s mechanical occlusion of the external loop to establish a zero-flow condition. To monitor left ventricular oxygen extraction $[(a-v)O_2]$, the difference in oxygen concentrations between arterial and coronary sinus blood was measured. For this purpose, a polyethylene catheter was inserted into a jugular vein, passed into the vena cava, and positioned 1-2 cm into the coronary sinus, which does not result in contamination of coronary sinus blood with right atrial admixture (34). A second polyethylene catheter was connected to a side arm of the arterial Tygon loop. Blood from both sources was pumped by a Gilson minipuls 2 peristaltic pump at a rate of 7 ml/min through a continuous A-VOX oxygen difference analyzer (58). Blood was returned to the animal through the femoral vein catheter. Once the preparation had stabilized but before the experiment was begun, arterial and coronary sinus blood samples were withdrawn, and oxygen concentrations were determined using a Lex-O₂-Con oxygen concentration analyzer (Lexington Instruments). This procedure provided both a method for checking the calibration of the A-VOX oxygen

difference analyzer as well as a measure of the initial arterial oxygen concentration (CaO_2). Heart rate (HR) was electrically paced by electrodes placed in the right atrial appendage and attached to a Grass S9 stimulator. In order to maintain a low HR prior to pacing, the right vagus nerve was isolated in the neck, tied distally to the heart and cut. The cut nerve proximal to the heart was stimulated with pulses of 1.6 ms duration and 5V at 5-15 Hz using a Narco SI-10 stimulator. Vagal stimulation was continued during subsequent pacing experiments. The pulsatile arterial pressure signal triggered a tachograph for recording of HR. All variables were recorded on a Beckman R611 eight-channel Dynograph recorder.

Experimental Protocol.--When the preparation was completed, the heart was paced at a rate of 120 bpm. After all variables stabilized, pre-hyperemic values were recorded. Coronary functional hyperemia was then elicited by increasing the HR to 180 bpm. In approximately 3-5 min, LCBF and $(a-v)\text{O}_2$ stabilized at new levels, and all variables were recorded. During the pacing-induced hyperemia in 9 of 10 dogs, adenosine was infused directly into the left coronary loop at a rate of $10\ \mu\text{g}/\text{min}$ for 2-3 min. The increase in LCBF resulting from the exogenous adenosine (F_{AD}) was recorded. After completion of the adenosine infusion, HR was returned to the pre-hyperemic rate of 120 bpm. Aminophyl-

line (100 mg) was then administered through the femoral vein catheter over a 10 min period as reported by Bittar and Pauly (8) and Afonso (1). A period of 20 min was then allowed for recorded variables to return to the pre-aminophylline values. Aminophylline has a transient vasodilatory and a positive inotropic effect (approximately 15 min) that is separate from the longer lasting blockade of the coronary adenosine response. In 4 of the 10 animals, arterial and coronary sinus blood samples were again withdrawn for determination of oxygen concentrations. In all animals, pre-hyperemic values were recorded, and hyperemia was again elicited by increasing HR to 180 bpm. After hyperemic values had been recorded, adenosine infusion was repeated, and F_{AD} was recorded. Following each experiment the heart was excised, the left ventricle was cleared and weighed, and LCBF was normalized to 100g left ventricle. Left ventricular oxygen consumption ($M\dot{V}O_2$) was calculated by the Fick equation using $(a-v)O_2$ and normalized LCBF.

Data Analysis.--To analyze the effects of aminophylline on pre-hyperemic values, MAP, LCBF, $(a-v)O_2$, and $M\dot{V}O_2$ obtained 20 min after aminophylline were compared to the respective values obtained before aminophylline using Student's t-test for paired data (59). Also, the CaO_2 found in 4 dogs after aminophylline was compared to the initial value found in all 10 dogs before aminophylline using the unpaired t-test (59).

To analyze the effects of aminophylline on changes in variables when HR was increased from 120 bpm to 180 bpm, pacing-induced changes in LCBF (ΔLCBF), $(a-v)\text{O}_2$ [$\Delta(a-v)\text{O}_2$], and $\dot{\text{MVO}}_2$ ($\Delta\dot{\text{MVO}}_2$), after aminophylline were compared to the respective changes before aminophylline using the paired t-test. However, among the individual experiments, $\Delta\dot{\text{MVO}}_2$ after aminophylline was often different from that before aminophylline. Because of the close coupling between coronary blood flow and $\dot{\text{MVO}}_2$, the variation in $\Delta\dot{\text{MVO}}_2$ made analysis of the specific effect of aminophylline on LCBF difficult. Therefore, when HR was increased to 180 bpm, it was useful to characterize the LCBF response as the flow response slope $\Delta\text{LCBF}/\Delta\dot{\text{MVO}}_2$ having the units ml blood/ml O_2 , and the flow response slope after aminophylline was compared to that before aminophylline using the paired t-test. Likewise, the $(a-v)\text{O}_2$ response was characterized as the extraction response slope $\Delta(a-v)\text{O}_2/\Delta\dot{\text{MVO}}_2$ having the units $\text{min} \cdot 100\text{g}/\text{ml}$ blood, and the extraction response slope after aminophylline was compared to that before aminophylline using the paired t-test. In all analyses, statistical significance was accepted at $P < 0.05$.

To examine the effect of aminophylline on the coronary response to exogenous adenosine, FAD after aminophylline was compared to that before aminophylline using the paired t-test. Finally, it was useful to examine whether the magni-

tude of the aminophylline-induced attenuation of the response to exogenous adenosine was correlated with the attenuation of functional hyperemia. For this purpose, in those 9 dogs in which F_{AD} was obtained, the reduction in F_{AD} after aminophylline was related to the reduction in the flow response slope by linear regression using the least mean squares methods. A correlation coefficient (r) was calculated, and statistical significance of the correlation was tested (59).

CHAPTER IV

RESULTS

Figure 7 shows the results of a pacing experiment in one dog both before and after administration of aminophylline. All variables were allowed to stabilize with the HR paced at 120 bpm. When HR was increased from 120 to 180 bpm before administration of aminophylline, LCBF increased from 112.0 to 141.1 ml/min/100g ($\Delta\text{LCBF} = 29.1$ ml/min/100g). Accompanying the increase in LCBF was a slight increase in (a-v) O_2 [$\Delta(\text{a-v})\text{O}_2 = 0.4$ ml/100ml]. As a consequence, $\dot{\text{MVO}}_2$ increased from 10.6 to 14.0 ml/min/100g ($\Delta\dot{\text{MVO}}_2 = 3.4$ ml/min/100g). The flow response slope ($\Delta\text{LCBF}/\Delta\dot{\text{MVO}}_2$) was 8.6 ml blood/ml O_2 , and the extraction response slope [$\Delta(\text{a-v})\text{O}_2/\Delta\dot{\text{MVO}}_2$] was 0.1 min·100g/ml blood. It is also notable that during the functional hyperemic response before aminophylline, infusion of adenosine (Ad) elicited a further substantial increase in LCBF. Throughout the experiment MAP and CPP remained relatively constant both before and after administration of aminophylline. Following the administration of aminophylline, the increase in HR from 120 to 180 bpm again caused an increase in LCBF ($\Delta\text{LCBF} = 13.6$), although the increase was substantially less than that observed before administration of aminophylline. It is

Figure 7. Results of one pacing-induced functional hyperemia experiment showing tracings of heart rate (HR), mean arterial pressure (MAP), coronary perfusion pressure (CPP), left coronary blood flow (LCBF), and left ventricular oxygen extraction $[(a-v)O_2]$ before and after administration of aminophylline. Calculated left ventricular oxygen consumption ($M\dot{V}O_2$) before and during pacing-induced hyperemia, the changes in LCBF ($\Delta LCBF$) and $(a-v)O_2$ [$\Delta(a-v)O_2$] during hyperemia as well as the flow response slopes ($\Delta LCBF/\Delta M\dot{V}O_2$) and extraction response slopes are shown. The changes in LCBF elicited by intracoronary infusions of adenosine (Ad) can also be seen.

evident that the blunted increase in LCBF was associated with a greater increase in $(a-v)O_2$ than observed before administration of aminophylline [$\Delta(a-v)O_2 = 1.2 \text{ ml/100ml}$]. Consequently, $\dot{M}\dot{V}O_2$ was increased by 2.7 ml/min/100g during functional hyperemia, although the flow response slope was reduced ($\Delta\text{LCBF}/\Delta\dot{M}\dot{V}O_2 = 5.0 \text{ ml blood/ml } O_2$) and the extraction response slope was increased [$\Delta(a-v)O_2/\Delta\dot{M}\dot{V}O_2 = 0.4 \text{ min}\cdot\text{100g/ml blood}$] compared to the pre-aminophylline values. Also, after aminophylline administration, the response to exogenous adenosine infusion was attenuated indicating that aminophylline was successfully blocking the coronary vascular adenosine receptor.

Table II shows the individual and mean pre-hyperemic values of MAP, CaO_2 , LCBF, $(a-v)O_2$, and $\dot{M}\dot{V}O_2$ before and after administration of aminophylline. Aminophylline had no significant effect on any of the observed variables under pre-hyperemic conditions.

Table III shows the ΔLCBF , $\Delta(a-v)O_2$, and $\Delta\dot{M}\dot{V}O_2$ as well as the flow response and extraction response slope in each experiment when HR was increased from 120 to 180 bpm both before and after administration of aminophylline. Several points are noteworthy. First, aminophylline had no significant effect on mean $\Delta\dot{M}\dot{V}O_2$ ($P>0.05$). In some individual cases, $\Delta\dot{M}\dot{V}O_2$ was hardly changed after aminophylline, while in others it was either increased or decreased. Second, although, mean LCBF was numerically less after aminophyl-

TABLE II. Effects of aminophylline on variables at a heart rate of 120 bpm.

	MAP (mmHg)	CaO ₂ (ml/100ml)	LCBF (ml/min/100g)	(a-v)O ₂ (ml/100ml)	MVO ₂ (ml/min/100g)
Pre-Aminophylline	89 ±3	18.7 ±0.2	53.4 ±7.2	14.9 ±0.9	7.6 ±0.6
Post-Aminophylline	85 ±3	18.5 ±0.2	58.9 ±5.2	14.1 ±0.9	8.1 ±0.5

All values are mean $\pm$ SE. $n = 10$ for all cases except post-aminophylline CaO₂ where $n = 4$. There was no significant difference in any value before and after aminophylline ($P > 0.05$). Abbreviations: MAP = Mean arterial pressure; CaO₂ = arterial oxygen concentration; LCBF = left coronary blood flow; (a-v)O₂ = left ventricular oxygen extraction; MVO₂ = left ventricular myocardial oxygen consumption.

TABLE III. Effect of aminophylline on changes in variables when heart rate was increased to 180 bpm.

	Pre-Aminophylline					Post-Aminophylline				
Dog				ΔLCBF	$\frac{\Delta(a-v)\text{O}_2}{\Delta\dot{\text{MVO}}_2}$				ΔLCBF	$\frac{\Delta(a-v)\text{O}_2}{\Delta\dot{\text{MVO}}_2}$
No.	ΔLCBF	$\Delta(a-v)\text{O}_2$	$\Delta\dot{\text{MVO}}_2$	$\Delta\dot{\text{MVO}}_2$	$\Delta\dot{\text{MVO}}_2$	ΔLCBF	$\Delta(a-v)\text{O}_2$	$\Delta\dot{\text{MVO}}_2$	$\Delta\dot{\text{MVO}}_2$	$\Delta\dot{\text{MVO}}_2$
1	18.1	0	3.0	6.0	0	6.7	0.3	1.4	4.8	0.2
2	10.7	1.6	2.8	3.8	0.6	12.9	2.5	3.9	3.3	0.6
3	12.4	1.5	2.8	4.4	0.5	7.1	1.2	1.7	4.2	0.7
4	5.0	0.8	1.3	3.8	0.6	2.5	1.5	1.4	1.8	0.8
5	29.1	0.4	3.4	8.6	0.1	13.6	1.2	2.7	5.0	0.4
6	12.2	0.8	2.5	4.9	0.3	12.9	1.1	2.9	4.4	0.4
7	20.8	0.5	2.6	8.0	0.2	26.3	0.6	3.4	7.7	0.2
8	8.7	0.8	2.0	4.4	0.4	7.6	1.0	1.9	4.0	0.5
9	19.9	0.3	3.0	6.6	0.1	6.4	0.9	1.3	4.9	0.7
10	5.0	0.3	0.9	5.6	0.3	3.0	0.8	0.8	3.8	1.0
Mean	14.2	0.7	2.4	5.6	0.3	9.9	1.1*	2.1	4.4*	0.6*
$\pm\text{SE}$	± 2.4	± 0.2	± 0.3	± 0.5	± 0.1	± 2.2	± 0.2	± 0.3	± 0.5	± 0.1

LCBF, $(a-v)\text{O}_2$, and $\dot{\text{MVO}}_2$ are expressed in same units shown in Table 1. The flow response slope $\Delta\text{LCBF}/\Delta\dot{\text{MVO}}_2$ is the ratio of the change in LCBF to the change in $\dot{\text{MVO}}_2$ and has the units ml blood/ml oxygen. The extraction response slope $\Delta(a-v)\text{O}_2/\Delta\dot{\text{MVO}}_2$ is the ratio of the change in $(a-v)\text{O}_2$ to the change in $\dot{\text{MVO}}_2$ and has the units $\text{min} \cdot 100\text{g/ml}$ blood.

* Indicates that the change in a variable after aminophylline was significantly different from the change before aminophylline ($P < 0.05$).

line than before aminophylline, the difference was not significant ($P=0.08$), possibly due to the variation in ΔLCBF secondary to the variation in $\Delta\dot{M}\dot{V}\text{O}_2$ among the individual experiments. Third, despite the lack of a significant difference in ΔLCBF , $\Delta(a-v)\text{O}_2$ after aminophylline was significantly greater than that before aminophylline. Indeed, all dogs except one (dog no. 3) showed an increase in $\Delta(a-v)\text{O}_2$ after aminophylline. Finally, it is extremely important to note the consistent differences in the flow and extraction response slopes after aminophylline compared to those before aminophylline. Thus, in all 10 experiments, the flow response slope after aminophylline was less than that before aminophylline. The mean flow response slope was reduced by about 20 per cent, and the reduction was significantly significant. At the same time, the extraction response slope was increased after aminophylline in 8 dogs, while in 2 dogs there was no difference in the slope when compared to the pre-aminophylline condition. The mean extraction response slope was increased by 100 per cent, and this increase was also statistically significant. The effects of aminophylline on the flow and extraction response slopes over the observed range of $\Delta\dot{M}\dot{V}\text{O}_2$ are graphically summarized in Figure 8.

Aminophylline significantly attenuated the coronary response to exogenous adenosine observed during pacing-induced functional hyperemia. The mean F_{AD} before

Figure 8. Graphic summary of flow response slope $\pm$ 1 SE (top graph) and extraction response slope $\pm$ 1 SE (bottom graph) when heart rate was increased to 180 bpm both before and after aminophylline. After partial blockade of the coronary response to exogenous adenosine with aminophylline, the flow response slope was significantly decreased, and the extraction response slope was significantly increased.

aminophylline was 15.9 ± 2.7 ml/min/100g compared to 4.9 ± 1.4 ml/min/100g after aminophylline ($P < 0.05$). Since the magnitude of the aminophylline-induced reduction in F_{AD} was variable from experiment to experiment, it was warranted to discern whether the reduction in the flow response slope after aminophylline was related to the reduction in F_{AD} . Figure 9 illustrates a linear correlation between the aminophylline-induced reduction in F_{AD} and the reduction in the flow response slope. The correlation was statistically significant and suggests that the attenuation of the coronary response to increased HR was due to the adenosine-antagonizing action of aminophylline.

Figure 9. Relationship between the reduction in the flow response slope ($\Delta\text{LCBF}/\Delta\text{M}\dot{\text{V}}\text{O}_2$) and reduction in the coronary response to adenosine (F_{AD}) caused by aminophylline. Each point represents the results of one experiment, and the line is the linear regression of all points ($P < 0.05$).

CHAPTER V

DISCUSSION

Early studies of coronary blood flow regulation examined the reactive hyperemic response of the coronary circulation and observed large increases in coronary blood flow associated with a proportional increase in some index of interstitial adenosine concentration (1,8,14,32,44,48, 53). The evidence from these studies supports the hypothesis that adenosine is a primary mediator of coronary reactive hyperemia. Later studies examined a more physiological manifestation of coronary blood flow regulation, functional hyperemia. These studies examined the role of adenosine in the response of coronary blood flow to an increase in myocardial oxygen demand, rather than to a decrease in oxygen supply as seen with reactive hyperemia. Many of these studies showed strong correlations between myocardial oxygen requirements and coronary blood flow (18,38,41,55,63). These correlations have been shown to be consistent throughout a wide variety of methods used to alter myocardial oxygen consumption (33). Furthermore, these studies suggest that the primary mediator of coronary blood flow regulation, and thus the link between myocardial oxygen demand and supply, is adenosine. The results of all these

studies support the popular hypothesis that adenosine is the primary mediator of coronary blood flow.

The classic adenosine hypothesis is summarized in the model shown in Figure 10. Upon examination of Figure 10 it is apparent that one very important point in this regulatory loop is the comparison between oxygen supply and demand. According to this hypothesis, it is the imbalance between these two variables which determines the magnitude of the "error signal" which in turn determines the production rate of adenosine, $d[A]/dt(p)$. The "error signal" represents the intracellular mechanism coupling the oxygen supply-demand balance of the myocardium to the degradation-production of the purine nucleotides (ATP, ADP and 5'-AMP). Although the mechanism remains unclear, it has been shown that 5'-AMP increases during a negative oxygen balance (due to ATP degradation), and thus, a negative "error signal" results in an increase in 5'-AMP. Adenosine is formed at the myocardial cell membrane by the action of 5'-nucleotidase which cleaves the phosphate group from 5'-AMP. Adenosine then diffuses into the interstitial fluid across the myocardial cell membrane. The concentration of adenosine in the interstitium is determined by the rate of adenosine production minus the rates of reuptake of adenosine by the myocardial cells and by red blood cells $[-d[A]/dt(u)]$, and washout of adenosine by coronary blood flow $[-d[A]/dt(w)]$. The adenosine concentration in the interstitium affects the number of

Figure 10. Model of the classic adenosine hypothesis. Net production of adenosine (n) is the result of adenosine production (p), uptake (u) and washout by blood flow (w), and determines the interstitial adenosine concentration $[A]$. Adenosine is shown to have an effect on coronary conductance (C). For a given pressure gradient (ΔP), any increase in C results in an increase in coronary flow.

vascular smooth muscle adenosine receptors which are activated and, therefore, the degree of vasodilation. The conductance (C) of the coronary bed increases with the vasodilation, and for any given pressure gradient (ΔP), coronary blood flow will also increase resulting in an increase in oxygen supply, thus, restoring the balance between oxygen supply and demand.

The negative feedback loop shown in Figure 10 would have to be applicable to each manifestation of coronary blood flow regulation (reactive hyperemia and functional hyperemia) if it is to fully describe this regulation. If the data from the studies previously mentioned were applied to the model, it can be seen that for both the reactive hyperemia and functional hyperemia responses it would be expected to observe correlations between myocardial oxygen consumption, the increase in adenosine concentration and coronary flow following a decrease in oxygen supply or an increase in cardiac activity. Such correlations indeed exist, but do not necessarily show a direct cause-effect relationship.

In the present study, the adenosine antagonist aminophylline was used to actually test the role of adenosine in coronary functional hyperemia induced by one specific type of perturbation, an increase in HR. During the increase in $\dot{MVO}_2$ elicited by the increase in HR, aminophylline was employed to open the negative feedback loop by blocking the

coronary vascular adenosine receptor. If adenosine is indeed an important metabolic signal in coronary functional hyperemia, blocking the vascular action of adenosine would be expected to attenuate the increase in coronary blood flow that normally accompanies the increase in $\dot{MVO}_2$. The results of the present experiments support the hypothesis that adenosine is an important factor involved in this regulatory system. Thus, after partial but significant blockade of the coronary response to exogenous adenosine, there was also a significant attenuation of the coronary response to the pacing-induced increase in $\dot{MVO}_2$, as indicated by a reduction in the flow response slope $\Delta LCBF/\Delta \dot{MVO}_2$ by about 20 per cent. Accompanying this aminophylline-induced attenuation of the coronary blood flow response, there was an increase in the extraction response slope $\Delta(a-v)O_2/\Delta \dot{MVO}_2$ by 100 per cent. This observation suggests that for a given increase in $\dot{MVO}_2$ after aminophylline, the myocardium relied more heavily on an increase in oxygen extraction. It is emphasized that the present results do not provide information regarding the precise quantitative contribution of adenosine to pacing-induced hyperemia since the blockade of the coronary response to exogenous adenosine was only partial. However, these results strongly imply that adenosine is involved in pacing-induced functional hyperemia and is necessary for its full expression.

The results of the present study are in marked contrast to the results of previous experiments from this laboratory (28) suggesting that adenosine was not necessary for the coronary functional hyperemia accompanying an increase in left ventricular inotropic state. These previous experiments used an experimental preparation identical to the preparation used in the present study differing only in the type of cardiac stimulus. In the previous experiments, instead of pacing the heart, a positive inotropic action in the left ventricle was elicited by infusion of norepinephrine into the left coronary circulation. The results demonstrated that the flow response slope was unaffected by aminophylline, although the coronary response to exogenous adenosine was almost totally abolished. According to the model shown in Figure 10, the failure of aminophylline to attenuate the norepinephrine-induced functional hyperemia could possibly be explained if adenosine production was greater in the presence of aminophylline. If aminophylline were added to the model following an inotropic stimulus, it would initially block the increase in conductance (C) of the coronary bed preventing an increase in oxygen supply. Thus, if oxygen demand remains elevated, any imbalance in oxygen supply and demand would remain. In other words, the "error signal" would persist and adenosine production would increase to the point that interstitial concentrations of adenosine could compete with aminophylline for the vascular

adenosine receptors. This would result in an increase in coronary conductance and oxygen supply to the myocardium, and would reestablish the balance between oxygen supply and demand.

To determine if adenosine production actually did increase in the presence of aminophylline, a second set of experiments was performed in the previous study to measure myocardial adenosine concentrations before and during nor-epinephrine-induced hyperemia with and without the presence of aminophylline. Four groups of dogs were used. The results of these experiments are shown in Figures 3 and 4. These figures show that the relationships between myocardial adenosine, $\dot{MVO}_2$ and LCBF were not altered by the presence of aminophylline during these experiments. Thus, the failure of aminophylline to attenuate the inotropically-induced functional hyperemia was therefore not attributable to greater concentrations of adenosine displacing aminophylline at the coronary vascular receptor. In addition, the linear relationships between myocardial adenosine concentration and $\dot{MVO}_2$, and between myocardial adenosine concentration and LCBF were no different from those before aminophylline. In this regard, it is interesting to note that these linear relationships observed in our previous experiments are compatible with the results by others (18,33,38,41,55,63) and indicate the potential for adenosine involvement in the

functional hyperemia accompanying an increase in inotropic state, although our results further indicated that adenosine was not necessary for the response.

The design of the present and previous studies was based on the ability of aminophylline to block the vascular action of adenosine. In this regard, experiments in intact hearts (1,8,14,27,43) as well as in isolated coronary vessels (29) have demonstrated that the methylxanthines block the vascular adenosine receptor. Additionally, the present experiment demonstrated that during the actual conditions of the functional hyperemia, aminophylline was effective in attenuating the coronary response to exogenous adenosine. Nevertheless, it is recognized that the methylxanthines exert actions in addition to the action on the vascular adenosine receptor (47). Furthermore, these actions may introduce a degree of uncertainty into the interpretations of the present results. However, since the aminophylline-induced reduction in the flow response slope was significantly correlated with the reduction in the coronary response to exogenous adenosine, it is felt that the effect of aminophylline on the coronary functional hyperemic response is primarily related to its action on the coronary adenosine receptor.

The basis for the difference between the present results and our previous results (28) is unclear. Thus, if the difference between oxygen demand by the myocardium and

oxygen supply to the myocardium (by the coronary circulation) were the sole "error signal" which initiates the formation of some vasodilator metabolite, the mechanism by which oxygen demand is increased would have no bearing on the nature of the metabolic signal. In this case, aminophylline should have the same effect on coronary functional hyperemia whether $\dot{MVO}_2$ was increased by an inotropic or a chronotropic stimulus. However, such was not the case. As an alternative hypothesis, it may be suggested that an increase in cardiac activity per se in some ways causes formation of several vasodilator substances. A similar possibility has been suggested by Olsson and Steinhart (45). In this case, it is more likely that the manner in which cardiac activity is increased determines the relative contribution of the various vasodilators to the functional hyperemia. The results of the present experiments in conjunction with our previous results (28) are more compatible with this alternative hypothesis. Thus, with an increase in cardiac activity by increased heart rate, adenosine may be necessary for full expression of the functional hyperemic response. However, with an increase in cardiac activity by increased myocardial contractile performance, the other metabolites may assume an equally important role as that of the adenosine. These other vasodilator substances may be sufficiently powerful to elicit a full

hyperemic response even in the absence of an adenosine effect.

Figure 11 illustrates a modified model of the classic adenosine hypothesis shown in Figure 10. This new model is more compatible with our new results. Note that the difference between this model and the classic model (Figure 10) is an additional loop of other vasodilator metabolites which are released in response to an increase in cardiac activity, and act on the coronary arteries to increase flow. With the addition of this loop, cardiac activity per se becomes a primary determinant of which vasodilators will have the primary role in regulating the coronary functional hyperemic response rather than the imbalance between oxygen supply and demand. Thus, according to the results of our two studies, when cardiac activity is increased by an inotropic stimulus, other vasodilator metabolites (M) are released that are equipotent to adenosine in increasing coronary flow without an imbalance between oxygen supply and demand being necessary. The increase in cardiac activity also increases $\dot{MVO}_2$ which results in an imbalance between oxygen supply and demand and leads to the production of adenosine. In the presence of aminophylline the vasodilatory response to adenosine is blocked and thus, is prevented from increasing coronary flow. However, the response to the other vasodilator metabolites is not blocked and therefore, coronary functional hyperemia is not attenuated. On the other hand,

Figure 11. Modified model of the classic adenosine hypothesis. This model illustrates the release of other vasodilator metabolites [M] in response to an increase in cardiac activity. Interstitial adenosine [A] may also be involved in the dilatory response.

if cardiac activity is increased by a chronotropic stimulus, the role of the other vasodilator metabolites seems to be less important than adenosine. If the vasodilatory response to adenosine is blocked by aminophylline, the vasodilatory response to the other vasodilator metabolites is insufficient to increase coronary flow to meet the oxygen demand. Thus, the myocardium increases oxygen extraction from the blood $[(a-v)O_2]$ in an attempt to increase oxygen supply.

The primary difference between the present study and our previous study was the stimulus used to increase cardiac activity. Perhaps differences in the metabolic mechanisms of each stimulus resulted in different means of coronary flow regulation. A chronotropic stimulus involves the rate of depolarization of the myocardial cell membranes and increases cardiac activity by increasing the number of beats per minute. Whereas, an inotropic stimulus involves the regulation of the contractile proteins and increases the rate of myosin ATPase activity, and thus increases cardiac activity by increasing the velocity and force of contraction. Although both stimuli increase cardiac activity, each of these processes utilizes the purine nucleotides in a different manner. Also, it has been suggested by Olsson et al. (45) that an intracellular protein-bound adenosine pool exist in addition to the purine nucleotide pool. Schrader et al. (57) and Schutz et al. (58) have investigated the possible physiological function of this intracellular

adenosine pool. Therefore, it is possible that an increase in cardiac metabolism caused by either an inotropic or a chronotropic stimulus may involve different mechanisms for coronary blood flow regulation.

Neither our previous experiments (28) nor the present study examined the roles of other specific vasodilator metabolites. Since coronary sinus P_{O_2} , P_{CO_2} , and pH were not measured, it cannot be discounted that differences in these variables between the two studies explain the difference in the results. In this regard, it may be pointed out that the initial Ca_{O_2} and $(a-v)O_2$ were similar between the two studies, and the data suggest that these factors under non-hyperemic conditions did not vary appreciably during the course of the protocol (Table 1). Therefore, it seems unlikely that differences in tissue P_{O_2} can account for the differences in results observed.

Manfredi and Sparks (37) recently reported that the hyperemia elicited by norepinephrine into the dog coronary circulation was accompanied by an increased adenosine release by the heart, but that the hyperemia elicited by atrial pacing was not. Our previous results (28) showing an increased myocardial adenosine level with norepinephrine infusion are in concert with the results of Manfredi and Sparks, although our results questioned the role of adenosine in the functional hyperemia. On the other hand, the

present results supporting a role for adenosine in pacing-induced hyperemia are difficult to reconcile with the atrial pacing data of Manfredi and Sparks. Thus, if adenosine is involved in the hyperemia observed with an increase in heart rate, it is reasonable to predict that interstitial adenosine concentration in the region of the coronary microvessels also increases. Although these seemingly disparate results have no readily apparent resolution, it should be pointed out that other researchers have reported an increased myocardial (54) and pericardial perfusate (33) adenosine level during atrial pacing.

The modification of the adenosine hypothesis is supported by the results of other studies which have found that the classic hypothesis is inadequate to fully explain the regulation of coronary blood flow. Lammerant and Becsei (36) also demonstrated an inhibition of pacing-induced hyperemia during infusion of aminophylline in anesthetized closed-chest dogs. These results are in concert with those from our pacing study. Downey et al. (16) examined non-ischemic myocardial hypoxia in anesthetized dogs and found that after 3 minutes of perfusion coronary flow had increased to the level of peak hyperemic flow following the release of an occlusion. The most interesting point of this study was that tissue adenosine and inosine concentrations decreased, rather than increased during the hypoxia. These results suggest that adenosine was not responsible for the

vasodilation and that some other mechanism, perhaps a direct relaxant effect of O_2 was involved. Two other studies have indicated that adenosine may not be the only mechanism involved in the vasodilatory response. In the first study (21), labeled adenosine deaminase was infused into conscious domestic swine with severe coronary artery stenosis. The concentration of adenosine deaminase in the myocardial interstitium was calculated to be at least one order of magnitude greater than that required to degrade all adenosine released in response to a 15-30 occlusion. Regional coronary flow, measured by microspheres, was reduced only slightly in absence of adenosine and may have been compensated by other mechanisms. The second study suggests that the release of adenosine in response to an inotropic stimulus may be a phasic release (15). Isolated guinea pig hearts were used to demonstrate that coronary perfusate adenosine levels peaked soon after the inotropic stimulus and then waned, although coronary flow and MVO_2 remained elevated. This suggests that adenosine may not maintain sustained metabolic dilation, and that other mechanisms may be involved in sustaining the increase in flow. Each of these studies has demonstrated that the imbalance between oxygen demand and supply is insufficient to fully explain the regulation of coronary blood flow. And furthermore, they suggest the role of other vasodilator mechanisms.

Several questions have been generated from the results of our two studies. First, which vasodilator metabolites are involved in inotropically-induced functional hyperemia, and how does the increase in cardiac activity signal their release? Many possible metabolic vasodilators have been previously suggested to play a role in the regulation of coronary blood flow (5). Some of these possibilities are: pH, CO₂, K⁺, lactate, O₂ tension, and others. Second, what role does the intracellular adenosine pool play, and what are its mechanisms for release? And finally, does adenosine have a regulatory role in cardiac metabolism in addition to its vasodilator role? Adenosine has been shown to have regulatory functions in other organs as well as the heart (7). Perhaps understanding the interrelationships of these functions will elucidate the mechanism by which cardiac activity signals the release of adenosine and the other metabolites.

Conclusions.--The results of the present study have shown that adenosine is necessary for full expression of coronary functional hyperemia induced by an increase in heart rate. These results differ from those of our previous study which indicated that adenosine was not necessary for full expression of coronary functional hyperemia induced by intracoronary norepinephrine infusion. Comparison of the

two studies suggests that the classic adenosine hypothesis should be modified as shown in Figure 11.

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