AMINOPHYLLINE AND SUSTAINED EXERCISE HYPEREMIA

By

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ABSTRACT

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Adenosine has been proposed as a metabolic vasoregulator of skeletal muscle blood flow. This study was designed to examine the role of endogenously released adenosine in mediating sustained exercise hyperemia in blood perfused canine skeletal muscle. It was hypothesized that adenosine released from skeletal muscle in response to an increase in metabolic demand contributes to sustained hyperemia during muscle contraction. This was tested by measuring the flow response to 3 Hz exercise in the presence and absence of aminophylline, an adenosine receptor antagonist. Adenosine release and plasma venous adenosine concentration were chosen as indices to assess changes in interstitial adenosine concentration in response to 3 Hz exercise.

Blood flow and oxygen consumption increased 5.6 fold and 26.2 fold in response to 3 Hz exercise. In the presence of an effective receptor blockade, aminophylline had no effect on the oxygen consumption/flow relationship during rest or sustained exercise. Adenosine release was significantly elevated above rest in response to 3 Hz stimulation (-0.25 ± 0.09 vs 1.24 ± 0.30 nmol/min/100g) while plasma venous concentration was not significantly different (0.060 ± 0.008 vs 0.077 ± 0.017 uM).

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Using a mathematical model to describe transcapillary exchange of adenosine, the relationship between interstitial concentration and adenosine release or plasma venous concentration was examined. The model indicates the relationship between interstitial concentration and adenosine release is much more dependent on flow, capillary permeability-surface area (PS), and arterial adenosine concentration than is the relationship between interstitial and venous concentration. For given values of flow, arterial and venous plasma adenosine concentration, and assumed values of PS, the model predicts no significant change in interstitial levels in response to 3 Hz exercise (0.69 ± 0.10 vs 0.90 ± 0.19 uM). The sensitivity of adenosine release to physiologic changes in these variables explains the increased release in the absence of enhanced interstitial levels. Under the conditions of these experiments, the model indicates that venous adenosine concentration is a better index of interstitial adenosine concentration than adenosine release. Since venous concentration did not increase in response to 3 Hz exercise, we attribute the lack of effect of aminophylline on sustained hyperemia to a lack of increase in interstitial adenosine concentration. We conclude that adenosine does not mediate sustained hyperemia during 3 Hz free flow exercise.

Dedicated to my parents,
Loren and Janet Thompson,

and sisters and brothers,
Karen, Gail, Don, and Jim,

for their love, support, and strength.

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INTRODUCTION

The control of skeletal muscle blood flow is important in maintaining proper nutrition of skeletal muscle and cardiovascular homeostasis. Since Gaskell's 1877 observation that changes in blood flow were associated with changes in muscle effort, investigators have studied the close feedback relationship between muscle metabolism and blood flow. Gaskell proposed that substances released from contracting muscle are responsible for the flow changes observed in that muscle. His work provided the original evidence for the metabolic hypothesis for control of organ blood flow.

The metabolic hypothesis states that regional blood flow is regulated by changes in metabolite concentration (Gaskell, 1877; Haddy and Scott, 1968; Sparks and Belloni, 1978). This hypothesis proposes that an increase in interstitial concentration of vasodilator substance(s), proportional to the metabolic rate of the tissue, is responsible for relaxation of vascular smooth muscle. The interstitial concentration is dependent upon the balance between the rate of vasodilator release and the rate of removal. The processes responsible for interstitial metabolite removal are cellular uptake, biochemical degration, and flow washout into the capillary compartment. The contribution of each process differs depending on the metabolite released. Elucidating the mechanisms responsible for

metabolic control of blood flow will lead to an understanding of how the cellular mechanisms of different cell types interact to provide homeostasis at the tissue level.

Increases in oxygen consumption are linearly related to increases in flow in skeletal muscle (Kramer et al., 1939). Skeletal muscle derives its energy from oxygen, an electron acceptor in oxidative processes of cellular respiration (Jöbsis, 1977). Although vasoactive metabolites are produced during oxidative processes, the chemical link between these metabolites and oxygen consumption and flow has not been demonstrated. However, vasodilator metabolites from both oxidative and non oxidative processes may mediate resistance changes. The relative contribution of aerobic versus anaerobic metabolism of the tissue would determine the relative importance of oxidative and non oxidative metabolites.

Mechanisms responsible for vascular relaxation depend upon tissue oxygen levels. This was demonstrated by examining the changes in vascular resistance concomitant with changes in venous oxygen content of canine muscles during brief tetanic contractions (Mohrman and Sparks, 1973). At low constant flow (25 ± 7 ml/100g/min), changes in venous oxygen content in response to sinusoidal changes in contraction (between 0.5 to 1.0 Hz) precede changes in vascular resistance. This finding suggests that vascular relaxation is related to changes in tissue oxygen. However, the relationship between venous oxygen content and vascular resistance can be altered by changing the oxygen supply to the muscles. At high constant flow (56 ± 8 ml/100g/min) changes in vascular resistance are faster than changes of venous oxygen content. These data suggest that at high

constant flow when the oxygen supply is in excess of demand, the effect of changes in tissue oxygen on vascular resistance can be reduced. These results indicate that during muscle contraction, both oxidative and non oxidative processes may be initiated, but vasodilator metabolites may contribute differently to the change in vascular resistance.

Identification of the local vascular control mechanisms responsible for regulating skeletal muscle blood flow is complicated. This is due to differences in muscle fiber composition between various preparations of the same species and similar preparations of different species. Since local feedback control is dependent on metabolic products released from the cell, differences in the ability of muscles to regulate blood flow in response to changes in oxygen consumption may originate in metabolic processes (Folkow and Halicka, 1968; Hilton et al., 1970; Hudlicka, 1969); Hudlicka et al., 1973). Histochemical studies and biochemical profiles of muscle fiber type have been used to explain the differences in oxygen consumption between various muscle groups (Folkow and Halick, 1968; Bockman and McKenzie, 1983). Skeletal muscle metabolism is dependent upon the proportion of white fast twitch and red slow twitch fibers (Maxwell et al., 1977; Karlson et al., 1972). Fibers classified as white fast twitch rely predominantly on glycolysis for energy, whereas red slow twitch fibers rely on oxidative phosphorylation. During exercise the proportion of vasodilator metabolites released may vary depending on the aerobic capacity of the muscle. Skeletal muscles which are predominantly white fast twitch fibers may have different blood flow control mechanisms than muscles containing a greater proportion of

red fibers. Therefore, differences in muscle fiber composition and oxidative capacity should be considered when studying regulation of muscle blood flow.

Bockman, McKenzie, and Ferguson (1980) showed that in muscles of different fiber composition (cat soleus [100% high oxidative] and gracilis [70% low oxidative]) the percent increase in blood flow in response to varied frequencies (0.25, 0.5, and 1.0 Hz) of contraction was not different. These results indicate that the regulation of blood flow in different feline muscles may be mediated by similar vascular mechanisms. However, since the oxygen consumption/flow relationship during contraction was not examined it is difficult to determine if the flow response differed quantitatively with changes in oxygen consumption.

In addition to differences in fiber composition and major metabolic pathways, metabolic regulation of exercise hyperemia is dependent upon the type of muscle contraction (tetanic, twitch, or sustained), duration of exercise, and rate of oxygen delivery to muscle preparations (Sparks, 1978). The mechanisms responsible for regulation of vascular relaxation in tetanic contraction and constant flow exercise have been extensively studied. However, little evidence is available to describe mechanisms mediating vasodilation in sustained free flow exercise. This study examines the possible role of adenosine in regulation of skeletal muscle blood flow in canine muscles (primarily oxidative fibers) perfused under free flow (constant pressure) conditions. Because of the potential for redundant vasodilator mechanisms it is likely that several

metabolites, rather than a single substance, are responsible for the sustained hyperemic response measured during continuous contraction.

PROPOSED PHYSIOLOGICAL REGULATORS OF MUSCLE BLOOD FLOW

Oxygen is a key factor in local control of blood flow. There is a close relationship between tissue gas exchange, tissue metabolism, and vascular smooth muscle function. The vascular response to reduced arterial oxygen supply may be mediated directly by reduced vessel wall oxygen tension (PO_2) or indirectly by reduced tissue PO_2. Considerable evidence favors the view that vascular relaxation occurs in response to reduced tissue PO_2 and is mediated by vasodilator substances released from parenchymal cells (Berne et al., 1957; Duling, 1974; Duling and Pittman, 1975; Stowe et al., 1975) rather than a direct effect of reduced PO_2 on vascular smooth muscle (Chang and Detar, 1980; Jackson and Duling, 1983).

The effect of reduced tissue PO_2 was examined in hamster cheek pouch preparations by increasing the oxygen content of the suffusion solution and measuring changes in arteriolar diameter (Duling, 1974). The direct effect of oxygen on the vascular wall was examined by micropipette application of solutions containing a high oxygen content relative to the perivascular space of arterioles. Global increases (10 to 150 mmHg) in tissue PO_2 produced larger decreases in arteriolar diameter (7 ± 1 um) than local increases (186 ± 34 mmHg) in perivascular PO_2. These results suggest that the vascular smooth muscle is more sensitive to global PO_2 changes (in the parenchyma) than to local PO_2 changes (surrounding the arteriolar surface).

However, using isolated arterial strips (hog carotid artery) Pittman and Duling (1973) observed a direct effect of oxygen on vascular smooth muscle, demonstrating that the vascular strip relaxed in response to reduced bath PO_2. The vascular response to reduced oxygen in these experiments was attributed to unstirred layers surrounding the tissue and the large diffusion distance for oxygen which lead to the development of anoxic cores in the vascular wall. Under physiologic conditions the vascular wall PO_2 never decreases to levels producing anoxia (10-20 Torr); therefore the direct effect of oxygen on vascular smooth muscle was not considered important in local control of blood flow.

Chang and Detar (1980) calculated the vascular wall PO_2 for a given bath PO_2 in vascular segments from aorta, femoral artery, and intramuscular arteries of rabbit. These calculations included the effect of diffusion distances and unstirred layers. They showed that relaxation of large conducting arteries (2 mm) and small parenchymal arteries (300 um) occurred when vascular wall PO_2 fell below 50 Torr. This study suggests that vascular smooth muscle is sensitive to physiologic changes in PO_2. These data argue against a role for anoxic cores in dilation induced by lowered tissue PO_2 and suggest that vascular smooth muscle is sensitive to oxygen levels above the critical PO_2 for anoxia. These results suggest that a direct effect of oxygen may be significant in local control of blood flow.

The role of a direct effect of oxygen on vascular smooth muscle function has been a point of controversy for ten years. However, recent evidence provides support for a direct effect of oxygen on vascular smooth muscle and Jackson and Duling (1983) suggest that

arteriolar oxygen sensitivity may be greater than previously thought (Duling, 1974). In hamster cheek pouch preparations, the effect of oxygen on arteriolar smooth muscle was assessed in vessel segments with their surrounding parenchyma removed along a 1 mm length of vessel (Jackson and Duling, 1983). Graded increases (0, 10, 21, and 95%) in oxygen tension of the suffusion solution produced graded decreases (38, 31, 28, and 24 um) in arteriolar diameter. These results suggest that the parenchyma is not required for oxygen induced constriction and that arteriolar smooth muscle is responsive to changes in vessel wall PO_2. These results are in conflict with Duling's earlier observation (1974) that local changes in PO_2 do not alter arteriolar diameter. To explain these divergent results, Jackson and Duling (1983) suggest that local changes in PO_2 (induced by micropipette application of solutions) influence only a small portion of the vascular wall and therefore may be insufficient to produce changes in vessel diameter. Therefore, changes in vessel diameter in response to a direct effect of oxygen may only be apparent when a greater length of the vascular wall is uniformally exposed to changes in oxygen.

Arteriolar wall PO_2 is influenced by intravascular PO_2 to a greater extent than by tissue PO_2 (Duling and Pittman, 1975). This is because of the high oxygen carrying capacity of the blood and oxygen permeability of the arteriolar wall. The role of a direct effect of oxygen on arteriolar vascular smooth muscle during exercise is unknown, since arteriolar wall PO_2 may actually increase in response to an increased blood flow (Sparks, 1980). Gorczynski and Duling (1976) demonstrated that arteriolar wall PO_2 does not decrease

during muscle contraction. It is therefore unlikely that relaxation of vascular smooth muscle during exercise is mediated by a direct effect of PO_2 on arteriolar smooth muscle. Despite the ability of vascular smooth muscle to react to changes in vessel wall PO_2, changes in vessel wall PO_2 probably do not contribute to vascular relaxation under conditions of adequate oxygen supply.

Potassium (K^+) released from the interstitium has been proposed to mediate the increase in vascular conductance seen at the onset of exercise (Kjellmer, 1965; Haddy and Scott, 1975). Action potentials of skeletal muscle result from sodium (Na^+) influx into cells and potassium efflux into the interstitium. Potassium has been proposed to reduce vascular resistance by hyperpolarization of vascular smooth muscle via stimulation of the electrogenic Na^+/K^+ pump (Bonaccorsi et al., 1977). Intraarterial infusions of potassium cause an increase in flow (Dawes, 1941; Kjellmer, 1965) and produce changes equivalent to exercise in arteriolar resistance, capillary exchange of diffusible substances, and venous capacitance (Kjellmer, 1964; Kjellmer and Odelram, 1965). However, the magnitude of K^+ induced flow increase is less than that associated with exercise (Barcroft, 1968; Scott et al., 1970). Furthermore, during sustained muscle contraction, venous potassium concentration returns to control while flow remains elevated and resistance decreased (Stowe et al., 1975). Therefore, in canine muscles potassium may mediate the onset of increased conductance in response to exercise but may not be responsible for sustained hyperemia.

In feline gracilis muscles, ouabain, which inhibits the Na^+/K^+ pump, significantly reduced the hyperemic response induced by

isometric contraction (Bockman, 1983). This effect was not observed at the same contraction rate in feline soleus muscles. The change in blood flow correlated with the change in K^+ release in gracilis muscles ($r=0.95$, $P<0.001$) but not in soleus muscles. These results suggest that K^+ release in feline gracilis muscles may play a role in mediating sustained hyperemia in this preparation.

Increases in tissue osmolarity have been proposed to mediate exercise hyperemia. Tissue osmolarity was evaluated because of the increase in venous osmolarity observed during exercise (Mellander and Lundvall, 1971). However, the importance of osmolarity in mediating flow may vary with the type of muscle fiber (Mellander and Lundvall, 1971). During equivalent exercise, canine muscles (predominantly oxidative fibers) fail to show increases in venous osmolarity equivalent to feline muscle (glycolytic and oxidative fibers) or forearm muscles of man (glycolytic and oxidative fibers) (Scott et al., 1970). Continuous infusion of hypertonic solutions in resting canine muscles does not increase flow to the level of the exercise equivalent or cause sustained increases in flow. Furthermore, in sustained contraction of canine muscles, venous osmolarity returns to control within minutes while flow remains elevated (Stowe et al., 1975; Morganroth et al., 1975; Scott et al., 1970). However, in feline muscles, having a higher proportion of glycolytic fibers than canine muscle, venous osmolarity during exercise remains elevated above control throughout the contraction period (Scott et al., 1970). These studies suggest that the role of osmolarity in mediating sustained hyperemia during continuous contraction may be less important in muscles of predominantly oxidative fibers. Since the

capacity to generate osmotically active products from glycolytic processes is greater than oxidative processes, the role of osmolarity has been considered to be more important in muscles with a greater proportion of glycolytic fibers.

Since the vasodilator properties of potassium and osmolarity increase during hypoxia (Skinner and Costin, 1970; 1971) it is possible that the vascular sensitivity to these substances increases during exercise. This implies that lower levels of potassium and/or osmolarity are required to mediate the flow response, and could explain how flow is maintained in the presence of lower venous concentrations. Mohrman (1982) proposed that vascular sensitivity to potassium or osmolarity increases during exercise. To test this hypothesis he examined the oxygen consumption/flow relationship for canine hindlimb muscles during 0.0, 0.5, 1.0, 4.0, and 6.0 Hz contraction in the presence and absence of intraarterial infusion of potassium or hypertonic saline. There were no differences in flow at given exercise rates before or after potassium or hyperosmolar infusions. Furthermore, the oxygen consumption/flow relationship was the same regardless of the infusate, suggesting that neither potassium nor osmolarity alters the vascular sensitivity to other vasodilator metabolites released during exercise. Since there is no sustained dilator effect of potassium or osmolarity on exercising muscle, these results indicate that potassium and osmolarity cannot be responsible for sustained exercise hyperemia.

Hydrogen ion (Haddy and Scott, 1968; Scott et al., 1970), inorganic phosphate (Dobson et al., 1971; Hilton et al., 1970) and

magnesium ion (Haddy, 1960; Altura and Altura, 1978; Scott et al., 1970) have also been proposed to mediate exercise hyperemia. Although venous concentrations of these substances increase in response to exercise, their ability to vasodilate when infused in skeletal muscle is weak. Therefore, the role of these substances in mediating exercise hyperemia is considered to be negligible.

Prostaglandins have recently been examined as potential mediators of exercise hyperemia. Formation of prostaglandins occurs via metabolism of arachidonic acid in response to reduced oxygen levels (Samuelsson et al., 1978). Prostaglandins (PG) E_1, E_2, A_1, A_2, (Conway and Hutton, 1975; Greenberg and Sparks, 1969; Kadowitz, 1972) and I_2 (Dusting and Vane, 1980) are potent vasodilators when infused into skeletal muscle. Young and Sparks found increased release of PGE_2 from canine hindlimb preparations during free flow (1980) and restricted flow (1979) exercise. The hyperemic response during free flow exercise was not reduced when prostaglandin synthesis was inhibited by indomethacin, suggesting that prostaglandins may not play a role in mediating sustained hyperemia. However, during restricted flow exercise, the return of vascular resistance to control after cessation of exercise was slowed in the presence of indomethacin. This suggests that prostaglandins may be more important under conditions of impaired oxygen supply to muscle. The role of other metabolites of arachidonic acid, including leukotrienes, has not been thoroughly investigated. Until the biochemical control of arachidonic acid metabolism is understood, the possibility that these substances play a role in metabolic vasodilation cannot be eliminated.

Adenosine triphosphate (ATP) may also be involved in mediating exercise hyperemia. Venous concentration of ATP increases during skeletal muscle exercise in both man (Forrester and Lind, 1969) and dog (Chen et al., 1972). Intraarterial infusions of ATP result in increases in flow equivalent to that measured during exercise (Drury and Szent-Gyorgyi, 1929). The ATP induced vasodilation is attributed to arteriolar relaxation (Kjellmer and Odelram, 1965). However, Kjellmer and Odelram (1965) suggest that ATP should not be considered a physiological regulator of flow since ATP infusion also produces dilation of venous capacitance vessels. Venous capacitance vessels do not dilate during exercise.

In addition, it is unclear if the highly charge ATP molecule can be transported across cell membranes (Glynn, 1968; Dierterle et al., 1978; Chaudry, 1982). ATP has been shown to be released from nerve endings in brain as a cotransmitter with norepinephrine (Kuroda, 1978). Studies using double isotope labeled ATP (^{32}P and ^{14}C) suggest that ATP is capable of being taken up by cells as ATP, without being degraded to adenosine (Chaudry and Baue, 1980). Also, in the presence of probenecid (a transport inhibitor of organic acids) ATP uptake by rat hepatocytes is inhibited. Probenecid did not inhibit adenosine uptake (Chaudry and Clemens, 1982), further supporting the idea that ATP can cross cell membranes without first being degraded to adenosine. Because of the presence of ectonucleotidases (Pearson, Carelton, and Gordon, 1980), if ATP is released from muscle cells, it is likely that it would be rapidly degraded to adenosine diphosphate (ADP), adenosine monophosphate (AMP), or adenosine and therefore would not appear as ATP in the

venous effluent. Presently no evidence is available to rule out ATP or other adenine nucleotides as mediators of exercise blood flow. Therefore, ATP remains a viable candidate for mediating sustained hyperemia.

Acetate has also been considered as a potential mediator of exercise hyperemia. Tissue acetate content of canine gracilis muscle increases linearly with graded increases in twitch rate (0.5, 1.0, and 2.0 Hz) of canine gracilis muscle (Steffen et al., 1982). During exercise, increases in tissue acetate content, venous concentration, and acetate release correlate with decreased vascular resistance ($r=0.75$, $P<0.001$). In a separate study, intraarterial infusions of sodium acetate (at concentrations equivalent to venous exercise levels) produced increased tissue adenosine content (Steffen et al., 1983). Graded increases in twitch rate (0.5, 1.0, and 2.0 Hz) produced elevations in tissue content of both adenosine and acetate. The increase in tissue adenosine and acetate correlated negatively with vascular resistance ($r=-0.60$, $P<0.005$ and $r=-0.83$, $P<0.001$, respectively). These results indicate that acetate may act to increase interstitial adenosine during exercise, providing the link between metabolism and vascular smooth muscle.

ADENOSINE PRODUCTION IN SKELETAL MUSCLE

In 1963, Berne proposed the adenosine hypothesis for regulation of coronary blood flow. This hypothesis states that coronary blood flow is regulated by changes in the interstitial concentration of adenosine proportional to changes in tissue metabolism. The

similarity of cardiac and skeletal muscle blood flow responses to alterations in metabolism suggests that similar blood flow control mechanism may operate in both tissues (Rubio et al., 1973). Because of the close association between oxygen consumption and blood flow (Kramer et al., 1939) and the predominance of oxidative fiber types in canine skeletal muscle (Maxwell et al., 1977), adenosine (as a product of aerobic metabolism in skeletal muscle) has been proposed as a metabolic vasodilator in skeletal muscle.

The major pathway of adenosine formation is via dephosphorylation of adenosine monophosphate (AMP) by ecto 5'nucleotidase. The site of adenosine formation in skeletal muscle is controversial and central to the postulate that adenosine released from skeletal muscle cells causes relaxation of vascular smooth muscle. Histochemical localization of the enzymes important in adenine nucleotide metabolism (Borgers et al., 1971; Nakatsu and Drummond, 1972; Rubio et al., 1973) has been used to determine sites of adenosine formation. Previously, adenosine was thought to be formed only extracellularly since 5'nucleotidase was located in the plasma membrane with its active site facing outside of the cell (Rubio et al., 1973). Based on the rapid kinetics of adenylate kinase for incorporating adenosine into AMP, it was believed that intracellular adenosine was rapidly rephosphorylated to AMP and did not exist as adenosine inside cells. Tissue adenosine content was used as an index of interstitial adenosine, since it was thought that the existing adenosine was exclusively in the interstitial space. However, intracellular distribution of cytosolic 5'nucleotidase (Itoh et al., 1978; Fritzon, 1978) and S-adenosylhomocysteine

hydrolase (Hershfield and Kredich, 1978; Schrader et al., 1981; Schütz, Schrader, and Gerlach, 1981; Ueland, 1983) in cardiac myocytes indicates that adenosine can also be formed intracellularly. Studies in isolated perfused guinea pig hearts utilizing the nucleoside transport blocker, nitrobenzylthioinosine (NBMPR), demonstrate an increase in tissue adenosine content and a decrease in adenosine release. If adenosine was found exclusively in the interstitial space, inhibition of nucleoside uptake should increase adenosine release. However, the increase in tissue content and decrease in release indicate that adenosine can be formed intracellularly. Therefore, it is likely that adenosine is formed both intra and extracellularly. The site of formation of adenosine must be considered when choosing an index to reflex interstitial adenosine.

Skeletal muscle is capable of degrading adenine nucleotides to both adenosine and inosine monophosphate (IMP). In dog skeletal muscle (predominantly oxidative fibers), AMP is primarily degraded to adenosine by 5'nucleotidase, whereas in rat skeletal muscle (containing a large percentage of glycolytic fibers) (Winder et al., 1974) AMP is degraded primarily to IMP via AMP deaminase (Rubio et al., 1973). The capacity for adenosine formation in skeletal muscle may vary depending upon the predominate fiber type of the muscle group studied.

Bockman and McKenzie (1983) compared 5'nucleotidase and AMP deaminase activity in canine cardiac muscle and the skeletal muscle of dog and cat. They found an inverse relationship between AMP deaminase activity and the oxidative capacity of the muscle. In

contrast, 5'nucleotidase activity increased in direct proportion to oxidative capacity of the muscle. They concluded that the potential for adenosine production by muscle is dependent on the ratio of 5'nucleotidase to AMP deaminase activity. The relative activity of these enzymes was related to the oxidative capacity of the tissues. Dog gracilis muscle, which is made up of primarily oxidative fiber types, is intermediate in oxidative capacity to cat gracilis and dog cardiac muscle. Canine cardiac muscle has the highest capacity. Since the relative activity of 5'nucleotidase to AMP deaminase is lower in skeletal muscle than in cardiac muscle, these results suggest that the capacity for adenosine formation may be less in muscles with a lower oxidative capacity (Bockman and McKenzie, 1983). These results indicate that the role of adenosine in mediating flow in different muscle preparations may be dependent upon the capacity for adenosine formation in those tissues.

EVIDENCE FOR ADENOSINE IN REGULATION OF MUSCLE BLOOD FLOW

The role of adenosine in mediating blood flow is unknown. Since earlier techniques for measuring adenosine in biological samples lacked the sensitivity of present techiques, the role of adenosine was originally tested under extreme conditions. These initial studies, measuring adenosine concentration under severe experimental conditions, established that skeletal muscle has the metabolic capacity to produce adenosine. With improvement in the techniques for measuring adenosine, the role of adenosine in mediating changes

in vascular resistance has been studied under physiological conditions.

Adenosine has a vasodilator effect when infused into skeletal muscle (Drury and Szent-Gyorgi, 1929). Because of this vasodilator effect, adenosine is considered a potential physiological regulator of skeletal muscle blood flow (Anrep, 1935; Dawes, 1971). Initial studies rejected adenosine as a metabolic vasodilator during exercise, since the vasodilator activity of reperfused venous blood remained after standing for 20-30 minutes (Anrep, 1935) despite the presence of degradative enzymes capable of removing the dilator activity of adenosine by deamination. However, since venous adenosine concentration was not measured, it was unknown if the adenosine concentration was sufficient to cause vasodilation. This would be possible if adenine nucleotides were released from blood elements and dephosphorylated to adenosine. Adenosine's role in mediating vasodilation was not reexamined until Berne (1963) proposed that adenosine formation could increase in response to hypoxia. Hypoxia increased adenosine formation by the intracellular breakdown of adenine nucleotides. This observation lead to extensive investigations of adenosine as a metabolic vasodilator in cardiac and skeletal muscle. In the following sections, the role of adenosine in mediating skeletal muscle blood flow is examined.

The presence of adenosine in the venous effluent of exercising skeletal muscle was first demonstrated in 1965 using the kidney and a resting skeletal muscle as bioassay organs (Scott, et al, 1965). Infusion of adenosine or AMP into the renal artery produce constriction, but identical infusion into skeletal muscle produce

dilation. Perfusion of the kidney with the venous effluent from contracting muscles (2.5 Hz) caused constriction of the renal vasculature, whereas perfusion of the noncontracting skeletal muscle preparation caused dilatation. Since adenosine and AMP were the only known endogenous substances to show this differential effect, it was reasoned that these substances were released from contracting muscle and could be responsible for mediating the hyperemia.

Adenosine metabolism in skeletal muscle was studied by measuring tissue content of adenine nucleotides and nucleosides in ischemic tissue (Imai et al., 1964). In canine skeletal muscles subjected to prolonged periods of ischemia (up to 30 minutes) there was continual decline in tissue ATP content, although tissue ADP and AMP did not decrease. Concentrations of tissue IMP, inosine, and hypoxanthine increased with the duration of ischemia. However, tissue adenosine was not measurable even after prolonged (30 minutes) periods of ischemia. With the development of more sensitive assay techniques for adenosine, Berne et al. (1971) found an increase in adenosine content in rat skeletal muscle (4 nmoles/g to 8 nmoles/g) with 5 Hz electrical stimulation. In addition, a significant increase in IMP content from 0.035 umoles/g to 2.035 umoles/g was measured during ischemia and 5 Hz stimulation. This study also demonstrated a four fold increase in adenosine concentration in the venous blood during ischemic muscle contraction and thus provided the first direct evidence that skeletal muscle was capable of producing adenosine in response to reduced oxygen delivery.

The ability of skeletal muscle to increase adenosine production in response to an increase in metabolism was demonstrated by Dobson

and coworkers (1971). Isolated canine skeletal muscle preparations were stimulated to contract at 20-30 Hz for 5 minutes while arterial inflow was completely occluded. Following the period of ischemic contraction, tissue and venous blood samples were collected and analyzed for tissue adenosine content and venous concentration, respectively. Tissue adenosine increased from 0.7 to 1.5 nmoles/g and venous adenosine concentration increased from 0.03 to 0.23 uM. These results indicate that skeletal muscle is capable of increasing adenosine production in response to the enhanced metabolic demand of severe exercise during ischemia.

Bockman et al. (1975) examined adenosine release from rat skeletal muscle perfused with Krebs bicarbonate buffer solution and stimulated for 3 minutes at varying frequencies. They demonstrated an increase in venous adenosine concentration proportional to the stimulation frequency. There was a continual increase in adenosine release during rest. The increased adenosine release was proportional to the duration of perfusion, suggesting that these preparations were hypoxic. Therefore, these results demonstrate the ability of skeletal muscle to increase adenosine release when there is an imbalance between oxygen supply and oxygen demand. These investigators suggest that adenosine could be a potential regulator of skeletal muscle blood flow.

To measure adenosine release from skeletal muscle under physiological conditions, canine skeletal muscle preparations were blood perfused at constant flow (Bockman et al., 1976). Arterial and venous concentration of adenosine were measured prior to a 30 minute contraction period (2-4 Hz) and 2 and 20 minutes following the

beginning of contraction. After 5 and 25 minutes of contraction, muscle samples were obtained for determination of tissue adenosine content. These investigators demonstrated that muscle adenosine content was significantly elevated after 10 minutes of contraction (1.97 to 8.35 nmoles/g) and remained elevated above control for the duration of the contraction period. Since the venous plasma adenosine levels did not change during the contraction period, they suggest that an increase in adenosine concentration in the venous effluent may only be detectable under conditions of severe stress. Interpreting the increase in tissue adenosine content as an increase in interstitial adenosine concentration during exercise, these investigators concluded that adenosine could mediate metabolic vasodilation.

The contribution of adenosine in mediating the vascular response that follows constant flow exercise has also been examined in an attempt to characterize the prolonged vasodilation which immediately follows the contraction period. In 1979, Belloni et al. showed a correlation between the change in tissue adenosine content and an increase in vascular resistance in canine skeletal muscle. During restricted flow exercise (4 twitches per second for 22 minutes) tissue adenosine was significantly greater than at rest (22.5 nmole/g versus 2.3 nmole/g). Immediately after the cessation of exercise, the previously elevated tissue levels fell, with a time course similar to the increase in vascular resistance. However, five minutes after cessation of exercise the time courses of adenosine content and the return of vascular resistance were no longer similar, suggesting that the effect of adenosine in regulating vascular

resistance following restricted flow exercise may predominate only immediately following the end of exercise. Elevated tissue levels of adenosine during contraction are consistent with a role for adenosine in mediating the sustained vasodilation.

Phair and Sparks (1979) examined the role of adenosine in sustained hyperemia during free flow exercise of canine muscle. Tissue adenosine content was measured during rest (2.84 and 1.13 nmol/g prior to 2 and 6 Hz, respectively) and sustained twitch rates of 2 Hz (2.88 nmol/g) and 6 Hz (1.55 nmol/g). They were unable to measure increases in tissue adenosine content during steady state exercise hyperemia. However, as arterial inflow was progressively restricted, tissue adenosine content increased, reaching 55 nmoles/g with complete ischemia. Therefore, although tissue levels did not increase under free flow conditions, adenosine content increased as oxygen supply was reduced. These results indicate that during free flow exercise, when oxygen supply to the muscle is not impaired, there is no correlation between tissue adenosine content and oxygen consumption. This suggests that interstitial adenosine concentration does not increase during exercise.

Contrary to these results (Phair and Sparks, 1979), Steffen et al. (1983) have recently shown that tissue adenosine of canine gracilis muscle content increases during natural flow exercise. Graded increases in twitch rates (0.5, 1.0, and 2.0 Hz) produced graded increases in tissue adenosine content at 1.0 and 2.0 Hz but not at 0.5 Hz. The increase in tissue adenosine content correlated with a decrease in vascular resistance ($r=0.57$, $P<0.001$). The authors of this study attribute the difference between their results

and those obtained by Phair and Sparks (1979) to a shorter freezing time after taking muscle biopsies (1 vs 5 seconds).

It was previously assumed that adenosine was formed only extracellularly from AMP by ecto 5'nucleotidase. Therefore tissue adenosine content was thought to be an adequate index of interstitial adenosine. Since nucleoside uptake (Olsson et al., 1972) and incorporation of adenosine into nucleotides is rapid, it was presumed that free intracellular adenosine did not exist. However, with the recent evidence that 5' nucleotidase (Itoh et al., 1978; Fritzon, 1978) and S-adenosylhomocysteine hydrolase (Hershfield and Kredich, 1978; Schütz, Schrader, and Gerlach, 1981; Ueland, 1983) exist within the cytosol, this assumption has been questioned.

Measurements of tissue adenosine content represent adenosine existing both intra and extracellularly. It is unknown whether intracellular adenosine is free or bound to intracellular adenosine levels, it is possible that changes in the interstitial concentraton (measured as tissue content) are undetectable, since interstitial adenosine represents a small fraction of total tissue adenosine (Sparks and Fuchs, 1983).

Adenosine concentration in arterial and venous plasma was measured during free flow exercise and used to calculate adenosine release from the tissue (Sparks and Fuchs, 1983). These measurements are independent of intracellular adenosine and are assumed to reflect the changes in extracellular adenosine responsible for vasodilation. The role of adenosine in mediating sustained hyperemia during 6 Hz exercise was examined using canine hindlimb preparations. Oxygen consumption and flow increased from 9.5 to 129.0 ml/min/100g and 0.26

to 15.0 ml/min/100g, respectively. Adenosine release during 6 Hz exercise (6.1 nmole/100g/min) was significantly ($P<0.05$) greater than the resting value (-0.1 nmol/100g/min). These results indicate that during free flow exercise, adenosine release increases concomitant with increases in oxygen consumption and blood flow.

Phair and Sparks (1979) were unable to demonstrate an increase in tissue adenosine content during 6 Hz exercise, suggesting that interstitial levels did not increase. These studies, using adenosine release (Sparks and Fuchs, 1983) and tissue adenosine content (Phair and Sparks, 1979), differ in the parameter chosen as an index of interstitial concentration. The use of venous concentration and release to detect changes in interstitial levels, independent of intracellular adenosine levels, has provided indirect evidence for the role of adenosine in mediating blood flow.

Pharmacological agents have been used to alter adenosine metabolism and test the direct effect of endogenous adenosine on vascular smooth muscle. In 1977, Tabaie et al. measured changes in perfusion pressure in response to contracting canine gracilis muscle preparations perfused at constant flow. The effect of theophylline (10^{-3}M) (an adenosine receptor antagonist) on the reduction in perfusion pressure was examined during brief (30 sec.) intervals on contraction at 1 and 6 Hz frequency. In the presence of theophylline, the maximal fall in pressure during 1 and 6 Hz exercise was reduced from control by 49% (45 to 23 mmHg) and 31% (74 to 51 mmHg), respectively. The diminished response in perfusion pressure in the presence of adenosine receptor blockade indicates that endogenous adenosine may mediate vascular relaxation during

constant flow exercise. However, in this study norepinephrine was administered to prevent the reduction in vascular resistance that occurs secondary to theophylline infusion. To test whether the attenuated responses during theophylline were due to a nonspecific effect of norepinephrine, dilator responses to injected acetylcholine and ATP were examined. These responses were not different from control responses during norepinephrine and theophylline infusion. This suggests that the reduction in exercise dilation during theophylline and norepinephrine is not due to a nonspecific norepinephrine effect that reduces the vascular sensitivity to other vasodilator metabolites.

Honig and Frierson (1980) also used theophylline (10^{-3}M) to examine the role of adenosine in mediation of exercise vasodilation. The effect of theophylline on the change in resistance was studied in canine gracilis muscles perfused at constant flow and electrically stimulated at 0.75 Hz. Changes in resistance in response to exercise were measured in the presence and absence of theophylline. Since the change in resistance was normalized to the resting levels, these investigators avoided the use of a constrictor agent to return resistance to control levels. In the presence of exogenous adenosine, theophylline reduced the relative change in resistance. However, during exercise the relationship between the change in resistance and initial resistance was unaltered by theophylline. These data indicate that adenosine does not mediate the sustained change in resistance which accompanies constant flow exercise. This result is in opposition to the observations made by Tabaie et al.

(1979). Therefore, the question of adenosine's contribution in vascular relaxation is unresolved.

The role of adenosine in mediating postexercise vasodilation following constant flow exercise was examined by altering adenosine metabolism. Dipyridamole, which blocks cellular uptake of adenosine, was infused into dog gracilis muscles (Klabunde, 1983). The effect of dipyridamole alone and dipyridamople plus adenosine deaminase during 1, 3, and 5 minutes of ischemia was examined to determine if adenosine mediates postischemic vasodilation. Dipyridamole infusion increased the tissue adenosine content above control during all periods of ischemia. The addition of adenosine deaminase attenuated this increase, returning adenosine content to control levels. In the presence of dipyridamole and adenosine deaminase there was no reduction of the postischemic vasodilation. These data do no support the hypothesis that accumulation of adenosine in the interstitium mediates the vasodilation accompanying ischemia.

The controversial issue of adenosine's role during tetanic contraction was studied by Hester et al. (1982). These investigators infused high (mM) concentrations of adenosine intraarterially for 1-3 hours to resting dog gracilis muscles. Although a hyperemic response was observed during adenosine infusion, blood flow returned to resting levels after 150 minutes of adenosine infusion. In the presence of the high exogenous adenosine concentration, with blood flow equivalent to preadenosine flow, the muscles were stimulated to contract at 10 and 20 Hz for 5 seconds. No difference in the hyperemic response to muscle contraction in the presence or absence

of the high adenosine concentrations was noted. These investigators reasoned that after 150 minutes the vascular smooth muscle was no longer responsive to exogenous adenosine and since there was no alteration in the hyperemic response during tetanic contraction, endogenous adenosine could not mediate the flow response.

Contrary to the previous study (Hester et al., 1982), Kille and Klabunde (1984) showed that adenosine may play a role in mediating the hyperemic response during tetanic contraction. These investigators measured the effect of altering adenosine metabolism on flow responses in canine gracilis muscles. Flow responses were measured during varying durations (1, 3, 5, and 10 seconds) of tetanic contraction (40 Hz). Changes in the excess oxygen consumption (amount of oxygen consumption above control) versus excess flow (area under the blood flow tracing above control) following the tetanic contraction period were determined in the presence and absence of dipyridamole (an adenosine uptake blocker), erythro-9-(2-hydroxy-3-nonyl)-adenine (EHNA) (inhibitor of adenosine deaminase), and alpha, beta-methylene adenosine 5'-diphosphate (AOPCP) (inhibitor of 5' nucleotidase). Dipyridamole and EHNA significantly increased the slope of the line, while AOPCP reduced the slope. These results suggest that dipyridamole and EHNA potentiate the flow response due to elevated interstitial adenosine levels, while AOPCP reduces the response by decreasing interstitial levels. Although no index of interstitial adenosine concentration was measured, these data support a role for adenosine in mediating the flow response during tetanic contraction.

Several reasons exist for the different result obtained by the previous groups during tetanic contraction. First, the stimulus pattern (10 and 20 Hz) used by Hester et al. (1982) may not be severe enough to induce measurable adenosine formation, while at 40 Hz (Kille and Klabunde, 1984) adenosine production may be greater. Second, since oxygen consumption was not measured in the study by Hester et al. (1982), it is unknown if tissue metabolism is altered by long term high dose adenosine infusion. Prolonged infusions of adenosine in dog hindlimb have been shown to increase the glycolytic rate of muscle. This is accompanied by a fall in oxygen consumption and an increase in venous hydrogen ion concentration and carbon dioxide tension (Weissel, Raberger, and Kraupp, 1973). Therefore, if tissue metabolism were altered by the long term high dose adenosine infusion, the metabolic status of the muscle may be different from the muscles studied by Kille and Klabunde (1984).

The role of adenosine has also been tested in cremaster muscle preparations. The advantage of this preparation is that it allows measurement of changes in individual arteriolar diameter in response to muscle fiber contraction. Proctor and Duling (1982) showed that in the presence of adenosine deaminase there is a 20% decrease in arteriolar diameter in response to muscle fiber stimulation of 1 Hz frequency. Unfortunately, neither arterial oxygen tension nor oxygen consumption was measured. It is therefore unknown if the decrease in diameter during adenosine deaminase infusion was due to a change in metabolism, an effect of adenosine deaminase, or adenosine released from hypoxic tissue. The reduction in functional diameter of the arteriole by adenosine deaminase was not different if the preparation

was perfused with 0% O_2 or 10% O_2. This suggests that adenosine release during muscle fiber contraction is independent of oxidative mechanisms mediating vasodilation. It is unknown if exercise dilation in this preparation is mediated by factors during hypoxia.

SUMMARY

The role of adenosine in mediating the hyperemic response accompanying different types of skeletal muscle contraction has been extensively studied. It has been established that skeletal muscle has the ability to produce adenosine. The site(s) of adenosine production remain obscure and controversial. However, using data inferred from cardiac muscle, there is general agreement that adenosine exists both intracellularly and extracellularly in skeletal muscle. It has been demonstrated that adenosine production by skeletal muscle increases during ischemia (Berne, 1971), ischemic contraction (Dobson et al., 1971; Berne, 1971), hypoxic perfusion (Bockman et al., 1975), and restricted flow exercise (Bockman et al., 1975; 1976; Belloni et al., 1980; Klabunde, 1982). The significance of adenosine production in regulating the relaxation of vascular smooth muscle during these conditions is unknown. The role of increased adenosine production in mediating vascular relaxation during constant flow exercise was examined by blocking adenosine receptors with theophylline (Honig and Frierson, 1980; Tabaie et al., 1977). Interstitial adenosine was not assessed in these studies (Honig and Frierson, 1980; Tabaie et al., 1977). Therefore, since divergent results were obtained, it is difficult to draw any

conclusions about the role of adenosine in mediating exercise vasodilation. Until 1979, the role of adenosine in mediating sustained exercise hyperemia during muscle contraction had not been examined. Mechanisms for sustained exercise hyperemia had been proposed from studies examining blood flow control mechanisms under completely different metabolic conditions. Contrary to results of Steffen et al. (1983), Phair and Sparks (1979) were unable to demonstrate an increase in adenosine release. Although these studies do not resolve the controversy surrounding the role of adenosine in mediating exercise hyperemia, they provide direct evidence regarding adenosine production by skeletal muscle under free flow conditions.

This study was designed to examine the role of endogenously released adenosine in mediating sustained exercise hyperemia in blood perfused canine skeletal muscle. Adenosine release and plasma venous adenosine concentration were chosen as indices to assess changes in interstitial adenosine concentration in response to exercise. The uniqueness of this study is the use of pharmacological blockade of adenosine receptors with measurements of adenosine production from contracting muscles. It was hypothesized that adenosine released from skeletal muscle cells in response to an increase in the metabolic demand contributes to sustained hyperemia during muscle contraction. The hypothesis will be rejected if blockade of adenosine receptors fails to reduce the hyperemic response during 3 Hz exercise. In addition, the hypothesis will also be rejected if interstitial adenosine concentration fails to increase in response to muscle contraction.

METHODS

ANIMAL PREPARATION

Male mongrel dogs weighing 20-45 kg were used in all experiments. Animals were initially anesthetized intravenously with sodium pentobarbital (30 mg/kg)(Sigma Pharm. Co.) and supplemented with 50 mg intravenously as needed. Anesthetic depth was assessed by observing ventilatory responses and reflex responses to tactile stimulation. Following the initial anesthetic period, animals were intubated with an endotracheal tube and ventilated by a Harvard respirator pump with room air supplemented with 100% oxygen (O_2). To maintain arterial oxygen tension (PO_2), carbon dioxide tension (PCO_2), and hydrogen ion concentration (pH) within the normal physiological range, the O_2 supplement flow rate, respiratory rate, and tidal volume were adjusted. Isotonic sodium bicarbonate solution was drip infused intravenously if metabolic acidosis was present. Physiological levels of arterial PO_2, PCO_2, and pH were 74-108 mmHg, 32-47 mmHg, and 7.386-7.462 pH units, respectively (Feigl and D'Alecy, 1972). Body temperature was maintained between 37-39 C with the use of electric heating pads and a Versa-Therm Electronic Temperature Controller (Model 2158) and monitored by a YSI esophageal probe and telethermometer (Yellow Springs Instruments).

SURGICAL PREPARATION

Canine hindlimb muscle preparations were used in all experiments. The preparation consisted of the following muscles: tibialis cranialis, extensor digitorum longus, gastrocnemius, fibularis longus, flexor hallucis longus, and flexor digitorum superficialis. Surgical preparation is described in the following sections and is illustrated in Figure 1.

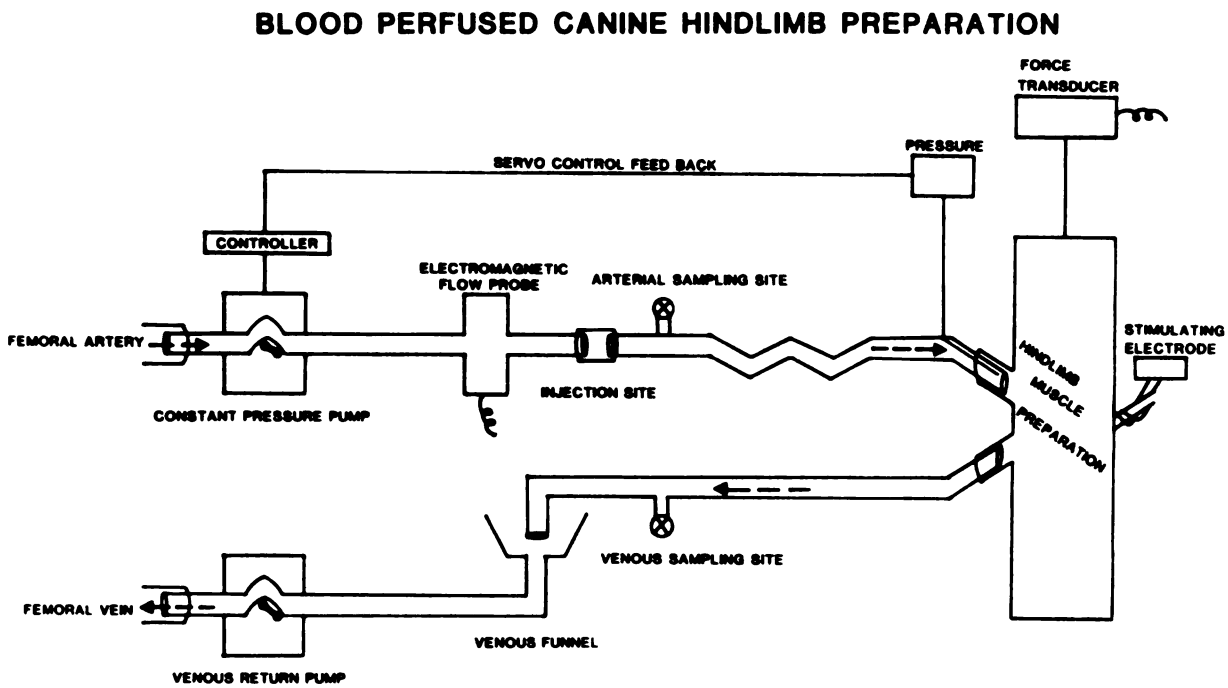

Figure 1. Schematic representation of blood perfused canine hindlimb preparation.

Using a cautery gun, a skin incision completely surrounding the leg was made at the distal end of the femur. The skin was removed from the incision to the level of the calcaneus. Collateral vascular branches perfusing the skin from the underlying tissue were ligated and cut. The dorsal pedal artery perfusing the paw was ligated and cut. A padded clamp was placed around this area of the tibia to prevent venous drainage from the paw. A longitudinal incision was made through the fascial layer overlying the tibialis cranialis from its insertion and extending to its origin. The fascial layer was separated from the underlying tissue on both medial and lateral sides thereby exposing the major muscle groups of the hindlimb. On the lateral side, collaterals to other muscle groups were ligated and cut. The lateral muscles were separated to isolate a branch of the sciatic nerve. The peroneal nerve, a mixed nerve carrying both somatic and sympathetic fibers, was carefully dissected from proximal branches, ligated and cut. The distal branch of the peroneal nerve was placed on a stimulating electrode to activate motor fibers for muscle contraction. The nerve was coated with mineral oil and wrapped with saline soaked gauze and Saran Wrap to minimize dehydration and prevent current spread during stimulation. On the medial side, the fascia was separated from the underlying muscle groups. Collateral branches of the popliteal artery and vein were ligated and cut in order to assure vascular isolation of arterial inflow and venous outflow from the preparation.

The tendon of the gastrocnemius was removed from the bone and secured to a force transducer by means of a wire. Increases in muscle tension development subsequent to nerve stimulation were

recorded and used as an index of sustained contractile activity. The muscle preparation was supported in an elevated and horizontal position by a vertical brace screwed into the distal end of the femur and a support clamp holding the paw. Support braces holding the paw clamp and femur were attached to the stationary dog table adding further stability to the preparation during twitch contraction. To minimize dehydration the hindlimb preparation was covered with saline soaked gauze and Saran Wrap. An incandescent lamp was used as a radiative heat source to prevent cooling of the muscle.

The popliteal artery, supplying arterial inflow to the muscle preparation, was cannulated using PE tubing (Clay Adams PE#330). Prior to cannulation, all animals received, an initial intravenous dose of sodium heparin (1000 U/kg)(Sigma) and were supplemented hourly with 750 U/kg. The preparation was perfused with blood from the contralateral femoral artery. From the contralateral femoral artery, blood was directed to a servo-control feedback pump through silastic tubing (Dow Corning #601-485). An inline (1/4" i.d.) flow probe (Zepeda Instruments, Inc.) was placed between the perfusion pump and the muscle preparation. Right angle bends were placed after the flow probe to insure proper mixing of drugs prior to infusion into the muscle. Flow rate was measured with a SWF-4 Flow Meter (Zepeda Instruments, Inc.). Perfusion pressure of arterial inflow was measured by a catheter (Clay Adams PE#50) inserted to the tip of the arterial cannula and monitored using a Gould Statham P23Db pressure transducer. The servo-control feedback pump was set to perfuse the preparation at a constant pressure of 100 mmHg. Changes in perfusion pressure were detected by the pressure transducer.

Adjustments in pump speed were made by the pump controller to offset any deviation from 100 mmHg. This resulted in constant pressure perfusion despite changes in vascular resistance or systemic blood pressure.

The venous outflow was directed to a reservoir through 5/16" (i.d.) tygon tubing. The tip of the outflow tubing was at the same level as the cannulated end of the popliteal vein, thereby making venous outflow pressure equal to atmospheric pressure. Blood was returned to the contralateral femoral vein by way of a venous return pump. This tubing arrangement allowed for timed collection of flow and enabled calibration of the flow probe throughout the experiment.

Arterial blood pressure was continuously measured with a Statham P23AC pressure transducer via a cannula (Clay Adams PE#280) inserted into the right brachial artery. Calibration of the pressure transducer was made prior to the experiments with the use of a mercury sphygmomanometer (Baumanometer, Inc.). The following variables were continuously recorded on a Grass Polygraph Model 7 (Grass Instruments, Inc.): brachial artery pressure, contractile tension development, arterial blood flow, and arterial perfusion pressure.

BLOOD GAS ANALYSIS

PCO_2, PO_2, and pH were analyzed using a Corning 165/2 pH/Blood Gas Analyzer. To determine the acid-base status of the animal, blood samples were periodically collected from the brachial artery catheter. Adjustments were made in the rate and depth of ventilation

and the flow rate of the oxygen supplement until blood gas values had stabilized within the normal range for anesthetized dogs. Drip infusions of sodium bicarbonate were added to the venous reservoir as needed to correct metabolic acidosis.

To calculate oxygen consumption, blood was simultaneously collected from the arterial inflow and venous outflow tubing. The following variables were measured: PO_2, PCO_2, pH, hematocrit (HCT), and hemoglobin (Hb) content. To determine hematocrit, heparinized micro-hematocrit capillary tubes were filled with blood and spun in an IEC MB Centrifuge (Damon, Inc.) for 2 minutes at maximal speed. The HCT (red blood cell volume relative to total blood volume) was determined from the capillary tubes using a Adams Micro-Hematocrit Reader (Clay Adams). Quantitive determination of hemoglobin in arterial and venous blood was done spectrophotometrically at a wavelength of 540 nm after samples were prepared by the Hycel Cyanmethemaglobin Method. Table 1 shows the average steady state values of these variables obtained in 7 dogs under conditions of rest and 3 Hz exercise.

Oxygen consumption (VO_2)(ml O_2/min/100g) was calculated by the following equation:

$$VO_2 = \text{Blood Flow (ml/min/100g)} \times (\text{A-V } O_2 \text{ Difference})$$

where A-V O_2 Difference (ml O_2/100 ml blood) is the difference between the arterial (A) and venous (V) O_2 content of blood as indicated in the following equation:

Table 1. Summary of blood gas and pH values

		PREAMINOPHYLLINE		POSTAMINOPHYLLINE	
		Rest	Exercise	Rest	Exercise
P_aO_2	(mmHg)	101±8	104±7	95±5	94±6
P_vO_2	(mmHg)	53±3	26±1*	50±3	24±1*
P_aCO_2	(mmHg)	38±1	40±1	40±1	43±2
P_vCO_2	(mmHg)	39±2	52±3*	41±2	57±3*
pH_a	(log units)	7.383 ±.009	7.385 ±.013	7.373 ±.015	7.341 ±.018
pH_v	(log units)	7.363 ±.013	7.302 ±.021	7.344 ±.020	7.228* ±.040
HCT	(%)	44±1	43±2	44±1	44±1

Steady state values were determined during rest and 3 Hz exercise before and after aminophylline (PRE- and POSTAMINOPHYLLINE, respectively; 10 mg/kg). Values are MEAN±SEM. *= $P<0.05$ vs. Rest, n=7; a= arterial; v= venous; HCT= hematocrit.

A-V O_2 Difference = A O_2 Content - V O_2 Content

Oxygen content (ml O_2/100 ml blood) was determined as the product of the oxygen carrying capacity and percent oxygen saturation of Hb. The small percent of O_2 dissolved in plasma (0.3%) was considered negligible and not included in the following calculation:

O_2 Content = O_2 Capacity of blood x % O_2 Saturation

The O_2 capacity of blood (ml O_2/100 ml blood) is the product of Hb content (gm Hb/100 ml blood) and O_2 carrying capacity of Hb:

O_2 Capacity = Hb content x 1.39 ml O_2/gm Hb

Percent O_2 saturation of Hb was determined using a nomogram relating PO_2, pH, and blood temperature for dog blood (Rossing and Cain, 1966).

DETERMINATION OF PLASMA ADENOSINE CONCENTRATION

Sample Collection

Arterial and venous blood samples were collected for determination of adenosine concentration by the following procedure. Prior to obtaining samples, 5 ml of blood was removed from the dead space of the tubing. Subsequently, blood samples of approximately 3 ml were simultaneously collected from inflow and outflow tubing sites and were immediately placed in test tubes containing a 250 ul

collecting solution to prevent uptake and degradation of adenosine. The collecting solution contained 26 uM dipyridamole (DIP)(Boerhinger Ingleheim), 3 uM erythro- 9-(2-hydroxy-3-nonyl) adenine hydrochloride (EHNA)(Burroughs Welcome), and 5% ethanol in isotonic saline. Test tubes containing collecting solution were preweighed to quantitate the sample volume and stored on ice until use for sample collection. Dipyridamole (Roos and Pfleger, 1972) and EHNA (Agarwal et al., 1976) were added to the solution to prevent uptake of adenosine by erythrocytes and deamination of adenosine to inosine by adenosine deaminase, respectively. After samples were thoroughly mixed with collecting solution they were immediately placed in a refrigerated (4 C) centrifuge and spun for 4.5 minutes at 2800 rpm (1360 x g).

To determine percent recovery of the assay, samples spiked with adenosine were also analyzed. These samples were processed in parallel with experimental samples. Average recovery was 85.3 $\pm$ 28.2% (MEAN $\pm$ SD, n=7) (see APPENDIX C for calculation).

Sample Processing

An aliquot of the plasma (1000 ul) was removed from each test tube and placed in a separate tube containing 250 ul of 35% perchloric acid. The tubes were vortexed and then spun in a refrigerated (4 C) centrifuge (Sorvall Model RC2-B) at 17,500 rpm (32,000 x g) for 15 minutes. One ml aliquots of the supernatant were transferred to another set of test tubes and stored overnight at 0 C. On the following day, samples were neutralized (to pH 6.5-7.5) with 110 ul K_2CO_3 (1 g/ml) centrifuged, decanted, and frozen.

Sample Fractionation

Adenosine was assayed by reversed-phase high pressure liquid chromatography (HPLC, Waters, Assoc.). An adenosine fraction of each sample was collected and deaminated to inosine to maximize the resolution of adenosine in samples containing substances co-migrating with adenosine.

1. Adenosine Fraction

Two hundred ul samples of neutralized plasma extract were injected onto a 5u Ultrasphere-ODS Beckman column or a 5u Radial Pak Cartridge (Nova Pak C18, Waters, Assoc.) using a WISP 710B automatic injector (Waters, Assoc.). Adenosine was separated from other sample substances using an isocratic (1.2 ml/min) elution mixture containing 90% 4 mM potassium phosphate (KH_2PO_4) and 10% 70/30 methanol/water. The elution time of each sample was 35 minutes which includes a wash period of 5-10 minutes. The retention time of adenosine was determined by processing standard solutions containing adenosine and inosine. Absorbance of the eluent was continuously monitored by a Waters Absorbance Detector (Model 440) at 254 nm and detection was recorded on a OmniScribe strip chart recorder (Houston Inst.). With this elution condition a typical retention time for adenosine was 15 minutes as indicated in Figure 2. A fraction of the effluent bracketing the retention time for adenosine by 3 minutes was collected (Eldex Universal Fraction Collector). Following fraction collection, samples were evaporated to concentrate the adenosine

Figure 2. Chromatogram of standard solution and plasma sample. The time axis indicates the retention times for adenosine (ADO), inosine (INO), and hypoxanthine (HYPO). A standard solution (upper left panel) and plasma sample (upper right panel) were injected onto a Ultrasphere-ODS Beckman column using an isoscratic elution. Sample fractions were collected at time intervals indicated by the bracketed horizontal arrow. The adenosine fraction was deaminated to inosine by adenosine deaminase and rechromatographed as shown in the lower figures. Quantitation of samples were done by relating absorbance units to a standard curve of known inosine amount.

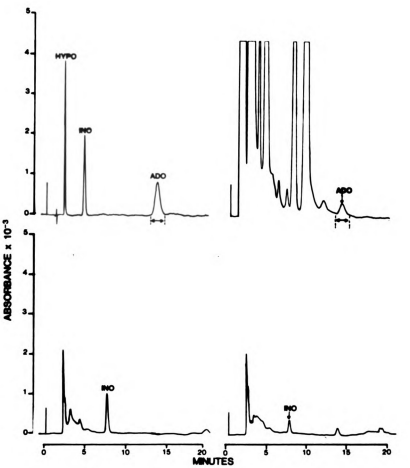

Figure 2. Chromatogram of standard solution and plasma sample.

sample. Samples were then reconstituted with 500 ul deionized distilled water (ultrapure grade) and adenosine was deaminated to inosine for 20 minutes using 5 ul of adenosine deaminase (Sigma Type III, 1:10 dilution). After this period, 500 ul of methanol (ultrapure grade) was added to stop enzyme activity. Samples were again evaporated to dryness.

2. Inosine Fraction

To reconstitute the dried sample 250 ul of elution solvent were added to each test tube. Two hundred ul from this volume was injected onto a 5u Ultrasphere-ODS Beckman column for quantitation of inosine. Inosine samples were separated with an isocratic elution of 90:10 4mM $K_2H_4PO_4$:70/30 methanol/water and monitored by a Waters Absorbance Detector (Model 440). The retention time for inosine was typically 7 minutes (Figure 2). Peak areas of inosine were integrated and recorded by a Waters Data Module.

EXPERIMENTAL PROTOCOL FOR ADENOSINE RELEASE SERIES

A length-tension relationship was established prior to the start of each experiment. Muscles were electrically stimulated to contract using a supramaximal stimulus (1-3 volts, 0.2 msec) and a constant frequency (1 Hz). Muscle length was adjusted until maximal tension was developed. The frequency of stimulation was increased to 3 Hz for exercise periods, while muscle length previously determined was maintained constant throughout the experimental period.

Kjellmer (1965) demonstrated that motor fibers in mixed nerves can be selectively activated with stimuli of low voltage and duration. In our preparations, the voltage pulses required to activate all motor fibers (judged by maximum isometric twitch tension development) ranged from 1 to 3 volts (0.2 msec duration). As determined in a previous study (Thompson and Mohrman, 1983), these stimulus parameters did not produce a flow response in muscles after the myoneural junction was blocked with decamethonium bromide (0.5 mg/kg, i.v.). The threshold for sympathetic fiber activation (judged by a reduction in blood flow) was approximately 30 volts and a maximum response was achieved with 60 to 80 volt pulses.

All animals were allowed a 30 minute stabilization period prior to the start of the experimental period. During this 30 minute period blood gas values, body temperature, and blood flow reached steady state values within physiological range. The protocol for the experimental period is shown in Figure 3.

To demonstrate flow responses to vasoactive substances during rest, adenosine (ADO) and adenosine triphosphate (ATP) solutions were infused. Adenosine (10^{-3} M, Sigma grade) dissolved in saline was infused into the arterial inflow line at the injection site prior to the right angle bends (Figure 1). The infusion rate was adjusted to a rate that increased flow to the flow equivalent measured during 3 Hz exercise. The infusion was given for a time period that established a steady flow and was then maintained for 2-3 minutes. The delivered plasma adenosine concentration causing a 3 Hz equivalent flow increase was approximately 10 uM. Blood flow returned to control after the infusion was stopped. The same

Figure 3. Diagram of experimental protocol.
Flow responses to adenosine (10 uM) (ADO) and ATP (1 uM) infusions were measured during rest and 3 Hz exercise. Aminophylline (10 mg/kg) was administered intraarterially. After the infusion was stopped and resting flow returned to control levels, ADO and ATP were infused to test the efficacy and specificity, respectively, of the adenosine receptor blockade.

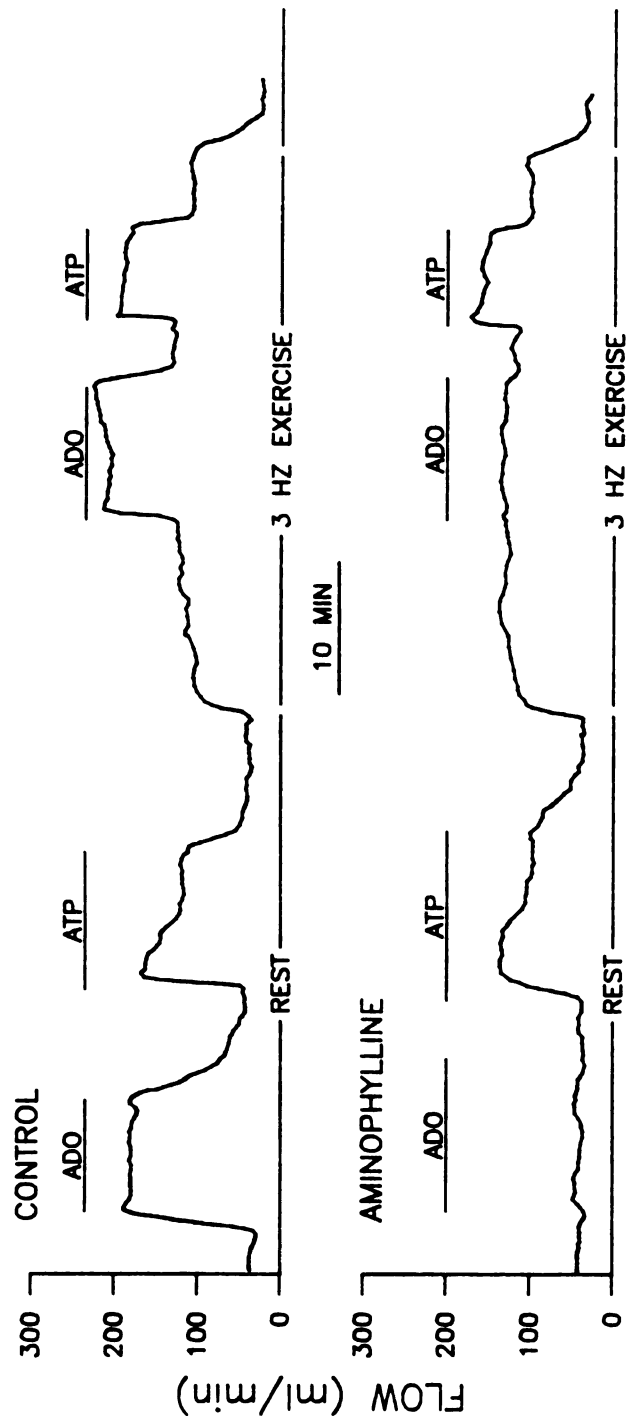

Figure 3. Diagram of experimental protocol.

infusion protocol was used for ATP dissolved in saline. Blood flow again returned to control after the infusion was stopped. The delivered concentration of ATP causing the flow equivalent to 3 Hz exercise was approximately 1 uM.

Following infusions, arterial and venous blood samples were collected for determination of blood gases, pH, HCT, and Hb. From these values, oxygen consumption was calculated for the resting period. Simultaneously obtained arterial and venous samples were collected for determination of plasma adenosine concentration. Adenosine release was calculated as the product of venous-arterial concentration difference and plasma flow.

Following the rest period, motor nerves were activated at a frequency of 3 Hz by the stimulating electrode. This activation initiated muscle contraction accompanied by an increase in tension development, oxygen consumption, and blood flow. Blood flow reached a steady state level ten minutes after the initiation of contraction. Simultaneous arterial and venous blood samples were collected 15 minutes after the onset of exercise for determination of plasma adenosine concentration and oxygen consumption. Following sample collection, the flow response to ADO and ATP infusions was measured during the exercise period. At 3 Hz twitch rate the vascular bed is capable of further dilation in the presence of ADO or ATP. Blood flow was allowed to return to steady state exercise flow before the infusion of the second agent. Since blood flow during exercise decreased over time, the flow level immediately prior to ADO or ATP infusions was used to calculate percent change in flow. After steady flow responses, the infusion of ADO or ATP was stopped and flow

returned to the preinfusion level. Flow returned to resting level within 3-5 minutes after cessation of motor nerve stimulation.

Following the protocol described above, aminophylline (Sigma grade) was administered intraarterially by a Harvard infusion pump. Aminophylline was dissolved in saline to a concentration of 1.2×10^{-2} M and infused at a rate of 1-2 ml/min for a total dose of 10 mg/kg body weight. The plasma infusate concentration of aminophylline was approximately 1×10^{-4} M. Infusion of aminophylline resulted in an increase in blood flow lasting the duration of the infusion period. At the end of infusion, flow was allowed to return to control resting levels prior to the start of the next experimental period. If flow did not return to the preaminophylline resting flow level the experiment was not continued. Adenosine receptor blockade by aminophylline was tested by infusing ADO during rest and exercise. Receptor blockade was considered acceptable if 90% of the flow response to ADO infusion was prevented. If the adenosine induced flow increase was not reduced by aminophylline, a subsequent infusion of aminophylline was given until a blockade was observed. To test for the specificity of the blockade, ATP infusion was repeated during rest.

For determination of muscle oxygen consumption and plasma ADO concentration, paired samples were simultaneously withdrawn from arterial and venous sampling sites during the postaminophylline rest period. Muscles were stimulated to contract at 3 twitches per second. Following a steady state period of sustained muscle contraction, arterial and venous blood samples were collected for determination of oxygen consumption and plasma adenosine

concentration. The effectiveness and specificity of the adenosine receptor blockade were tested using ADO and ATP infusions, respectively. The protocol for infusion was the same as that used during the preaminophylline period.

EXPERIMENTAL PROTOCOL FOR DOSE RESPONSE SERIES

To quantitate the effectiveness of adenosine receptor blockade in blood perfused skeletal muscles, dose response curves for infused adenosine were determined in the presence and absence of aminophylline. The muscle preparation used in this series was identical to that in the previously described adenosine release series. Five dogs were studied to determine the dose response relationship to exogenous adenosine.

Adenosine was dissolved in saline to obtain infusate concentrations of 1.0×10^{-6} M to 1.0×10^{-3} M. Differing adenosine concentrations were infused intraarterially by means of a roller pump perfusion system, which was different than the infusion system used in the first series of experiments. A single pump head (Masterflex Model 7106) was placed on top of the roller pump head perfusing blood to the preparation. The two pump heads rotated simultaneously. With this arrangement the delivery rate of ADO increases in proportion to the increase in flow. This prevents the dilutional effect which occurs with increased flow and allows stepwise increases in adenosine concentration. Infusions of low (10^{-6} M) to high concentration (10^{-3} M) of ADO were given to determine threshold and maximal flow responses.

The dose response relationship to infused adenosine was then determined in the presence of aminophylline (10 mg/kg). The protocol for aminophylline administration was identical to that used in the adenosine release studies. After aminophylline administration was completed, increasing adenosine concentrations were infused until maximal flow was achieved.

MODEL OF CAPILLARY TRANSPORT OF ADENOSINE

Purpose of Model

Interstitial adenosine concentration was calculated using a 21 compartment mathematical model of capillary transport of adenosine. Interstitial adenosine concentration was calculated for conditions of rest and 3 Hz exercise. The model was not used to predict the absolute levels of interstitial adenosine but rather to provide a conceptual framework to compare the relationship between interstitial concentration and adenosine release and venous adenosine concentration. Model simulations were performed with the IBM System/360 Continuous System Modeling Program on the Amdahl V8 Computer at Wayne State University in Detroit, Michigan.

Structure of Model

The model is based on a unit capillary which is assumed to be identical to all other capillaries in the muscle (Figure 4).

Figure 4. Schematic representation of transcapillary exchange of adenosine.
A 21 compartment model was used to calculate interstitial adenosine concentration.

F_{pl} =plasma flow
C^{a} =plasma arterial adenosine concentration
C^{c} =plasma capillary adenosine concentration
C^{v} =plasma venous adenosine concentration
C_{isf} =interstitial adenosine concentration
PS_{g} =permeability-surface area product for diffusible solutes between endothelial cells
PS_{ec} =permeability-surface area product of adenosine for endothelial cell uptake
P_{ado} =parenchymal cell production of adenosine
V^{c} =capillary volume
V_{isf} =interstitial fluid volume of distribution

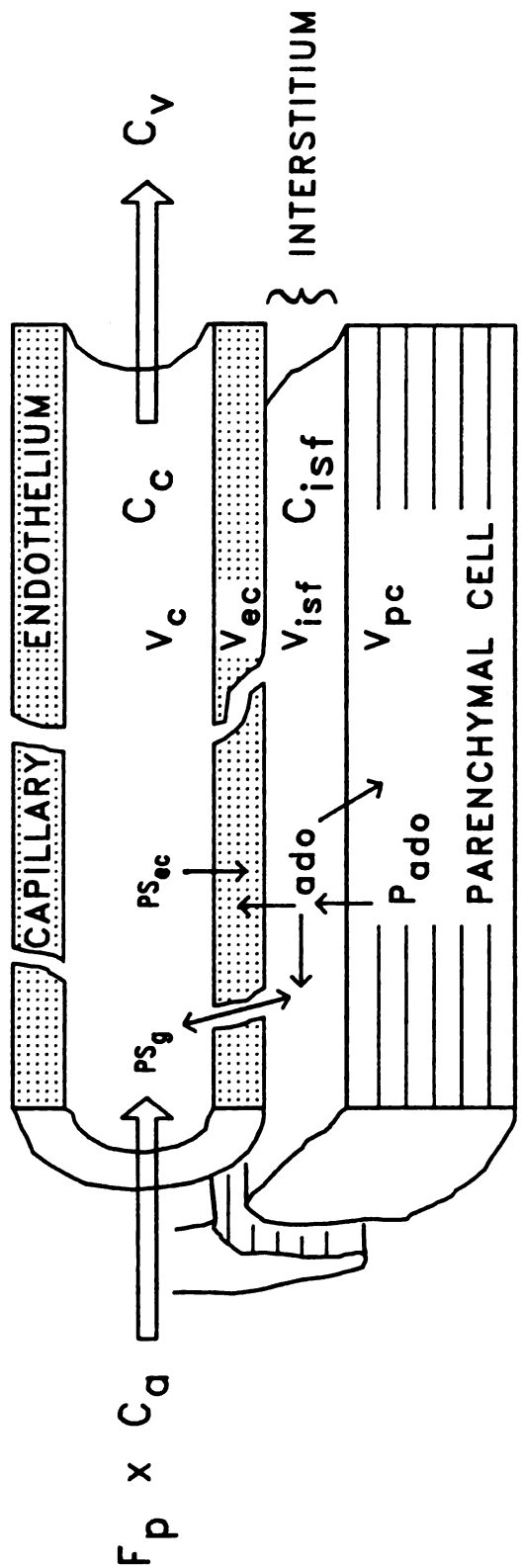

Figure 4. Schematic representation of transcapillary exchange of adenosine.

The main assumptions of the model are as follows: 1) adenosine crosses the capillary wall via simple diffusion through interendothelial clefts, 2) capillary endothelium acts as a metabolic sink for adenosine, and 3) the only source of endogenous adenosine is from the interstitium.

The capillary is divided into ten compartments placed in series. Plasma flows from one compartment to the next by bulk flow. This is equivalent to plugs of plasma separated by red blood cells (Prothero and Burton, 1961; Jacquez, 1972). The ten compartments represent well mixed plugs of plasma moving down the capillary. Parallel to the plasma compartments are ten interstitial compartments, which define the concentration profile of adenosine in the interstitium as it exchanges along the length of the capillary. The interstitial fluid space is composed of an interfibrillar matrix made up of a polysaccharide network (Laurent, 1970). However, since knowledge is lacking about the distribution and absolute local concentrations of polysaccharides it is difficult to assess the actual importance of the connective tissue matrix in solute transport through the interstitium. In this model, we assumed a homogeneous distribution of the fiber network and assumed that solute transport through the interstitial fluid space is via simple diffusion.

Entry of adenosine into the arterial end of the first plasma compartment is described in terms of the product of arterial plasma adenosine concentration and plasma flow. Adenosine may leave this compartment by at least five routes: (1) convective transfer to the second capillary segment, (2) simple diffusion to the interstitium through interendothelial clefts, (3) uptake by endothelial cells,

(4) uptake by cellular elements of blood, and (5) deamination to inosine by plasma adenosine deaminase. Subsequent plasma compartments are handled in an analogous fashion. The net flux of adenosine in and out of each compartment determines its instantaneous mass (see Appendix A for transfer equations).

Carrier mediated uptake and enzymatic destruction of adenosine are defined in terms of Michaelis-Menten kinetics. The kinetic parameters for adenosine uptake (V_{max}=90 nmol/min/cell and K_m=3 uM) were obtained from cultured porcine aortic endothelial cells (Pearson et al., 1978). In the model, the V_{max} (250 nmol/min/cell) for cellular uptake by capillary endothelium was increased until extraction of arterial adenosine was 90%, equivalent to that observed by Thompson et al. (1983) in skeletal muscle for a continous infusion (20 min) of ^{3}H-adenosine (10^{-8} M). Kinetic parameters were also obtained for the disappearance rate of adenosine from plasma by formed blood elements (0.18/min) (Manfredi and Sparks, 1982) and degradation in the interstitium by adenosine deaminase (V_{max}= 4.8 nmol/min/100g (van Belle, 1969) and K_m=40 uM (Fox and Kelly, 1978). Because capillary transit time is short relative to the rate of degradation by adenosine deaminase and uptake by erythrocytes, these routes have a negligible effect on the calculations.

We assumed that adenosine diffuses across the capillary wall via simple diffusion through interendothelial clefts. The rate of diffusion through interendothelial clefts is dependent on cleft surface area, path length from the plasma to the interstitial fluid space, and the concentration difference between these compartments. The cleft surface area during rest is taken to be 1.32 cm^2/100g.

This value was calculated for given values of diffusion coefficient, path length for diffusion, and permeability-surface area product (PS) for 9-B-D arabinofuranosyl hypoxanthine (ara-H) (an adenosine analogue not transported by the membrane nucleoside carrier). The cleft surface area was calculated using the PS_{ara-H} value experimentally determined from canine skeletal muscle ($SA = (PS_{ara-H} \times \text{path length})/D_{ado}$). The diffusion coefficient of adenosine for plasma and interstitial fluid is taken to be the same as sucrose (1.06×10^{-4} cm^2/min) based on the similarity of molecular weights. The average path length of interendothelial clefts is taken to be 4.0×10^{-5} cm (0.4 um) from the capillary to interstitial spaces (Berne and Rubio, 1979). This length is two fold greater than the thickness of capillary endothelium (0.2-0.3 um) (Bruns and Palade, 1968) and accounts for cleft tortuosity. During exercise, cleft surface area is taken to be 4.1 $cm^2/100g$. The increase in cleft surface area during exercise is derived from the measured 3.1 fold increase in capillary surface area (Renkin et al., 1966; Casley-Smith et al., 1975; Duran, 1977).

Capillary surface area of skeletal muscle during rest is 70 cm^2/g (Landis and Pappenheimer, 1963) and increases to 220 cm^2/g during exercise (Casley-Smith et al., 1975) resulting from an increase in the number of open capillaries. Capillary plasma volume has been calculated from capillary surface area, radius, and length as 0.74 ml/100g during rest and 2.31 ml/100g during exercise (Casley-Smith et al., 1975). Capillary hematocrit is taken to be 20% of arterial hematocrit during rest and 80% during exercise (Klitzman and Duling, 1979).

Adenosine may leave the interstitial compartment by at least four ways: (1) parenchymal cell uptake, (2) deamination by interstitial adenosine deaminase, (3) diffusion into the plasma space through clefts, and (4) diffusion into the adjacent interstitial compartment. Conversely, adenosine may enter the interstitial compartment via (1) production by parenchymal cells, (2) diffusion from adjacent interstitial compartments, and (3) diffusion from the plasma space. The kinetic parameters of V_{max} and K_m for uptake of adenosine by skeletal muscle are taken to be 320 nmol/min/100g and 9.9 uM, respectively. These parameters were calculated from isolated platelet preparations (Sixma et al., 1976) and assigned to the parenchymal cell compartment since values for skeletal muscle cells remain unknown. (This study was used because it accounted for both intra- and extracellular nucleotides and nucleosides.) Adenosine leaves the capillary bed and enters the venous compartment by bulk flow. (Appendix B lists model parameters).

Application of Model to Experimental Results

The relationship between steady state adenosine release and calculated interstitial concentration was determined for individual animals. Interstitial concentrations in the presence and absence of aminophylline were calculated during rest and 3 Hz exercise. Measured experimental values were blood flow, hematocrit, and arterial and venous adenosine concentration. The interstitial fluid volume of skeletal muscle was taken to be 15 ml/100g tissue (Aukland and Nicolaysen, 1981).

Interstitial adenosine concentration for each experimental condition was adjusted by varying cellular adenosine production rate until the model predicted the measured venous adenosine concentration to within 1 x 10^{-9} M. Steady state interstitial adenosine concentration during rest and exercise was calculated as the average concentration of the ten individual compartments. The variation in concentration between the first interstitial compartment and the tenth compartment was approximatley 0.2%.

Parameter Sensitivity of the Model

The effect of different parameters (blood flow, permeability-surface area (PS), and arterial adenosine concentration) on the relationship between interstitial concentration and adenosine release and venous plasma adenosine concentration was examined. Changes in interstitial concentration were produced by changing adenosine production (10, 30, and 60 nmol/min/100g).

The effect of flow on the relationship between interstitial concentration and adenosine release and venous concentration was evaluated for three flow values (20, 80, and 160 ml/min/100g). For each flow value, capillary PS (3.5 ml/min/100g) and arterial concentration (.075 uM) remained constant. The effect of capillary PS (3.5, 11.0, and 21.0 ml/min/100g) on the relationship between interstitial concentration and release was examined at constant flow (80 ml/min/100g) and arterial concentration (.075 uM). Alterations in capillary PS were accompanied by necessary changes in capillary volume, endothelial cell surface area (and V_{max} for

adenosine uptake), and interendothelial cleft surface area. The effect of arterial adenosine concentration (0.05, 0.075, and 1.0 uM) on this relationship was determined in the presence of constant flow (80 ml/min/100g) and capillary PS (11.0 ml/min/100g).

STATISTICAL ANALYSIS

Statistical comparisons between preaminophylline and postaminophylline values were made using one way analysis of variance (within-subjects ANOVA). Differences between group means were tested using Student-Newman-Keuls' test for multiple comparisons (Linton and Gallo, 1975; Sokal and Rohlf, 1981). Multiple comparisons were only made if the calculated F statistic from ANOVA was greater than the critical F statistic (n=7, alpha= 0.05). All results are reported as MEAN $\pm$ SEM. Statistical significance between mean values were shown using a P value of 0.05.

Potency (ED_{50}) values were determined from probit-logarithm transformations (Goldstein, 1964) of dose response curves. Comparisons between ED_{50} values determined before and after aminophylline were made using Student's t-test for paired observations. The critical t-value at $P<0.05$ was used to determine statistical significance between means (n=5, df=4). Results are illustrated as MEAN $\pm$ SEM for selected concentration ranges.

Linear regression was used to show significant correlations between experimental and model computed values. Student t-test was used to determine if the slope of regression lines differed significantly from zero ($P<0.001$).

RESULTS

ADENOSINE RELEASE SERIES

FLOW MEASUREMENTS BEFORE AMINOPHYLLINE

The effect of aminophylline induced adenosine receptor blockade on the hyperemic response to exogenous adenosine, adenosine triphosphate (ATP), and 3 Hz exercise was tested in the first experimental series. Flow responses of 7 dogs to adenosine and ATP infusions were measured during rest and 3 Hz exercise and compared to responses after aminophylline (Table 2). Figure 5 shows the flow responses to adenosine and ATP infusions during rest, both before and after aminophylline infusion. The resting flow (MEAN$\pm$SEM) was 14.1$\pm$2.7 ml/min/100g. Intraarterial infusion of adenosine (10^{-5} M) produced a 7 fold increase in flow (94.9$\pm$10.8 ml/min/100g). ATP infusion (10^{-6} M) caused a 5.3 fold increase in flow (75.6$\pm$5.4 ml/min/100g). The increase in flow caused by infusion of either adenosine or ATP approximated the 5.3 fold flow increase measured during 3 Hz exercise (82.5$\pm$6.0 ml/min/100g). Arterial perfusion pressure was maintained constant at 100 mmHg. Therefore, increases in blood flow reflect a decrease in vascular resistance secondary to relaxation of vascular smooth muscle.

Table 2. Effect of aminophylline on blood flow.

EXP #	REST	REST+ADO	REST+ATP	EXER	EXER+ADO	EXER+ATP
PREAMINOPHYLLINE						
1	24.1	137.8	86.6	87.2	158.4	128.7
2	23.3	60.7	54.1	61.1	117.0	73.2
3	9.8	68.5	68.3	70.6	182.8	128.9
4	14.9	126.9	93.4	103.0	142.5	112.0
5	5.3	84.5	64.7	76.8	145.5	109.1
6	11.7	96.8	88.0	102.6	152.5	123.2
7	9.9	88.8	74.0	76.5	128.2	103.6
MEAN	14.1	94.9*	75.6*	82.5*	146.7*+	111.2*+
±SEM	2.7	10.8	5.4	6.0	8.0	7.4
POSTAMINOPHYLLINE						
1	11.4	33.5	48.6	80.9	90.8	91.9
2	11.7	17.6	65.9	49.7	54.0	79.5
3	14.9	20.1	42.6	57.3	61.5	89.0
4	14.2	22.4	68.7	97.0	108.3	209.7
5	4.8	12.1	56.6	68.7	76.8	95.0
6	11.7	17.6	70.4	123.2	129.0	149.6
7	7.4	12.4	61.7	76.5	83.8	98.6
MEAN	10.9	19.4#	59.2*	79.0*	86.3*#	116.2*+
±SEM	1.4	2.7	4.0	9.4	9.9	17.8

Adenosine (ADO, 10 uM) and adenosine triphosphate (ATP, 1 uM) were infused during rest and 3 Hz exercise (EXER) before and after aminophylline (10mg/kg, PRE- and POSTAMINOPHYLLINE, respectively). Significant differences between means were tested using one way analysis of variance and Student-Newman-Keuls test. (*= $P<0.05$ vs. REST; += $P<0.05$ vs. EXER; #= $P<0.05$ vs. PREAMINOPHYLLINE; n=7).

Figure 5. Effect of aminophylline on flow response to adenosine and ATP infusions during rest. PREINFUSION indicates period before adenosine (ADO)(10 uM) and ATP (1 uM) infusion. CONTROL indicates the period before aminophylline (10 mg/kg) infusion. AMINOPHYLLINE indicates the period following aminophylline infusion. Values are MEAN $\pm$ SEM.

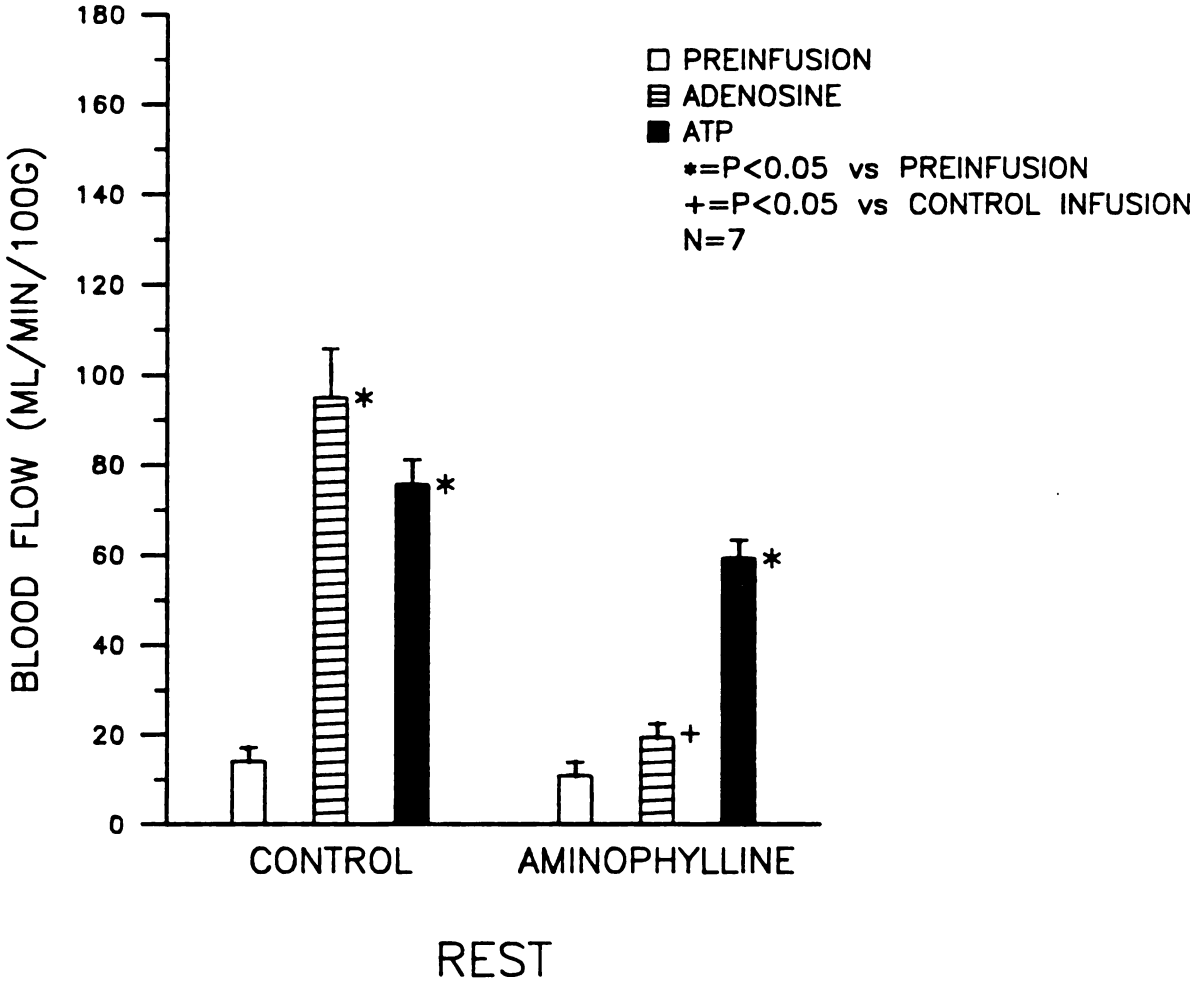

Figure 5. Effect of aminophylline on flow responses to adenosine and ATP infusions during rest.

FLOW MEASUREMENTS AFTER AMINOPHYLLINE

Following aminophylline infusion, the average blood flow before infusion of adenosine or ATP was 10.9±1.4 ml/min/100g (Figure 5 and Table 2). This flow was not significantly different from the average resting flow prior to infusion of aminophylline, indicating that aminophylline did not alter vascular resistance. Since aminophylline did not change resting flow, differences in flow responses to adenosine or ATP relative to preaminophylline conditions were not due to differences in resting vascular resistance. The flow response to adenosine infusion was profoundly reduced in the presence of aminophylline (19.4±2.7 ml/min/100g, $P<0.05$). However, the flow during ATP infusion (59.2±4.0 ml/min/100g) was signficantly greater ($P<0.05$) than resting flow (10.9±1.4); this flow was not different from the ATP induced flow response in the preaminophylline period (75.6±5.4 ml/min/100g). These data indicate that aminophylline specifically reduces the increase in flow induced by exogenous adenosine but does not inhibit the vasodilation by ATP.

The effect of aminophylline on the flow responses to adenosine and ATP infusions during 3 Hz exercise is illustrated in Figure 6. The average steady state flow after ten minutes of 3 Hz exercise was 82.5±6.0 ml/min/100g (Table 2). Flow was further increased with adenosine infusion during exercise (146.7±8.0 ml/min/100g). In the presence of ATP infusion, flow increased to 111.2±7.4 ml/min/100g. The delivery rates of adenosine and ATP were established separately during rest and continued at those rates during exercise. Since flow

Figure 6. Effect of aminophylline on flow responses to adenosine and ATP infusions during 3 Hz exercise. PREINFUSION indicates period before adenosine (10 uM)(ADO) and ATP (1 uM) infusion during 3 Hz exercise. CONTROL indicates periods before aminophylline (10 mg/kg) infusion. AMINOPHYLLINE indicates the period following aminophylline infusion. Values are MEAN $\pm$ SEM.

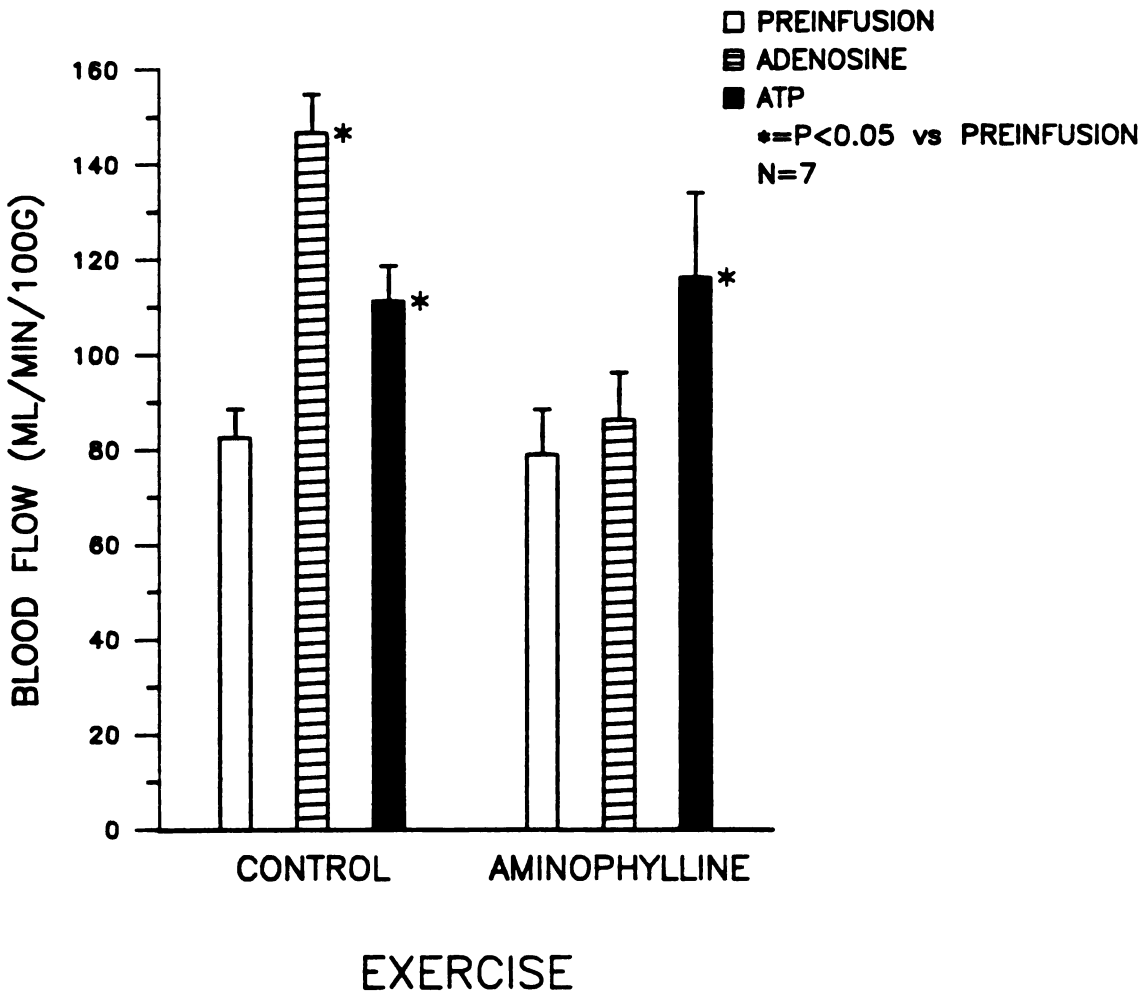

Figure 6. Effect of aminophylline on flow responses to adenosine and ATP infusions during 3 Hz exercise.

during exercise is higher, the infused concentration during exercise was less than that during rest.

In the presence of aminophylline, blood flow during 3 Hz exercise was not significantly different from the preaminophylline control. Exercise flow was 79.0±9.4 ml/min/100g after aminophylline, compared to 82.5±6.0 ml/min/100g during control. The flow response to adenosine infusion during exercise (86.3±9.9 ml/min/100g) was significantly reduced compared to its control value (146.7±8.0 ml/min/100g, $P<0.05$). However, ATP infusions during exercise caused flow to increase (116.2±17.8 ml/min/100g) above exercise levels. Aminophylline is effective in blocking the flow increase to exogenous adenosine during both rest and 3 Hz exercise. However, blockade of adenosine receptors has no effect on exercise hyperemia.

OXYGEN CONSUMPTION BEFORE AND AFTER AMINOPHYLLINE

Blood flow increases in response to increased metabolic activity. Oxygen consumption was determined to assess the metabolic rate during rest and 3 Hz exercise in the presence and absence of aminophylline (Figure 7). Resting oxygen consumption was 0.4±0.1 ml O_2/min/100g and increased to 10.5±0.9 ml O_2/min/100g during 3 Hz exercise. In the presence of aminophylline, resting oxygen consumption was 0.4±0.1 ml O_2/min/100g and increased to 11.2±1.3 ml O_2/min/100g.

In 4 separate animals, oxygen consumption during infusion of adenosine and ATP was determined during rest and exercise. As shown in Table 3, oxygen consumption during infusion of adenosine

Figure 7. Effect of aminophylline on oxygen consumption during rest and 3 Hz exercise (EXER). Steady state values were obtained prior to (PRE) and following (POST) aminophylline (10 mg/kg) infusion.
Values are MEAN $\pm$ SEM.

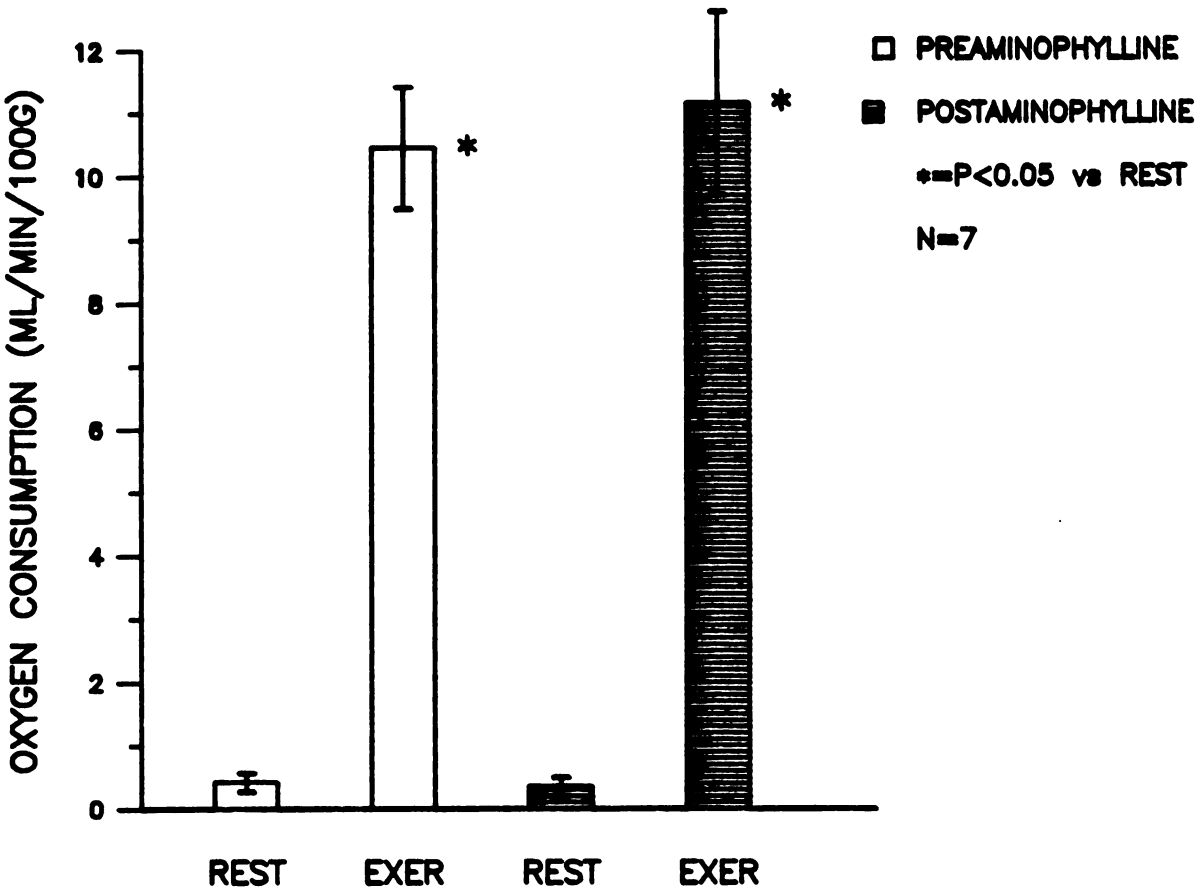

Figure 7. Effect of aminophylline on oxygen consumption during rest and 3 Hz exercise.

Table 3. Effect of aminophylline on oxygen consumption during adenosine and adenosine triphosphate infusions.

			PREAMINOPHYLLINE			
EXP #	REST	REST+ADO	REST+ATP	EXER	EXER+ADO	EXER+ATP
a	0.39	0.33	0.40	11.2	12.7	10.0
b	0.30	0.30	0.37	9.5	9.3	3.4
c	0.38	0.31	0.42	8.3	7.5	8.8
d	0.25	0.12	0.22	8.7	6.8	8.1
MEAN	0.33	0.27	0.35	9.4*	9.1*	7.6*
±SEM	0.03	0.05	0.04	0.6	1.3	1.4
			POSTAMINOPHYLLINE			
a	0.35	0.41	0.44	11.8	11.4	11.3
b	0.31	0.38	0.41	9.8	8.3	8.4
c	0.37	1.30	0.42	8.9	6.9	6.7
d	0.23	0.16	0.26	9.3	7.7	7.0
MEAN	0.32	0.56	0.38	10.0*	8.6*	8.4*
±SEM	0.03	0.25	0.04	0.6	1.0	1.1

Adenosine (ADO, 10 uM) and adenosine triphosphate (ATP, 1 uM) were infused at concentrations which increased flow to level equivalent to 3 Hz exercise (EXER). One way analysis of variance and Student-Newman-Keuls test were used for multiple comparisons between mean values. (* = $P<0.05$ vs. REST, n=4).

(0.27±0.05 ml O_2/min/100g) or ATP (0.35±0.04 ml O_2/min/100g) did not change from preinfusion control (0.33±0.03 ml O_2/min/100g). Oxygen consumption increased 28 fold above resting values (0.33±0.03 ml O_2/min/100g) during 3 Hz exercise (9.4±0.6 ml O2/min/100g). Oxygen consumption during exercise was not altered by the elevated flow observed during adenosine (9.1±1.3) or ATP (7.6±1.4) infusions.

The effect of aminophylline on the relationship between oxygen consumption and blood flow is shown in Figure 8 (see also Table 4). The ability of skeletal muscle to increase blood flow according to its oxidative requirements is summarized in this graph. This relationship is unaltered in the presence of an effective adenosine receptor blockade by aminophylline.

PLASMA ADENOSINE CONCENTRATION MEASUREMENTS

Arterial and venous blood samples were simultaneously collected during rest and exercise for determination of plasma adenosine concentration (n=7) (Figure 9 and Table 5). Venous concentration was used to assess changes in interstitial adenosine levels in response to exercise.

Before aminophylline, the mean arterial adenosine concentration during rest was 0.092±0.020 uM. Venous concentration (0.060±0.008 uM) was not significantly different from the arterial concentration. During exercise, there were no statistical differences between arterial adenosine concentration (0.049±0.011 uM) and venous concentration (0.077±0.017 uM) as compared to their resting control.

Table 4. Summary of blood flow and oxygen consumption during rest and 3 Hz exercise

	BLOOD FLOW				OXYGEN CONSUMPTION			
	CONTROL		AMINOPHYLLINE		CONTROL		AMINOPHYLLINE	
EXPERIMENT #	REST	EXER	REST	EXER	REST	EXER	REST	EXER
1	24.1	87.2	11.4	80.9	0.4	11.3	0.2	12.5
2	23.3	61.1	11.7	49.7	0.5	6.9	0.4	7.9
3	9.8	70.6	14.9	57.3	0.3	7.4	0.6	6.9
4	14.9	103.0	14.2	97.0	0.4	11.5	0.3	12.4
5	5.3	76.8	4.8	68.7	0.2	10.3	0.2	9.3
6	11.7	102.6	11.7	123.2	0.3	14.2	0.4	18.4
7	9.9	76.5	7.4	76.5	0.8	11.6	0.4	10.7
MEAN	14.1	82.5*	10.9	79.0*	0.4	10.5*	0.4	11.2*
±SEM	2.5	6.0	1.4	9.4	0.1	0.9	0.1	1.3

Steady state values of blood flow (ml/min/100g) and oxygen consumption (ml O_2/min/100g) obtained during rest and 3 Hz exercise. Aminophylline (10 mg/kg) was administered intraarterially. Data were analyzed by one way analysis of variance with Student-Newman-Keuls test to determine statistical significance (* = $P<0.05$ vs REST, n=7).

Figure 8. Effect of aminophylline on the oxygen consumption/flow relationship. Steady state values of blood flow and oxygen consumption were obtained before and after aminophylline (10 mg/kg) infusion. Values are MEAN $\pm$ SEM.

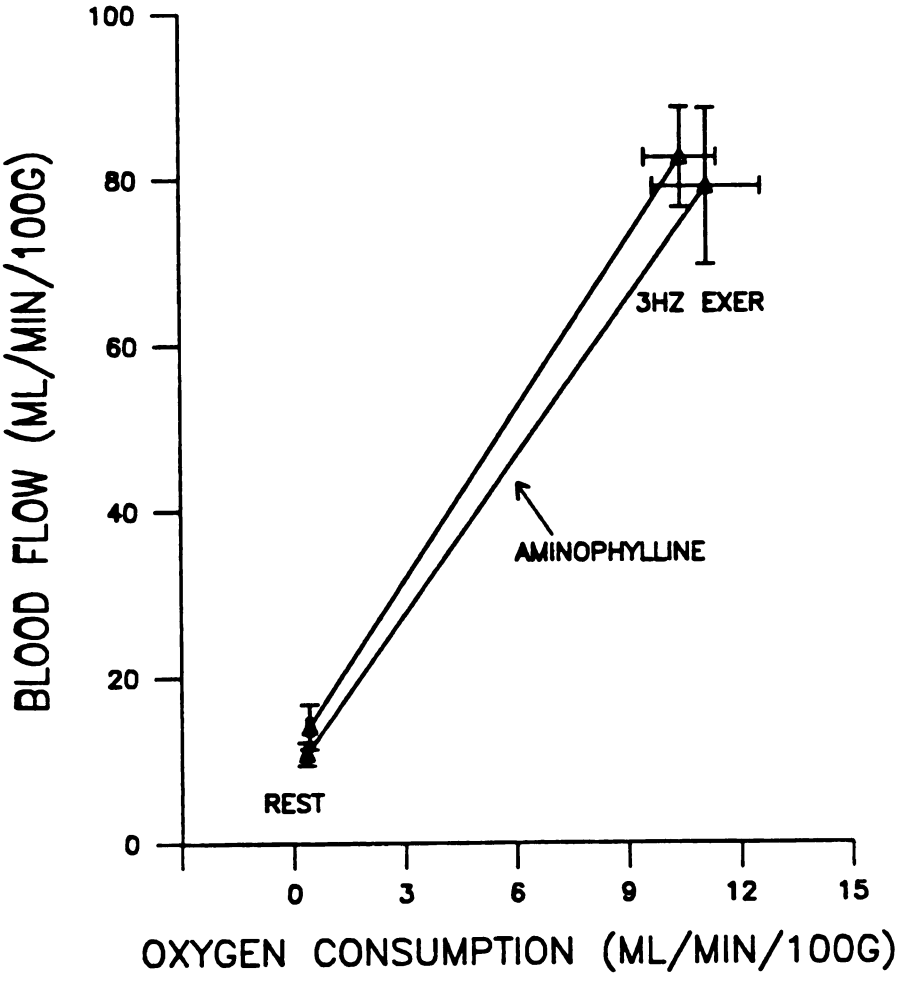

Figure 8. Effect of aminophylline on the oxygen consumption/flow relationship.

Table 5. Arterial and venous plasma adenosine concentration during rest and 3 Hz exercise.

	PREAMINOPHYLLINE				POSTAMINOPHYLLINE			
EXPERIMENT #	REST		EXERCISE		REST		EXERCISE	
	[ADO]a	[ADO]v	[ADO]a	[ADO]v	[ADO]a	[ADO]v	[ADO]a	[ADO]v
1	0.110	0.078	0.110	0.150	0.092	0.110	0.053	0.092
2	0.080	0.060	0.040	0.100	0.100	0.110	0.110	0.180
3	0.052	0.060	0.032	0.042	0.047	0.066	0.038	0.028
4	0.061	0.038	0.017	0.026	0.110	0.086	0.095	0.100
5	0.037	0.051	0.031	0.037	0.073	0.067	0.024	0.043
6	0.103	0.037	0.052	0.088	0.060	0.049	0.037	0.086
7	0.198	0.099	0.058	0.094	0.116	0.070	0.038	0.079
MEAN	0.092	0.060	0.049	0.077	0.085	0.080	0.056	0.087
±SEM	0.020	0.008	0.011	0.017	0.010	0.009	0.012	0.018

[ADO]a and [ADO]v indicates arterial and venous plasma adenosine concentration (uM), respectively; Aminophylline was intraarterially infused (10mg/kg). Each value represents a single sample or the average of two samples. No statistical difference was found between mean values.

The effect of aminophylline on circulating levels of adenosine was assessed by measuring arterial and venous plasma adenosine concentrations (Table 5). In the presence of aminophylline, arterial adenosine concentration at rest (0.085±0.010 uM) was not different from preaminophylline control (0.092±0.020 uM). Venous concentration (0.080±0.009 uM) was not significantly different from the preaminophylline value (0.060±0.008 uM).

During exercise, arterial adenosine concentration in the presence of aminophylline was 0.056±0.012 uM, a value not significantly different from the preaminophylline control (0.049±0.011 uM). Venous concentration was 0.087±0.018 uM but was not significantly different from venous concentration (0.017±0.017 uM) measured before aminophylline.

Plasma venous-arterial adenosine concentration differences during rest and exercise are shown in Table 6 and Figure 9. Negative values indicate net uptake of adenosine by the tissue. Before aminophylline, the average venous-arterial concentration difference during exercise (0.028±0.008 uM) is significantly greater than that measured during rest (-0.033±0.014 uM). In the presence of aminophylline, the average venous-arterial concentration difference increased from -0.006±0.009 uM to 0.030±0.010 uM. This increase was not statistically significant. In 12 out of 14 paired observations, venous-arterial differences increased in response to exercise. The individual differences, shown in Table 6, were used to calculate adenosine release as discussed in the next section.

Table 6. Differences between venous and arterial plasma adenosine concentration.

	PREAMINOPHYLLINE		POSTAMINOPHYLLINE	
EXP #	REST	EXERCISE (3 Hz)	REST	EXERCISE (3 Hz)
1	-0.032	0.040	0.018	0.039
2	-0.020	0.060	0.010	0.070
3	-0.008	0.010	0.019	-0.010
4	-0.023	0.009	-0.024	0.005
5	0.014	0.006	-0.006	0.019
6	-0.066	0.036	-0.011	0.049
7	-0.099	0.036	-0.046	0.041
MEAN	-0.033	0.028*	-0.006	0.030
±SEM	0.014	0.008	0.009	0.010

Differences were calculated from venous minus arterial plasma adenosine concentrations (uM). Negative values indicating adenosine uptake by tissue. One way analysis of variance and Students-Newman-Keuls test were used for multiple comparisons of mean values.(*= $P<0.05$ vs. REST, n=7).

Figure 9. Arterial and venous plasma adenosine concentration during rest and 3 Hz exercise. Plasma samples were obtained during steady state conditions of rest and 3 Hz exercise before and after aminophylline (10 mg/kg). Values are MEAN $\pm$ SEM.

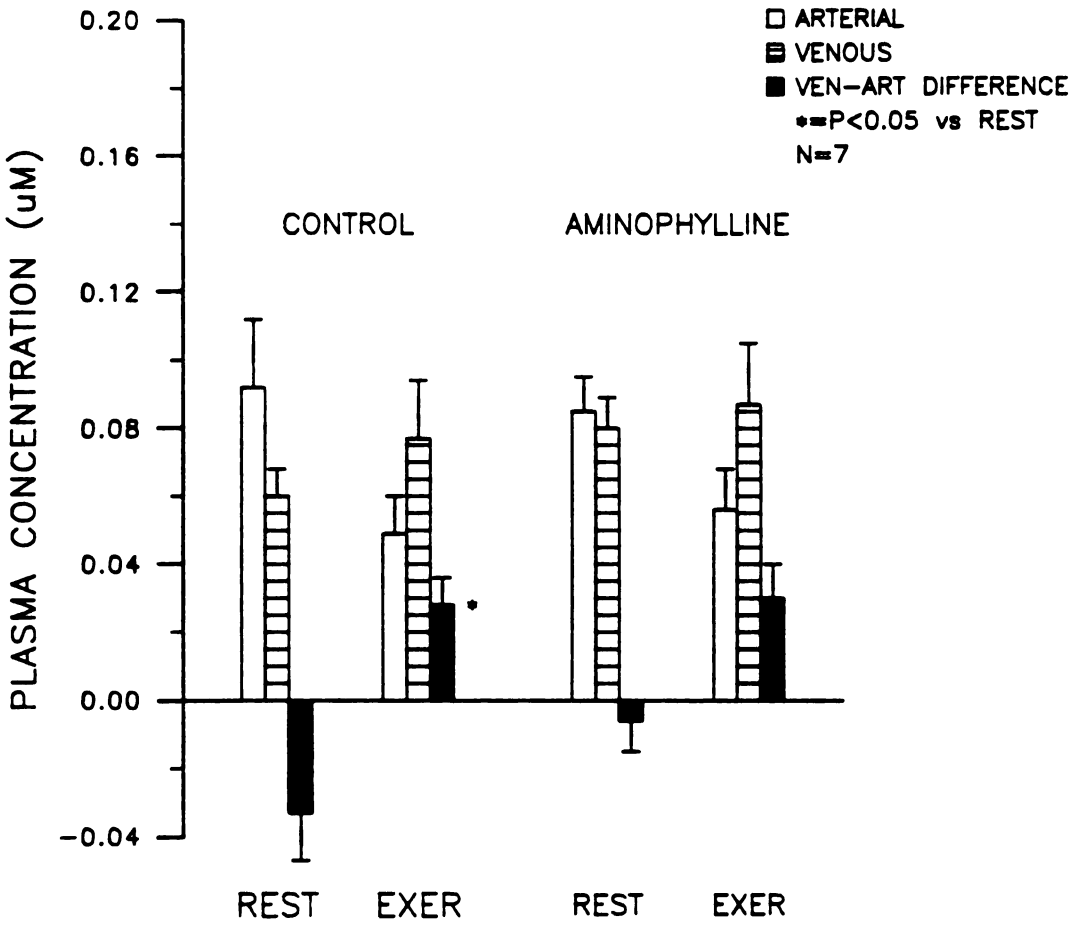

Figure 9. Arterial and venous plasma adenosine concentration during rest and 3 Hz exercise.

ADENOSINE RELEASE

Changes in adenosine release were used as an index of alterations in interstitial adenosine concentration. The net adenosine release was calculated as the difference between the flux of adenosine leaving the tissue and the flux of adenosine entering the tissue. Adenosine release was used to determine if interstitial levels increase in response to 3 Hz exercise. In addition, the effect of aminophylline on interstitial adenosine concentration was assessed using adenosine release.

The average adenosine release during rest and 3 Hz exercise is shown in Figure 10. Negative release values indicate that venous concentration is less than arterial concentration. The average release during rest was -0.25 ± 0.09 nmoles/min/100g (Table 7, n=7). During 3 Hz exercise, adenosine release increased significantly ($P<0.05$) above resting values to 1.24 ± 0.30 nmol/min/100g. Before aminophylline the exercise induced increase in release was accompanied by increases in venous-arterial concentration difference (Table 6) and blood flow (Tables 2 and 4). After aminophylline, the average resting adenosine release was -0.02 ± 0.06 nmol/min/100g (Table 7). This was not significantly different from preaminophylline resting release (-0.25 ± 0.09 nmol/min/100g). However, during exercise, adenosine release (1.35 ± 0.47 nmoles/min/100g) increased significantly above the resting release (-0.02 ± 0.06 nmol/min/100g), but was not different from the exercise value (1.24 ± 0.30 nmol/min/100g) before aminophylline. Despite the ability of aminophylline to block the flow response to exogenous

Table 7. Effect of aminophylline on adenosine release.

	PREAMINOPHYLLINE		POSTAMINOPHYLLINE	
EXP #	REST	EXERCISE (3 Hz)	REST	EXERCISE (3 Hz)
1	-0.40	1.93	0.13	1.84
2	-0.25	1.96	0.06	1.80
3	0.05	0.43	0.17	-0.33
4	-0.23	0.60	-0.19	0.24
5	0.04	0.26	-0.02	0.73
6	-0.42	1.99	-0.07	3.34
7	-0.55	1.52	-0.19	1.77
MEAN	-0.25	1.24*	-0.02	1.35*
±SEM	0.09	0.30	0.06	0.47

Adenosine release (nmol/min/100g) was determined during steady state periods of rest and 3 Hz exercise. One way analysis of variance and Student-Newman-Keuls test were used for multiple comparison between mean values. (*= $P<0.05$ vs. REST, n=7). PRE- and POSTAMINOPHYLLINE indicates before and after aminophylline (10mg/kg) administration.

Figure 10. Effect of aminophylline on adenosine release during rest and 3 Hz exercise. Adenosine release was calculated as the product of venous-arterial plasma concentration difference and plasma flow. Values are MEAN $\pm$ SEM.

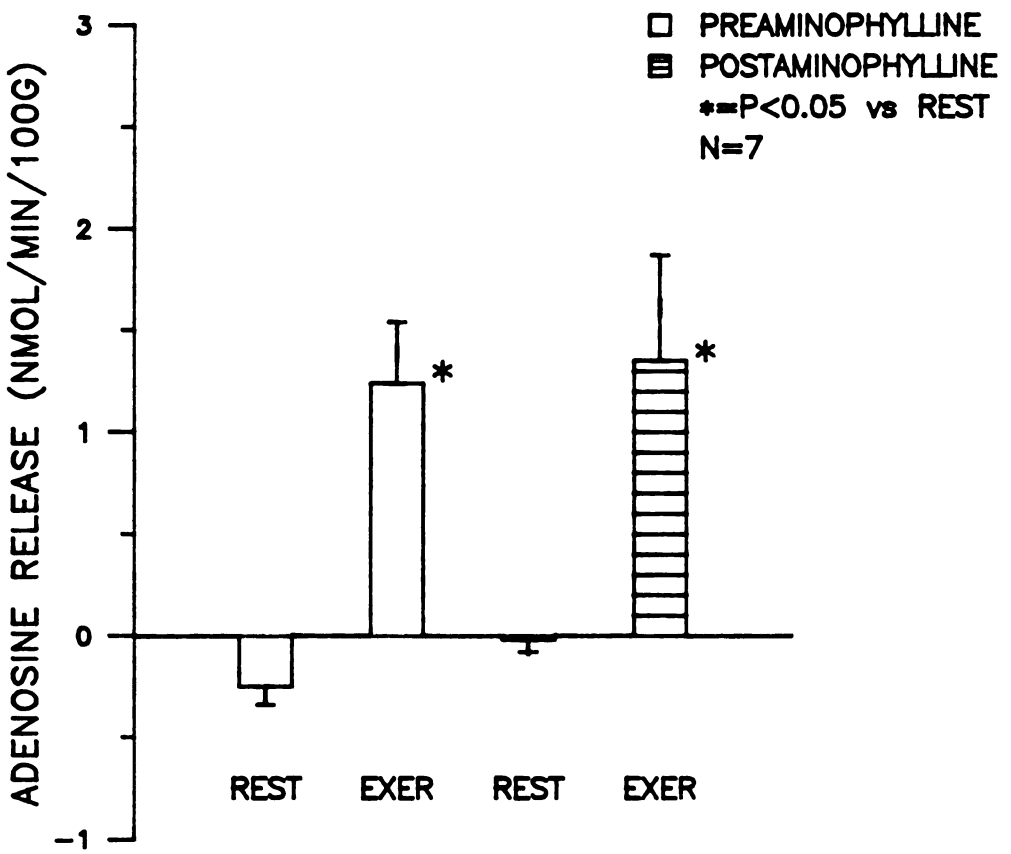

Figure 10. Effect of aminophylline on adenosine release during rest and 3 Hz exercise.

infusion of adenosine there is no effect of aminophylline on the exercise induced increases in blood flow, oxygen consumption, or adenosine release.

DOSE RESPONSE SERIES

In the second experimental series, the cumulative dose response relationship to varying concentrations of infused adenosine was determined (n=5). The effect of intrarterially infused aminophylline (10 mg/kg) on the adenosine induced hyperemia was assessed by the shift in the dose response curve (Figure 11). A parallel shift to the right along the abscissa indicates competitive inhibition by aminophylline and that an increase in adenosine concentration is necessary to overcome adenosine receptor blockade.

The effect of aminophylline on shifting the dose response curve was consistent in each animal, however, differences in the location of the dose response curve occurred between animals. The flow response to increasing adenosine concentration was measured as the percent of maximal flow above preinfusion flow. Prior to infusion, resting flow was 12.6±2.6 ml/min/100g. In the presence of infused adenosine, a dose dependent increase in flow occurred (202.7±12.3 ml/min/100g, 100% maximal flow). In the presence of aminophylline, resting flow (10.6±2.0) was not different from its preaminophylline control (12.6±2.6). Although higher concentrations of infused adenosine were required to increase flow, the maximal flow was not different from the preaminophylline level (208.7±12.1 ml/min/100g).

Figure 11. Effect of aminophylline on cumulative dose response relationship for adenosine. Flow responses to infused adenosine were measured before and after aminophylline (10 mg/kg). Values are MEAN $\pm$ SEM.

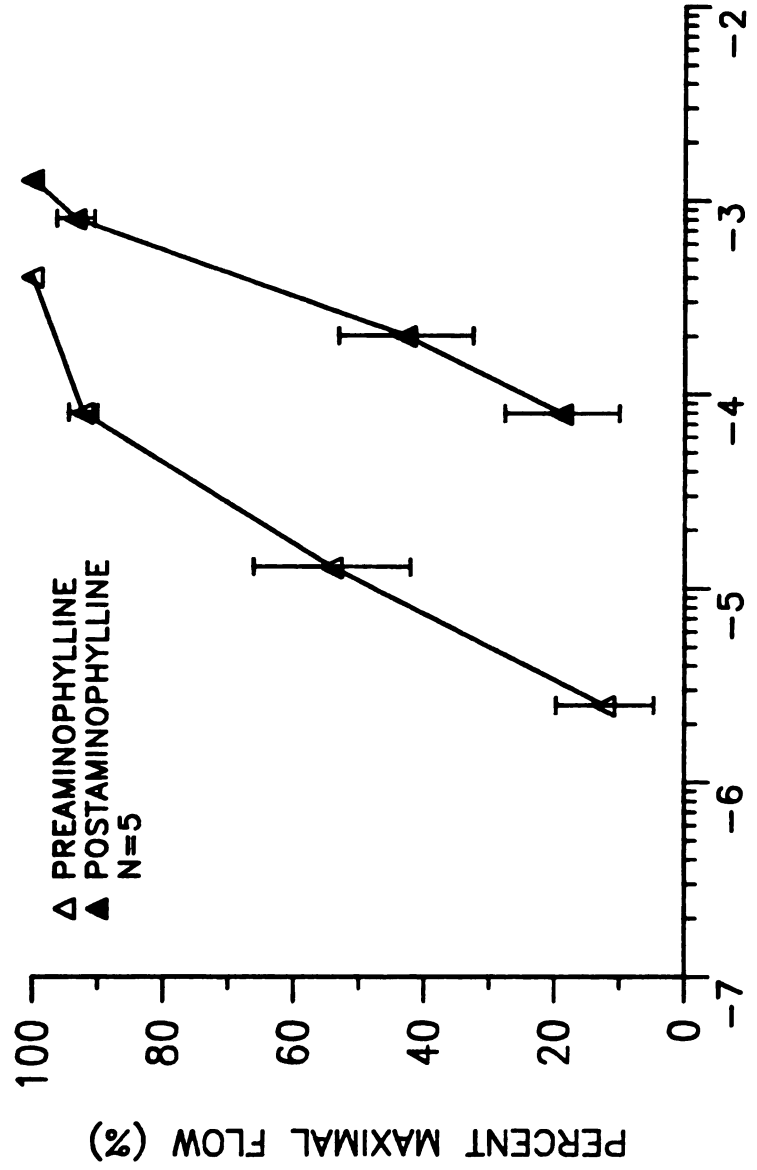

Figure 11. Effect of aminophylline on cumulative dose response relationship for adenosine.

The hyperemic response to 3 Hz exercise (82.5±6.0 ml/min/100g) was equivalent to 40% maximal flow.

Aminophylline shifted the dose response curve to the right in all animals. The average dose of adenosine which produced 50% maximal flow (ED_{50}) was $8.9 \pm 1.8 \times 10^{-6}$ M. The ED_{50} value ($1.7 \pm 0.6 \times 10^{-4}$ M) in the presence of aminophylline was significantly greater ($P<0.05$) than the preaminophylline control. To overcome aminophylline blockade, a 20 fold increase in infused adenosine concentration is required.

Percent extraction of infused adenosine was calculated to examine the effect of aminophylline on the uptake capacity of adenosine. A reduction in parenchymal cell uptake of adenosine could decrease removal from the interstitium. If aminophylline reduces adenosine uptake by parenchymal cells this could account for elevated interstitial levels capable of overcoming the aminophylline blockade. Before aminophylline, the average percent adenosine extraction over a concentration range of 1.0×10^{-6} to 1.0×10^{-4} M was 71.6±3.0%. Adenosine extraction was not significantly different (63.5±6.7%) after aminophylline administration.

INTERSTITIAL CONCENTRATION CALCULATED FROM CAPILLARY MODEL

INTERSTITIAL ADENOSINE CONCENTRATION AND ADENOSINE RELEASE

Interstitial adenosine concentration was calculated for rest and 3 Hz exercise using a 21 compartment model describing adenosine transport across capillaries. As shown in Table 8, experimental

Table 8. Calculated interstitial adenosine concentrations (ISF[ADO]) during rest and 3 Hz exercise.

		MODEL INPUT					MODEL OUTPUT		
EXP #	CONDITIONS	BF	HCT	[ADO]a	[ADO]v	REL	[ADO]v	REL	ISF[ADO]
1	REST	24.1	47	0.11	0.08	-.40	0.08	-.40	0.87
	EXER	87.2	46	0.11	0.15	1.93	0.15	1.93	1.73
	REST+AM	11.4	42	0.09	0.11	0.13	0.11	0.13	1.28
	EXER+AM	80.9	42	0.05	0.09	1.84	0.09	1.84	1.10
2	REST	23.3	47	0.08	0.06	-.25	0.06	-.25	0.67
	EXER	61.1	47	0.04	0.10	1.96	0.10	1.96	1.17
	REST+AM	11.7	48	0.10	0.11	0.06	0.11	0.06	1.26
	EXER+AM	49.7	48	0.11	0.18	1.80	0.18	1.80	2.00
3	REST	9.8	42	0.05	0.06	0.05	0.06	0.05	0.70
	EXER	70.6	39	0.03	0.04	0.43	0.04	0.43	0.49
	REST+AM	14.9	40	0.05	0.07	0.17	0.07	0.17	0.77
	EXER+AM	57.3	41	0.04	0.03	-.33	0.03	-.33	0.32
4	REST	14.9	36	0.06	0.04	-.23	0.04	-.23	0.41
	EXER	103.0	36	0.02	0.03	0.60	0.03	0.60	0.32
	REST+AM	14.2	44	0.11	0.09	-.19	0.09	-.19	0.98
	EXER+AM	97.0	46	0.10	0.10	0.24	0.10	0.24	1.13
5	REST	5.3	46	0.04	0.05	0.04	0.05	0.04	0.62
	EXER	76.8	44	0.03	0.04	0.26	0.04	0.26	0.43
	REST+AM	4.8	42	0.07	0.07	-.02	0.07	-.02	0.82
	EXER+AM	68.7	44	0.02	0.04	0.73	0.04	0.73	0.51
6	REST	11.7	45	0.10	0.04	-.42	0.04	-.42	0.43
	EXER	102.6	46	0.05	0.09	1.99	0.09	1.99	1.06
	REST+AM	11.7	44	0.06	0.05	-.07	0.05	-.07	0.57
	EXER+AM	123.2	44	0.04	0.09	3.34	0.09	3.34	1.11
7	REST	9.9	44	0.20	0.10	-.55	0.10	-.55	1.14
	EXER	76.5	45	0.06	0.09	1.52	0.09	1.52	1.10
	REST+AM	7.4	44	0.12	0.07	-.19	0.07	-.19	0.83
	EXER+AM	76.5	44	0.04	0.08	1.77	0.08	1.77	0.94

BF= blood flow (ml/min/100g); HCT= hematocrit (%); [ADO]a, [ADO]v= arterial, venous plasma conc. (uM), respectively; REL= release(nmol/min/100g); REST= resting condition; EXER= 3 Hz exercise; +AM= period after aminophylline infusion (10mg/kg).

measurement of blood flow, hematocrit, and plasma adenosine concentration from 7 dogs were used to calculate interstitial adenosine concentration. During rest, the values for capillary cleft surface area, endothelial cell surface area, and capillary volume were taken to be 1.32 cm^2/100g, 7.0 x 10^3 cm^2/100g, and 0.74 ml/100g, respectively (Casley-Smith et al., 1975). These parameters were increased 3.1 fold during exercise (4.1 cm^2/100g, 2.2 x 10^4 cm^2/100g, and 2.22 ml/100g, respectively). The individual values used to determine interstitial adenosine concentration for each experimental condition are shown in Table 8.

The correlation between individual values of calculated interstitial concentration and adenosine release is shown in Figure 12. During rest, there is a large variability among interstitial values corresponding to low values of adenosine release. In the presence of exercise, adenosine release increases, although the variability among the corresponding interstitial values remains. This results in a positive correlation ($r=0.514$, $P<0.005$) between adenosine release and interstitial concentration in response to 3 Hz exercise.

The average values of interstitial concentration (n=7) calculated for rest and 3 Hz exercise are summarized in Table 9. During rest, interstitial adenosine concentration was calculated as 0.69$\pm$0.10 uM, calculated using measured values for flow (14.1$\pm$2.5 ml/min/100g) and arterial (0.092$\pm$0.020 uM), and venous (0.060$\pm$0.008 uM) adenosine concentrations. During 3 Hz exercise, interstitial adenosine concentration was 0.90$\pm$0.19 uM but was not significantly different from calculated interstitial adenosine concentration at

Figure 12. Relationship between ISF[ADO] and release during rest and 3 Hz exercise. Interstitial adenosine concentration (ISF[ADO]) was calculated from a mathematical model describing transcapillary exchange of adenosine. Calculated ISF[ADO] is correlated with experimental values of adenosine release.

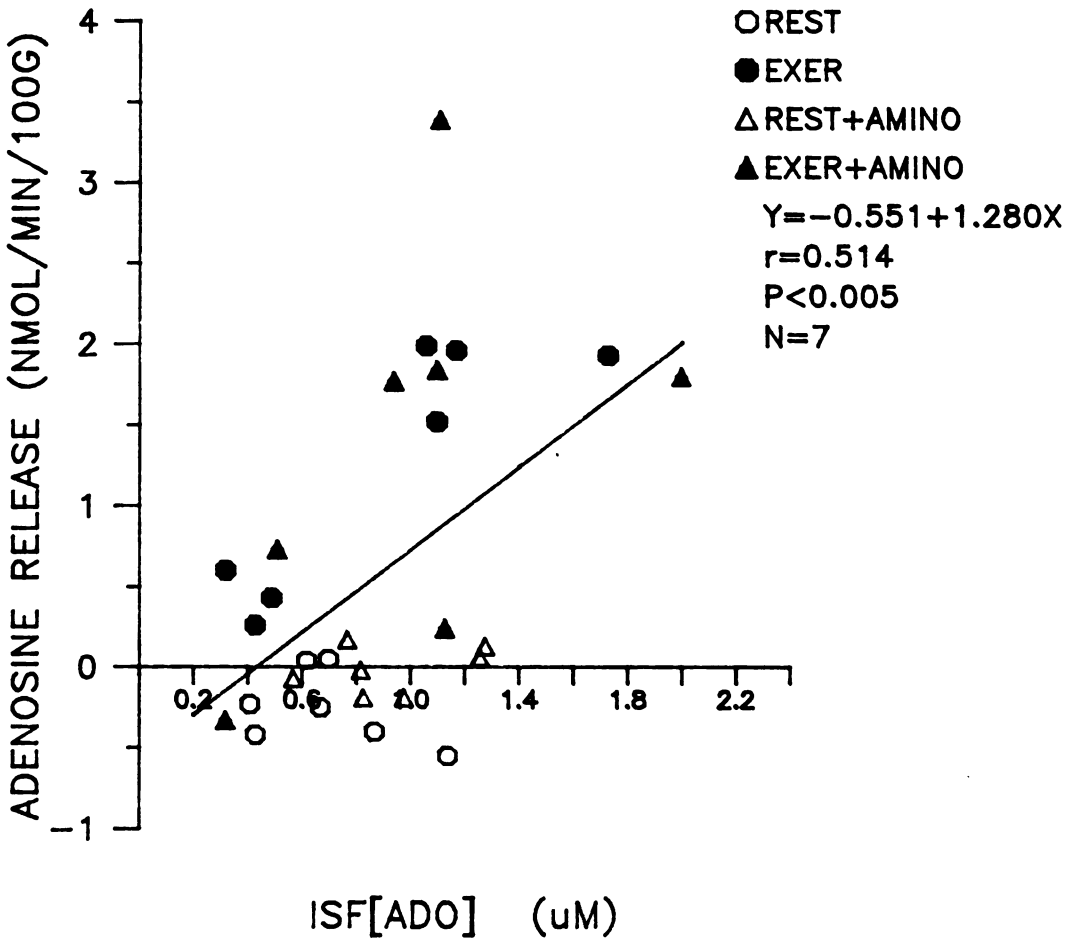

Figure 12. Relationship between ISF[ADO] and release during rest and 3 Hz exercise.

Table 9. Summary of average values used to calculate ISF[ADO].

	PREAMINOPHYLLINE		POSTAMINOPHYLLINE	
VALUES	REST	EXERCISE (3 Hz)	REST	EXERCISE (3 Hz)
ISF[ADO] (uM)	0.69±0.10	0.90±0.19	0.93±0.10	1.02±0.20
Blood flow (ml/min/100g)	14.1±2.5	82.5±6.0*	10.9±1.4	79.0±9.4*
[ADO]a (um)	.092±.020	.049±.011	.085±.010	.056±.012
[ADO]v (uM)	.060±.008	.077±.017	.080±.009	.087±.018
RADO (nmol/min/100g)	-0.25±0.09	1.24±0.30*	-0.016±0.055	1.35±0.47*

Values are MEAN±SEM (n=7). Significant differences ($P<0.05$) were determined using one way analysis of variance and Student-Newman-Keuls test for multiple comparisons between mean data. PRE- and POSTAMINOPHYLLINE indicate before and after aminophylline (10 mg/kg), respectively. (ISF[ADO], [ADO]a, [ADO]v= interstitial, arterial, venous adenosine concentration, respectively; RADO= adenosine release).

rest (0.69±0.10 uM). Despite an increase ($P<0.05$) in adenosine release (-0.25±0.09 nmol/min/100g to 1.24±0.30 nmol/min/100g) in response to 3 Hz exercise, interstitial concentration was not altered from resting values.

In the presence of aminophylline, the interstitial adenosine concentration was 0.93±0.10 uM during rest and 1.02±0.20 uM during 3 Hz exercise. Despite a significant increase in adenosine release (-0.02±0.06 to 1.35±0.47 nmol/min/100g), interstitial concentrations calculated for steady state rest and exercise were not significantly different. The results from the model indicate that calculated interstitial levels do not increase in response to exercise, nor are they elevated by aminophylline.

PARAMETER SENSITIVITY ON INTERSTITIAL ADENOSINE CONCENTRATION AND ADENOSINE RELEASE

We examined the influence of arterial adenosine concentration, flow, and capillary permeability-surface area (PS) on the relationship between interstitial adenosine concentration and adenosine release. The influences of these values on the relationship were examined to explain the poor correlation between the individual values (Figure 12). The relationship between interstitial adenosine concentration and adenosine release can be affected by arterial adenosine concentration (Figure 13). Blood flow and capillary PS are held constant as interstitial adenosine concentration is altered. A decrease in arterial adenosine concentration increases the adenosine release for a given

Figure 13. Effect of ART[ADO] on ISF[ADO] and release.
ISF[ADO] =interstitial adenosine concentration
ART[ADO] =arterial plasma adenosine concentration (uM)
BF =blood flow (ml/min/100g)
PS =permeability-surface area product (ml/min/100g)
Hematocrit =45%

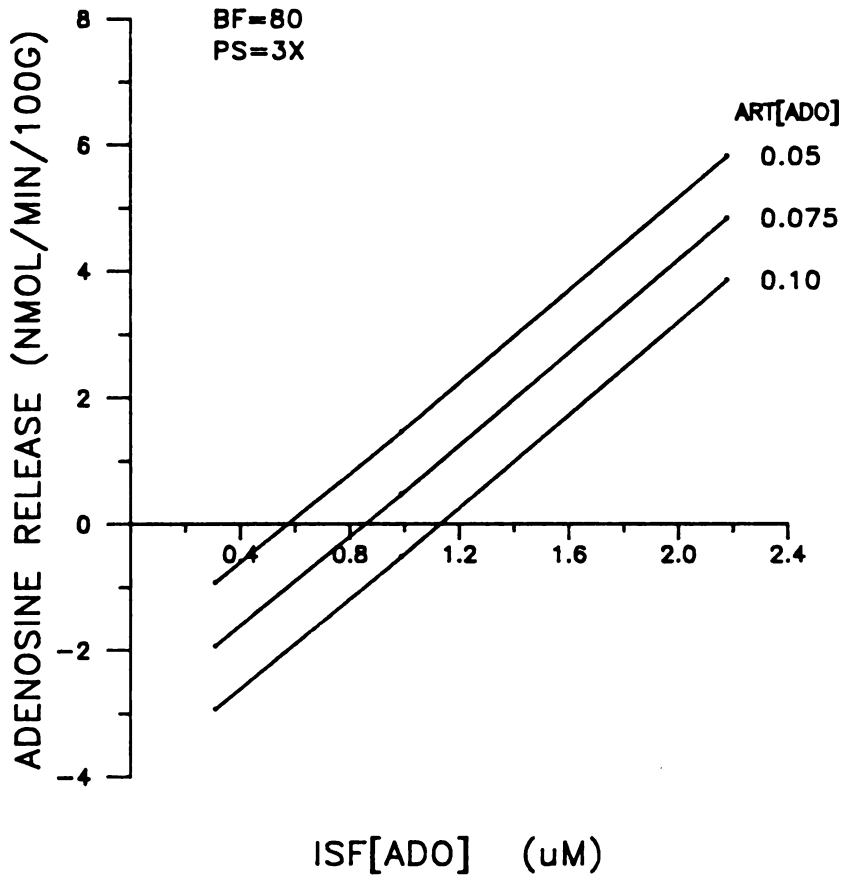

Figure 13. Effect of ART[ADO] on ISF[ADO] and release.

interstitial concentration. Since the slope of all lines is identical (3.6) sensitivity is not affected by changes in adenosine concentration. However, for a given interstitial concentration, increasing arterial concentration can reduce adenosine release by reducing the concentration gradient across the capillary wall.

The effect of blood flow on the relationship between adenosine release and interstitial concentration is shown in Figure 14. Arterial adenosine concentration and capillary PS were held constant as adenosine production was varied at different flow values. There is a linear relationship between adenosine release and interstitial adenosine concentration for a given flow. The sensitivity of this relationship can be measured as the slope of the line. For a flow of 20 ml/min/100g, adenosine release increases 1.0 nmol/min/100g with each 1 uM increase in interstitial concentration. An increase in flow from 20 to 80 ml/min/100g increases the sensitivity (slope) from 1.0 to 2.2 nmol/min/100g.uM^{-1}. The sensitivity is further increased to 5.6 nmol/min/100g.uM^{-1} for a flow of 160 ml/min/100g. Therefore, for a given interstitial concentration the increase in adenosine release is dependent on the flow.

The effect of increasing the capillary PS on adenosine release when arterial adenosine concentration (0.075 uM) and blood flow (80 ml/min/100g) are held constant is illustrated in Figure 15. There is a linear relationship between adenosine release and interstitial concentration. The sensitivity of release to changes in interstitial concentration increases with capillary PS. The slope of the line for a 1 fold increase (1X) in capillary PS is 2.3 nmol/min/100g.uM^{-1} and

Figure 14. Effect of blood flow on ISF[ADO] and release.
ISF[ADO] =interstitial adenosine concentration
ART[ADO] =arterial plasma adenosine concentration (uM)
BF =blood flow (ml/min/100g)
PS =permeability-surface area product (ml/min/100g)
Hematocrit =45%

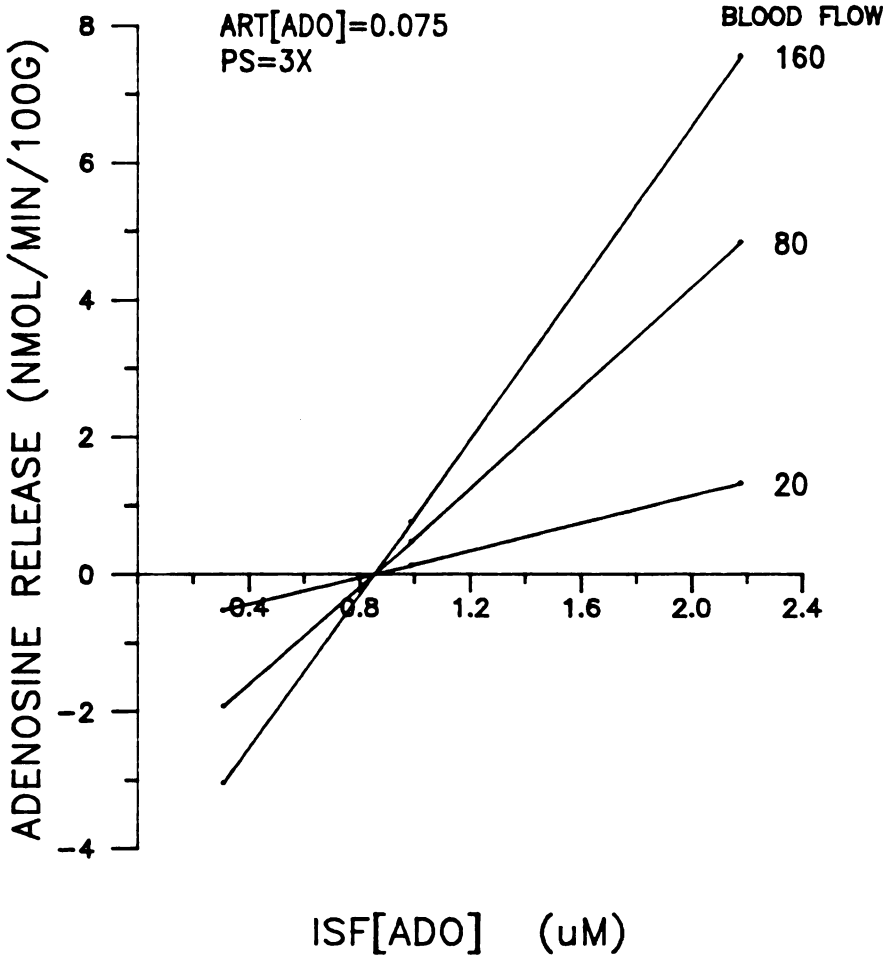

Figure 14. Effect of blood flow on ISF[ADO] and release.

Figure 15. Effect of capillary PS on ISF[ADO] and release.
ISF[ADO] =interstitial adenosine concentration
ART[ADO] =arterial plasma adenosine concentration (uM)
Blood flow =ml/min/100g
PS =permeability-surface area product (ml/min/100g)
Hematocrit =45%

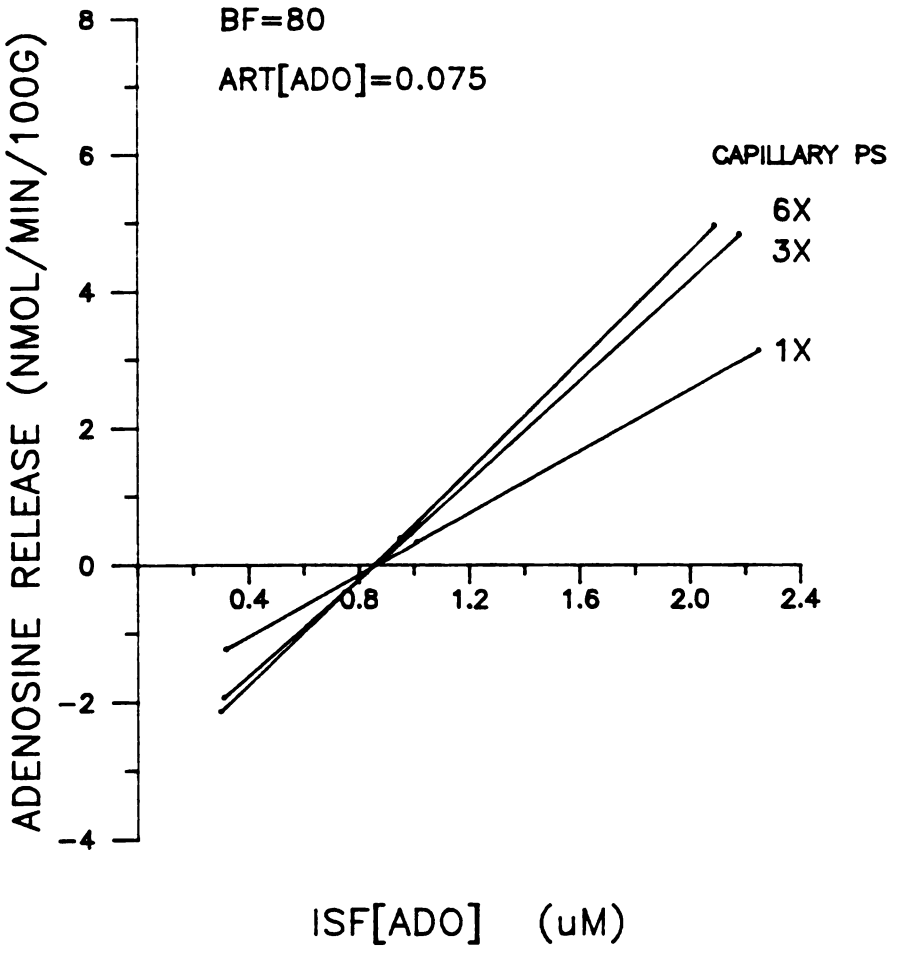

Figure 15. Effect of capillary PS on ISF[ADO] and release.

increases to 3.6 with a 3 fold increase (3X) in capillary PS. Increasing capillary PS to 6 times (6X) the control value, further increases the slope to 3.8. At an interstitial concentration of 1 uM, an increase in capillary PS from 1 to 3 fold produces an increase in adenosine release of approximately 0.2 nmol/min/100g. The 3 fold increase in capillary PS represents a physiological increase during 3 Hz exercise (Casley-Smith et al., 1975).

INTERSTITIAL AND VENOUS ADENOSINE CONCENTRATION

We also examined the relationship of individual values between interstitial and venous adenosine concentrations (Figure 16). Interstitial adenosine levels were 11 fold higher than venous levels (Table 9), indicating a significant barrier for adenosine movement across the capillary. Individual values of venous concentration are linearly related to calculated interstitial concentration during rest and 3 Hz exercise (Figure 16). There is a much better correlation between venous and interstitial concentration ($r=0.997$, $P<0.001$) than between release and interstitial concentration ($r=0.514$, $P<0.005$; Figure 12). These data demonstrate that the relationship between venous and interstitial concentration is less sensitive to changes in arterial concentration, flow, and capillary PS than release and interstitial concentration.

Figure 16. Relationship between ISF[ADO] and VEN[ADO] during rest and 3 Hz exercise. Interstitial adenosine concentration (ISF[ADO]) was calculated from a mathematical model describing transcapillary exchange of adenosine. Calculated ISF[ADO] is correlated with experimental values of plasma venous adenosine concentration (VEN[ADO]).

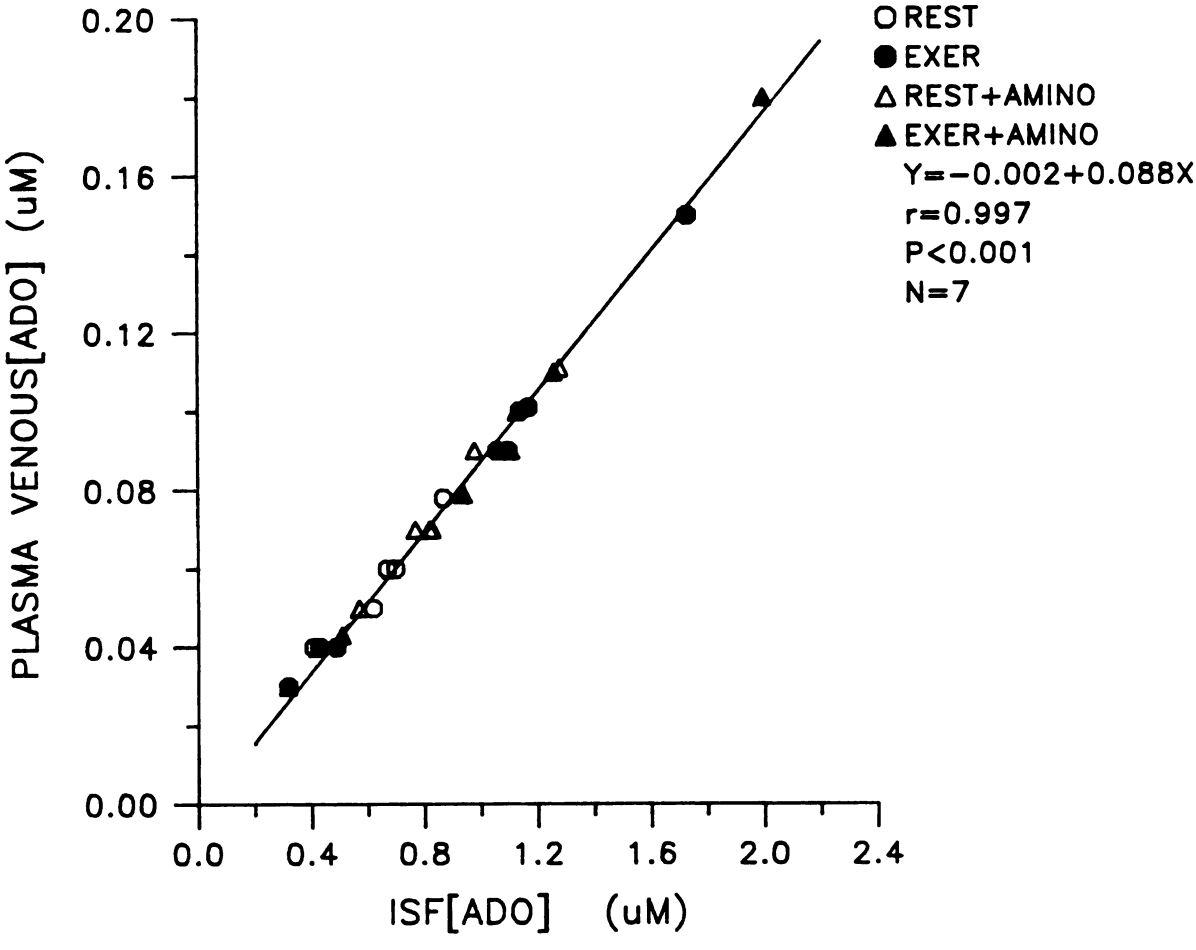

Figure 16. Relationship between ISF[ADO] and VEN[ADO] during rest and 3 Hz exercise.

PARAMETER SENSITIVITY ON INTERSTITIAL AND VENOUS ADENOSINE CONCENTRATION

The effects of arterial concentration, flow, and capillary PS on the relationship between venous and interstitial adenosine concentration are shown in Figures 17, 18, and 19, respectively. Changes in arterial adenosine concentration have little effect on venous concentration (Figure 17). For a given flow (80 ml/min/100g), capillary PS (3 fold rest), and arterial concentration (0.075 uM), the slope of the line is 0.082 uM_{ven}/uM_{isf}. Therefore, venous concentration is less than interstitial concentration by a factor of 0.082. If interstitial concentration increases 2 fold, venous concentration should increase by 0.16 uM. This relationship indicates that small changes in intersitial concentration should be measurable using plasma venous concentration.

At an interstitial concentration of 1 uM, venous concentration is independent of flow (Figure 18). At higher interstitial levels, the effect of flow on venous concentration is increased. However, even at interstitial concentrations 2 fold higher than those calculated for exercise, the effect of flow on venous concentration is minimal.

The effect of capilary PS on this relationship is also small relative to its effect on release (Figure 19). For a capillary PS that is 1 fold rest, the slope is 0.052 and increases to 0.082 for a 3 fold increase in capillary PS. At interstitial concentrations of 1 uM, the model predicts that venous concentration is insensitive to

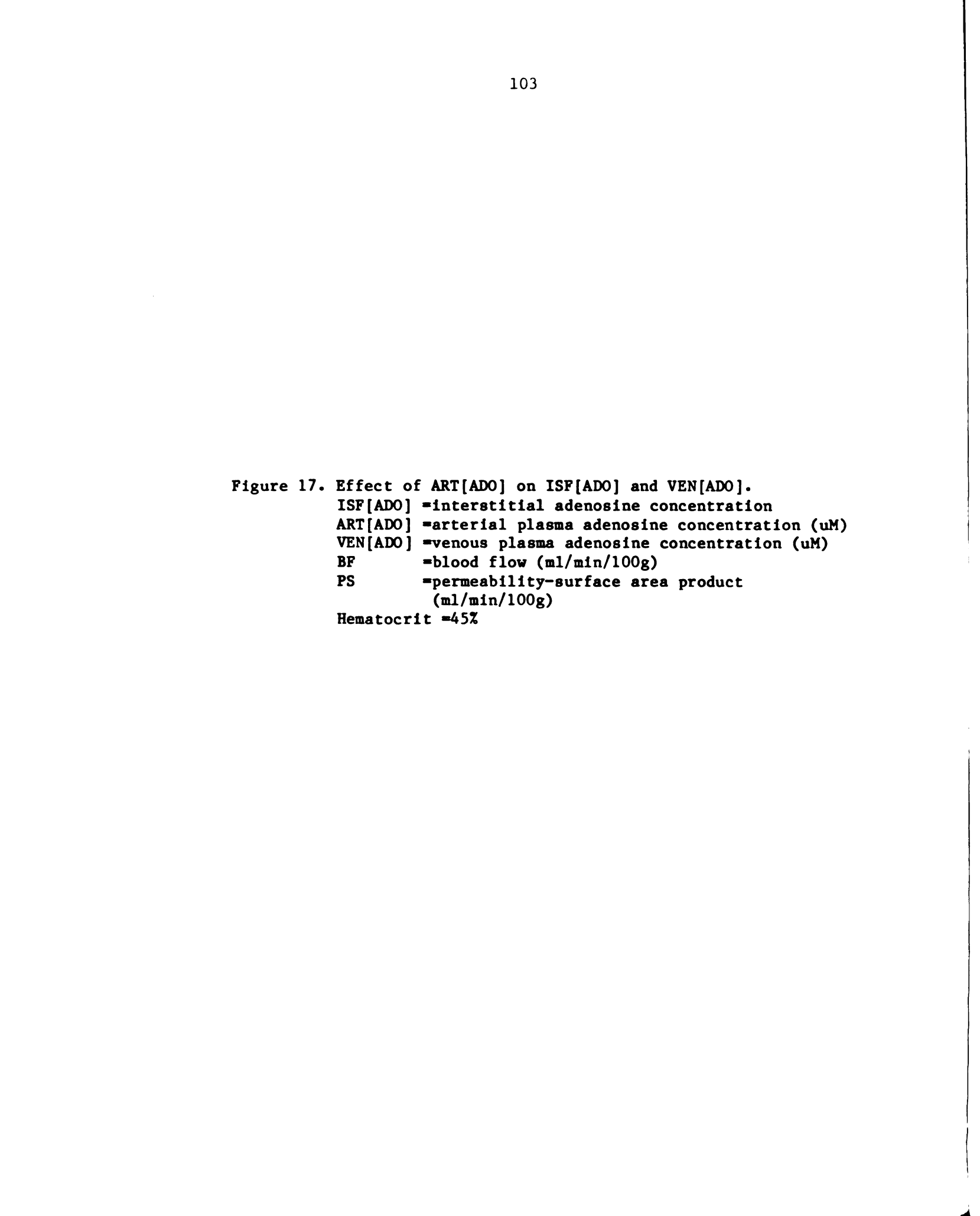

Figure 17. Effect of ART[ADO] on ISF[ADO] and VEN[ADO].
ISF[ADO] =interstitial adenosine concentration
ART[ADO] =arterial plasma adenosine concentration (uM)
VEN[ADO] =venous plasma adenosine concentration (uM)
BF =blood flow (ml/min/100g)
PS =permeability-surface area product (ml/min/100g)
Hematocrit =45%

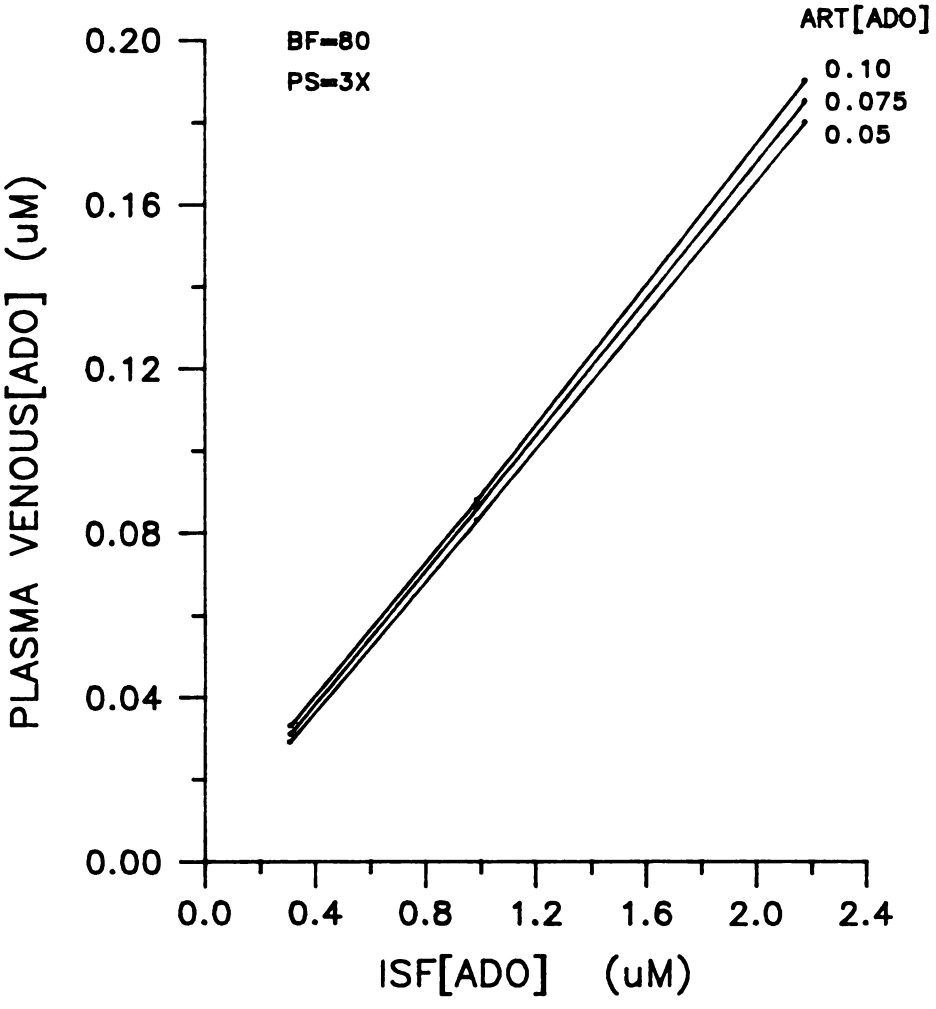

Figure 17. Effect of ART[ADO] on ISF[ADO] and VEN[ADO].

Figure 18. Effect of blood flow on ISF[ADO] and VEN[ADO].
ISF[ADO] =interstitial adenosine concentration
ART[ADO] =arterial plasma adenosine concentration (uM)
VEN[ADO] =venous plasma adenosine concentration (uM)
Blood flow =ml/min/100g
PS =permeability-surface area product (ml/min/100g)
Hematocrit =45%

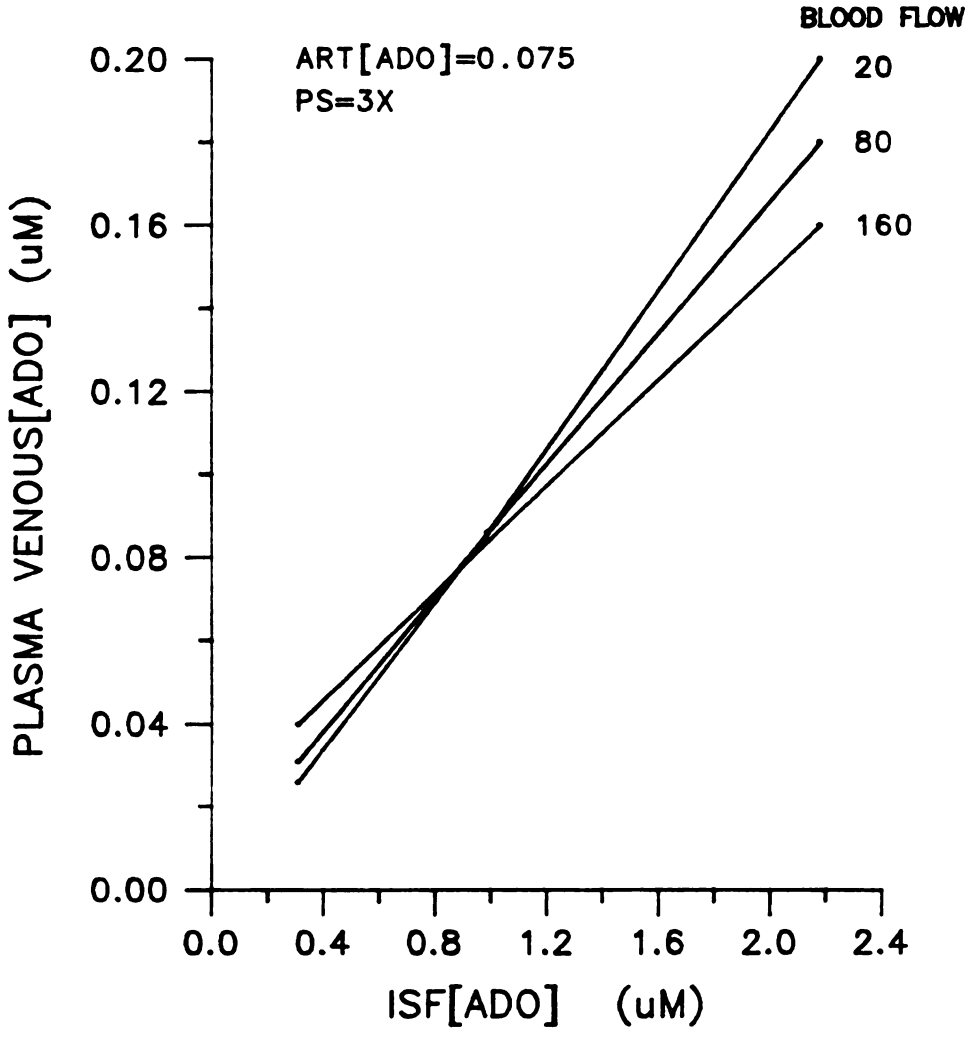

Figure 18. Effect of blood flow on ISF[ADO] and VEN[ADO].

Figure 19. Effect of capillary PS on ISF[ADO] and VEN[ADO].
ISF[ADO] =interstitial adenosine concentration
ART[ADO] =arterial plasma adenosine concentration (uM)
VEN[ADO] =venous plasma adenosine concentration (uM)
BF =blood flow (ml/min/100g)
PS =permeability-surface area product (ml/min/100g)
Hematocrit =45%

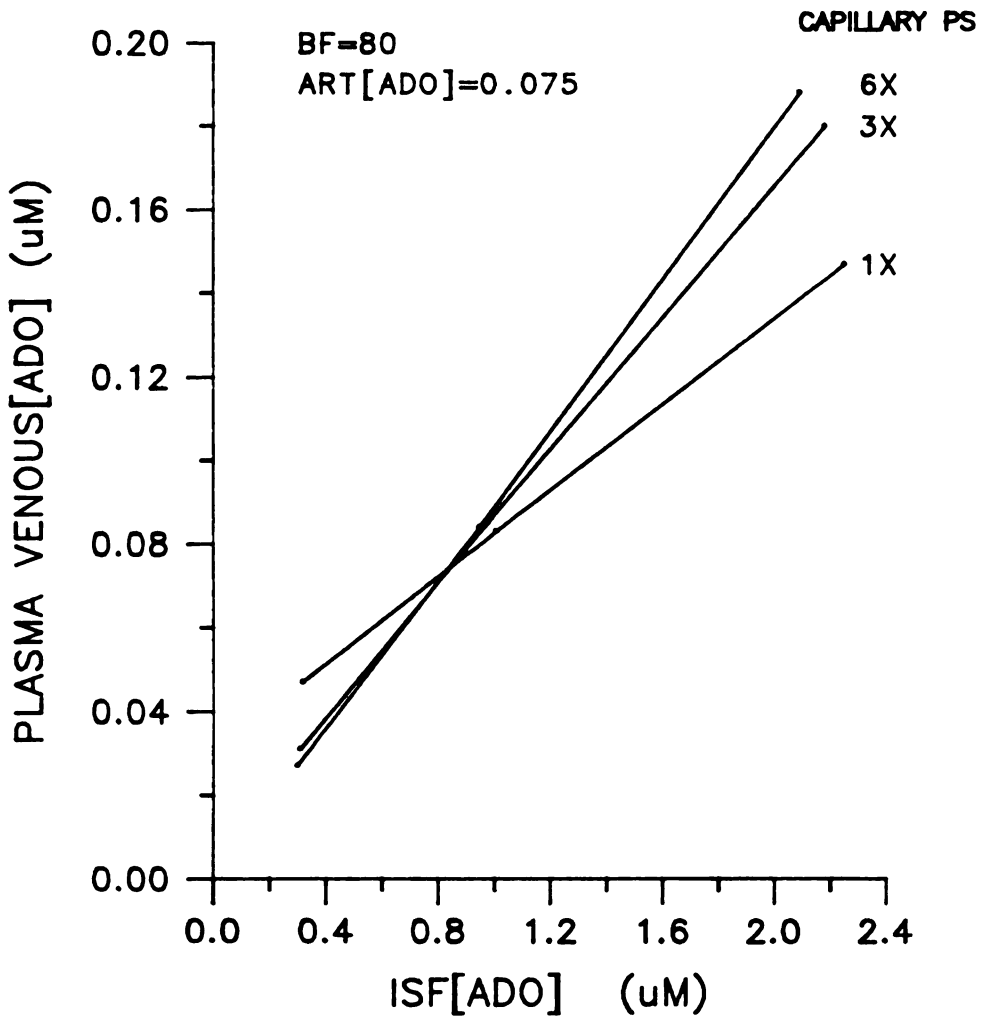

Figure 19. Effect of capillary PS on ISF[ADO] and VEN[ADO].

changes in capillary PS. Furthermore, even at higher interstitial concentrations, the effect of PS on venous concentration is small.

SUMMARY OF RESULTS

Blood flow and oxygen consumption increased 5.6 fold and 26.2 fold in response to 3 Hz exercise. Aminophylline had no effect on the oxygen consumption/flow relationship during rest or sustained 3 Hz exercise. Adenosine release was significantly elevated above rest in response to 3 Hz stimulation while plasma venous concentration was unchanged. In the presence of aminophylline, adenosine release and venous concentration during 3 Hz exercise were not different from the preaminophylline control.

Aminophylline is capable of blocking the flow response to adenosine infusion during both rest and 3 Hz exercise. However, the flow response to infusion of ATP was not reduced, indicating a selective blockade of adenosine receptors by aminophylline. The interstitial adenosine dose response curves indicate that a 20 fold increase in adenosine concentration is necessary to overcome aminophylline blockade. Therefore, a 20 fold increase in interstitial concentration is necessary to explain the lack of effect of aminophylline on exercise hyperemia. The relationship between interstitial concentration and adenosine release or plasma venous concentration was examined using a mathematical model. These relationships were examined to determine which measurement best reflects changes in interstitial concentration. There is a better correlation between interstitial adenosine concentration and venous

concentration than between interstitial concentration and adenosine release. This suggests venous adenosine concentration may be a better index of interstitial concentration.

DISCUSSION

Adenosine has been proposed as a metabolic vasoregulator of skeletal muscle blood flow. It has been demonstrated that in response to increased metabolic activity of skeletal muscle there is an increase in both tissue adenosine content (Berne et al., 1971; Dobson et al., 1971; Bockman et al., 1976; Belloni et al., 1979; Bockman and McKenzie, 1983; Steffen et al., 1983) and venous adenosine concentration (Bockman et al., 1975; Sparks and Fuchs, 1983). However, the relationship between increased tissue adenosine content or venous concentration and decreased vascular resistance has been examined primarily under conditions of impaired oxygen delivery. The vasoregulatory role of adenosine in skeletal muscle when oxygen demand is matched by sufficient oxygen delivery is largely unexplored.

We examined the role of adenosine in mediating sustained hyperemia during 3 Hz exercise in muscles perfused at constant pressure. An increase in oxygen consumption is accompanied by an increase in oxygen delivery. The balance between these variables is indicated by sustained contractile force during continuous exercise. The flow response to exercise was measured in the presence and absence of aminophylline. Adenosine release and venous concentration were used as indices of interstitial concentration. Although venous adenosine concentration does not increase above rest in response to exercise, release is significantly increased. Our results indicate that despite an effective adenosine receptor blockade, aminophylline

has no effect on exercise vasodilation. Adenosine is a well known vasodilator (Dawes, 1941; Drury and Szyent Gyorgi, 1929). If release indicates an elevated interstitial concentration, the lack of effect of aminophylline on exercise flow in the presence of elevated adenosine release is parodoxical.

Four hypotheses are proposed to account for this paradox:

(1) Intraarterially infused aminophylline does not reach the adenosine receptors that bind adenosine released by skeletal muscle.
(2) Increased vascular sensitivity to adenosine during exercise reduces the effectiveness of aminophylline.
(3) The effect of aminophylline is completely overcome by elevated interstitial adenosine concentration.
(4) Interstitial adenosine concentration does not increase in response to exercise.

Each hypothesis will be discussed in the following sections.

HYPOTHESIS 1: INFUSED AMINOPHYLLINE DOES NOT BLOCK ENDOGENOUS ADENOSINE

An intraarterial infusion of aminophylline was used to block receptor mediated vasodilation. Receptor blockade was tested by measuring the reduction in flow associated with an intraarterial adenosine infusion during rest and exercise. In the presence of aminophylline there was a 20 fold shift in the dose response curve to

exogenous adenosine. We assume that infused adenosine and aminophylline bind to the same receptors activated by endogenous adenosine. If this assumption is invalid, then the lack of effect of infused aminophylline on exercise hyperemia could be explained by an ineffective blockade of adenosine receptors accessible to endogenous adenosine. However, this assumption is thought to be valid for several reasons. First, adenosine is a small water soluble molecule which should distribute throughout the extracellular space of skeletal muscle. Second, there is evidence which supports the hypothesis that infused aminophylline is equally effective as extravascular administered aminophylline (Mohrman and Heller, 1982). Aminophylline present in the suffusion solution surrounding a cremaster muscle preparation blocks the vascular response to adenosine added to the suffusion solution. Third, there is evidence that infused aminophylline crosses the capillary wall to exert an antagonistic effect (Belardinelli et al., 1980; 1981; 1982).

The efficacy of infused aminophylline to block adenosine receptors exposed to interstitial adenosine was examined in the rat cremaster muscle preparation (Mohrman and Heller, 1982). Aminophylline was placed either in the suffusion solution, therefore having access to adenosine receptors via diffusion through the interstitium, or was infused intravenously into the whole animal, having access to receptors via diffusion across the capillary. Changes in arteriolar diameter were measured when adenosine was added to the suffusion solution. Intravascular and suffused aminophylline equally reduced adenosine induced dilation. These results indicate

that infused aminophylline can cross the capillary wall and inhibit adenosine receptors acted on by interstitial adenosine.

Further support for the ability of aminophylline to cross the capillary wall was obtained by Belardinelli et al. (1980, 1981, 1982). They demonstrated that adenosine (5×10^{-6} M) infused into the aortic cannula of isolated guinea pig hearts increases the atrioventricular (AV) conduction delay, indicating that infused adenosine must cross the capillary wall to exert its effect. In the presence of aminophylline (3×10^{-5} M), the adenosine induced AV conduction delay was eliminated. Therefore, aminophylline must cross the capillary wall to exert its antagonistic effect on the adenosine mediated response.

The ability of infused aminophylline to cross the capillary wall and block adenosine receptors exposed to endogenous adenosine was demonstrated using dipyridamole (Afonso, 1970). Dipyridamole has been proposed to mediate vasodilation by blocking cellular uptake of adenosine (Mustafa, 1979; Afonso and O'Brien, 1967). Afonso (1970) proposed that dipyridamole induced dilation occurred by accumulation of interstitial adenosine to levels capable of activating vascular adenosine receptors. Afonso (1970) examined the effect of aminophylline on the dipyridamole induced vasodilation in the canine coronary bed. Aminophylline, administered intravenously (50 or 100 mg) reduced the dilator effect of dipyridamole, indicating that aminophylline had access to receptors exposed to endogenous adenosine.

Intraarterial infusion of adenosine may produce dilation via vascular mechanisms initiated from the luminal side of the vessel. An intraluminal mediated mechanism for relaxation of large coronary

arteries by exogenous adenosine has recently been demonstrated by Hintze and Vatner (1984). Large artery dilation is blocked by intravenous administration of aminophylline (Hintze and Vatner, 1984). If dilation by exogenous adenosine occurs exclusively by an intraluminal mechanism, then attenuation of this response by infused aminophylline would not adequately test the efficacy of receptor blockade to endogenous adenosine. The significance of an intraluminal mediated mechanism in arterioles in mediating the flow response to infused adenosine is unknown. DeMey and Vanhoutte (1982) showed that in response to increasing exogenous adenosine concentration (1×10^{-6} to 1×10^{-3}M) the vascular relaxation of isolated canine arterial rings is the same in the presence or absence of the endothelium. This suggests that when adenosine is exposed to both vascular smooth muscle and endothelium, the intraluminal mediated mechanism for adenosine may be insignificant. Therefore, the predominant mechanism for vascular relaxation to infused adenosine in vivo is likely mediated by a direct effect on vascular smooth muscle. Since adenosine has access to both endothelium and vascular smooth muscle via the interstitium, aminophylline blockade of the adenosine induced flow response can not be explained by selective blockade of an intraluminal mediated mechanism. Because both adenosine and aminophylline are capable of entering the interstitium, we think infused aminophylline provides an effective blockade of receptors to endogenous adenosine. Therefore, the lack of effect of aminophylline on exercise hyperemia can not be explained by the inability of infused aminophylline to reach the same receptors exposed to endogenous adenosine.

HYPOTHESIS 2: INCREASED VASCULAR SENSITIVITY REDUCES EFFECT OF AMINOPHYLLINE

Vascular sensitivity to adenosine has been shown to increase in response to reduced pH (Merrill et al., 1978). In our experiments the venous pH during 3 Hz exercise was significantly reduced and within the range (7.42 to 6.89) tested by Merrill and co workers. Therefore, vascular sensitivity to adenosine may be increased during exercise. It could be argued that during exercise the effectiveness of aminophylline on adenosine receptor blockade is reduced. This would explain the lack of effect of aminophylline on exercise hyperemia. However, this is not supported by our observation that aminophylline reduces the flow response to infused adenosine during exercise. Furthermore, Merrill et al. (1978) found that competitive antagonism of adenosine induced dilation by theophylline (10^{-6} M to 10^{-4} M) is not altered when pH is reduced by hypercapnia. This suggests that despite a possible increase in vascular sensitivity to adenosine, aminophylline is still effective in blocking adenosine receptors.

HYPOTHESIS 3: ELEVATED INTERSTITIAL ADENOSINE CONCENTRATION OVERCOMES AMINOPHYLLINE BLOCKADE

Adenosine release, flow, and oxygen consumption increase from rest to exercise. The increase in these variables is the same in the presence and absence of aminophylline. If aminophylline blockade is

overcome by elevated interstitial adenosine concentration, adenosine release should increase above control. This would occur providing release is sensitive to increases in interstitial concentration. The following questions were considered: (1) Is aminophylline capable of increasing adenosine in skeletal muscle? (2) If so, does interstitial adenosine concentration increase to levels capable of overcoming aminophylline blockade? (3) Would increased interstitial levels be detectable from release measurements or plasma venous adenosine concentration?

Aminophylline blocks adenosine mediated vascular relaxation by competitive inhibition (Bünger et al., 1975). In the open chest dog preparation, simultaneous intracoronary infusions of theophylline and isoproterenol significantly increased tissue adenosine content and coronary sinus adenosine concentration above those observed during isoproterenol alone (McKenzie et al., 1981). The increase in adenosine in the presence of theophylline and isoproterenol was not due to changes in cardiac metabolism, since oxygen consumption and the lactate/pyruvate ratio were not different from the isoproterenol control. If blockade of adenosine receptors by theophylline or aminophylline is overcome by elevated interstitial adenosine, this could explain why aminophylline does not reduce exercise hyperemia in our study. However, this is unlikely for the following reasons: First, receptor blockade was tested during exercise and found to be effective in reducing the flow response to exogenous adenosine. Second, adenosine release and venous adenosine concentration during exercise were not elevated above the preaminophylline control,

indicating that during exercise interstitial adenosine is not potentiated by aminophylline.

To determine the adenosine concentration necessary to overcome aminophylline blockade, adenosine dose response curves in the presence and absence of aminophylline (Figure 11) were obtained. These results indicate that a 20 fold increase in interstitial concentration is necessary to overcome adenosine receptor blockade by aminophylline. Therefore, a 20 fold increase in interstitial concentration would be necessary to explain the lack of effect of aminophylline on exercise hyperemia. However, during exercise neither release nor venous concentration was elevated above preaminophylline controls. If measurements of release or venous concentration are insensitive to a 20 fold change in interstitial concentration, then aminophylline blockade could be overcome by elevated interstitial levels and not detected from these measurements. The lack of increase in flow during exercise can be explained if the exogenous adenosine was only a small fraction of interstitial concentration resulting in no further dilation. We used a mathematical model to determine if adenosine release and venous concentration would be sensitive to 20 fold changes in interstitial concentration.

SENSITIVITY OF ADENOSINE RELEASE AND VENOUS ADENOSINE CONCENTRATION TO CHANGES IN INTERSTITIAL LEVELS

We used a mathematical model for adenosine movement across the capillary to evaluate adenosine release and plasma venous adenosine concentration as indices of interstitial adenosine concentration. The model shows that the relationship between interstitial adenosine concentration and appearance of adenosine in the venous effluent is influenced by endothelial cell uptake, blood flow, capillary permeability-surface area (PS) product, and arterial adenosine concentration. We evaluated the effect of each of these parameters on the relationship between interstitial concentration and adenosine release or plasma venous adenosine concentration. The effect of increases in interstitial adenosine concentration on adenosine release and venous adenosine concentration are determined while holding all but one of these parameters constant. The parameters of blood flow, capillary PS, and arterial adenosine concentration were taken to be those which occurred during exercise. Figures 13, 14, and 15 illustrate the sensitivity of adenosine release to changes in interstitial concentration at various levels of arterial adenosine concentration, blood flow, and capillary PS, respectively. In Figures 17, 18, and 19, we examined the sensitivity of plasma venous adenosine concentration to changes in interstitial concentration for the same parameter values used in the evaluation of adenosine release.

As shown in Figure 13, for a given blood flow (80 ml/min/100g), arterial adenosine concentration (0.075 uM), and capillary PS (3 fold

rest), adenosine release increases 5 fold when interstitial concentration doubles. The model predicts that an increase in adenosine release would be easily detectable if a 20 fold increase in interstitial concentration occurred. As shown in Figure 17, for a given blood flow (80 ml/min/100g), arterial adenosine concentration (0.075 uM), and capillary PS (3 fold rest), plasma venous concentration increases two fold when interstitial concentration doubles. The model predicts that plasma venous adenosine concentration would easily detect 20 fold increases in interstitial concentration. At a given interstitial concentration, changes in blood flow and arterial adenosine concentration alter adenosine release and venous concentration. However, the influences of arterial adenosine concentration and blood flow can not be significant, since exercise blood flow and arterial adenosine concentration in the presence of aminophylline were not different from preaminophylline controls.

The effect of aminophylline on capillary PS is unknown. If aminophylline reduced capillary PS and thereby reduced adenosine flux across the capillary, interstitial concentration could theoretically increase in the absence of changes in adenosine release (Figure 15) or venous concentration (Figure 19). However, it is unlikely that aminophylline reduces capillary PS during exercise. Since aminophylline has vasodilator properties, the expected effect on capillary surface area would be an increase rather than a reduction in capillary PS. Although the effect of aminophylline on capillary PS should be determined experimentally, we do not believe that a reduction in PS sufficient to mask a 20 fold increase in interstitial

adenosine concentration could have occurred. Therefore, since adenosine release and venous concentration in the presence of aminophylline were not different from the preaminophylline controls, and the flow response to infused adenosine during exercise was blocked by aminophylline, it was concluded that receptor blockade was not overcome by an increased interstitial adenosine concentration.

We have argued that the ineffectiveness of adenosine receptor blockade on exercise hyperemia is not due to (1) a differential blockade of adenosine receptors inaccessible to endogenously released adenosine, (2) reduced effectiveness of blockade as a result of increased vascular sensitivity in the presence of reduced pH or (3) overcoming of aminophylline blockade due to elevated interstitial adenosine concentration. An alternative explanation for the inability of aminophylline to effect exercise hyperemia in the presence of elevated adenosine release is that an increase in release does not indicate an increase in interstitial concentration. The following section examines the relationship between adenosine release, interstitial and venous adenosine concentrations, and the determinants of adenosine movement across the capillary wall.

HYPOTHESIS 4: INTERSTITIAL ADENOSINE CONCENTRATION IS NOT ELEVATED DURING EXERCISE

Adenosine release was used as an index of interstitial adenosine concentration. We assumed that an increase in release reflects a proportional increase in the flux of adenosine across the capillary wall due to an increase in interstitial adenosine concentration.

Adenosine release increased from -0.25 nmol/min/100g at rest to 1.24 nmol/min/100g during 3 Hz exercise, concomitant with a 5.6 fold increase in flow and a 26.2 fold increase in oxygen consumption. The increase in release during exercise can be interpreted as an increased flux of adenosine across the capillary due to increased interstitial adenosine concentration. However, venous adenosine concentration has also been used as an index of interstitial concentration. In our experiments venous adenosine concentration did not change in response to 3 Hz exercise.

To determine which parameter best reflects changes in interstitial adenosine concentration, we used a mathematical model that describes adenosine movement across the capillary. We used the model to calculate interstitial adenosine concentration utilizing literature values for capillary PS (Casley-Smith et al., 1975) and endothelial cell uptake kinetics (Pearson et al., 1978) and individual experimental measurements of arterial and venous plasma adenosine concentrations, blood flow, and hematocrit. We varied interstitial adenosine concentration until the predicted venous concentration matched the experimentally measured venous concentration and release. Because arterial adenosine concentration and plasma flow were supplied as model parameters, a match of calculated and observed venous concentration also gives a match for adenosine release.

EFFECT OF ARTERIAL ADENOSINE CONCENTRATION ON THE MOVEMENT OF ADENOSINE ACROSS THE CAPILLARY

The arterial adenosine concentration decreased from 0.092 uM (rest) to 0.056 uM in response to 3 Hz exercise. We predicted that changes in arterial adenosine concentration could influence movement of adenosine across the capillary, since flux of adenosine is partially determined by the concentration gradient across the capillary wall. The effect of changes in arterial adenosine concentration, blood flow, and capillary PS on the relationship between interstitial adenosine concentration and adenosine release or plasma venous adenosine concentration was examined (Figures 20, 21, and 22). Each figure is a composite of four relationships. In each graph one parameter is varied while the other two parameters are held constant. The sensitivity of adenosine release and plasma venous concentration to changes in one of the three parameters was assessed for a range of interstitial adenosine concentrations (0.2-2.2 uM). In addition, the sensitivity of the relationship between interstitial adenosine concentration and adenosine release or venous concentration was evaluated in the presence and absence of endothelial cell uptake of adenosine.

Figure 20 shows the influence of arterial adenosine concentration on the relationship between interstitial adenosine concentration and appearance of adenosine in the venous effluent. In the absence of endothelial cell uptake (Figure 20A), our model indicates that arterial adenosine concentration has a minimal effect on this relationship. However, when endothelial cell uptake of

Figure 20. Effect of ART[ADO] on adenosine release and VEN[ADO] in the presence and absence of endothelial cell uptake. The upper panels show the relationship between interstitial adenosine concentration (ISF[ADO]) and adenosine release. The bottom panels show the relationship between ISF[ADO] and venous plasma adenosine concentration (VEN[ADO]). ART[ADO]= arterial plasma adenosine concentration (uM); BF= blood flow (ml/min/100g); PS= permeability-surface area product (ml/min/100g); hematocrit =45%.

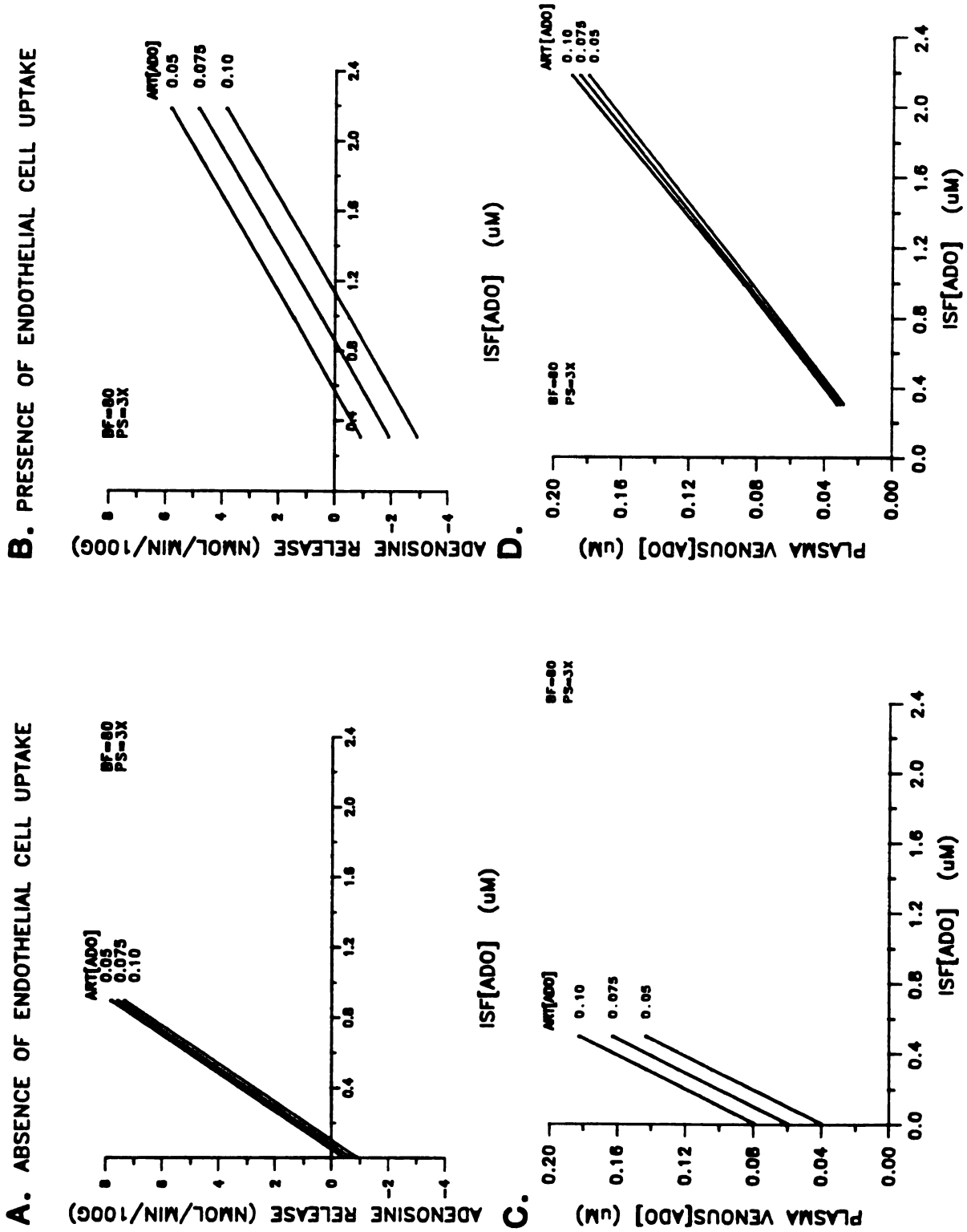
A. ABSENCE OF ENDOTHELIAL CELL UPTAKE
B. PRESENCE OF ENDOTHELIAL CELL UPTAKE
C.
D.
ADENOSINE RELEASE (NMOL/MIN/100G)
ISF[ADO] (uM)
PLASMA VENOUS[ADO] (uM)
ART[ADO]
0.05
0.075
0.10
BF=80
PS=3X

Figure 20

adenosine is included (Figure 20B), changes in arterial adenosine concentration can influence release independent of changes in interstitial concentration. At a given interstitial concentration, adenosine release will be greater at low arterial concentrations (0.05 uM) than at higher arterial concentrations (0.10 uM).

The effect of arterial concentration on venous adenosine concentration is shown in Figures 20C and 20D. In the absence of endothelial cell uptake, venous concentration is influenced by arterial levels of adenosine (Figure 20C). However, when endothelial cell uptake is included (Figure 20D), arterial concentration has little effect on venous concentration. Because of the large capacity of endothelial cells to take up adenosine, arterial adenosine is taken up by capillary endothelium and consequently does not influence venous concentration. For this reason we think that venous concentration is less sensitive than adenosine release to changes in arterial concentration and therefore should be a better index of changes in interstitial concentration.

EFFECT OF BLOOD FLOW ON MOVEMENT OF ADENOSINE ACROSS THE CAPILLARY

In Figure 21, the effect of blood flow on the relationship between interstitial concentration and adenosine release or plasma venous concentration was shown. Capillary PS (3 fold rest) and arterial adenosine concentration (0.075 uM) were held constant while blood flow was altered (20, 80, and 160 ml/min/100g). In the absence of endothelial cell uptake (Figure 21A), an increase in flow has a small effect on adenosine release at a given interstitial

Figure 21. Effect of blood flow on adenosine release and VEN[ADO] in the presence and absence of endothelial cell uptake. The upper panels show the relationship between interstitial adenosine concentration (ISF[ADO]) and adenosine release. The bottom panels show the relationship between ISF[ADO] and venous plasma adenosine concentration (VEN[ADO]). ART[ADO]= arterial plasma adenosine concentration (uM); PS= permeability-surface area (ml/min/100g); hematocrit =45%.

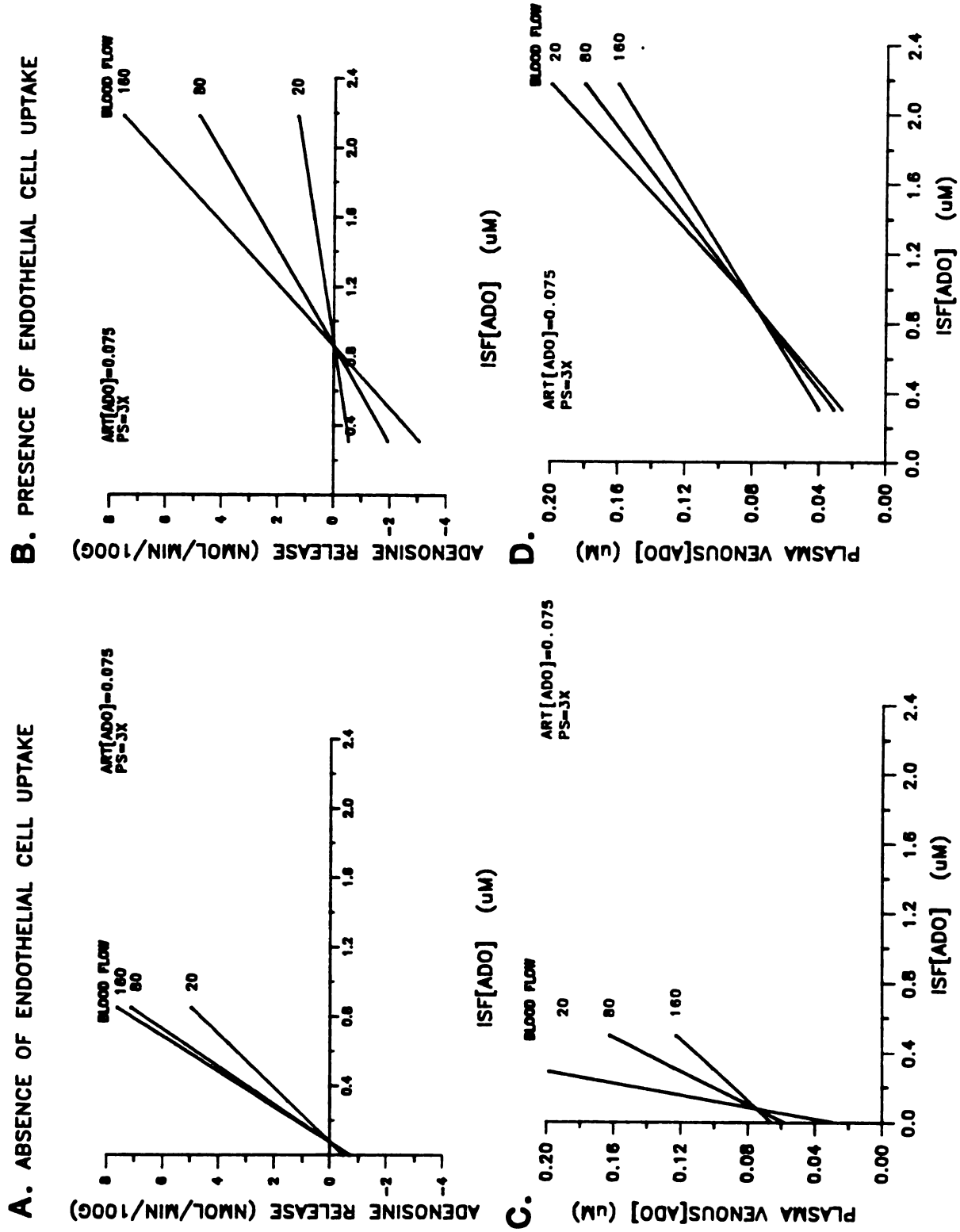
A. ABSENCE OF ENDOTHELIAL CELL UPTAKE
B. PRESENCE OF ENDOTHELIAL CELL UPTAKE
C.
D.
ADENOSINE RELEASE (NMOL/MIN/100G)
PLASMA VENOUS[ADO] (uM)
ISF[ADO] (uM)
ART[ADO]=0.075
PS=3X
BLOOD FLOW
160
80
20

Figure 21

concentration. However, the effect of an increase in flow on adenosine release is much greater in the presence of endothelial cell uptake (Figure 21B). Therefore, our model indicates that changes in blood flow can alter adenosine release independent of changes in interstitial concentration.

Figure 21C shows the effect of blood flow on plasma venous adenosine concentration in the absence of endothelial cell uptake. At a given interstitial concentration, an increase in blood flow reduces venous concentration due to a dilutional effect. However, the decrease in venous concentration is not proportional to the increase in flow, since arterial adenosine minimizes the dilutional effect. At interstitial concentrations near zero, increases in flow cause venous concentration to increase. This occurs because venous concentration is influenced more by arterial levels when interstitial adenosine concentration is near zero. In the presence of endothelial cell uptake (Figure 21D), increases in blood flow have a small effect on plasma venous adenosine concentration. Venous concentration is less affected by an increase in flow because the dilutional effect is minimized. Decreased cellular uptake of adenosine resulting from the reduced transit time through the capillary bed is responsible for the reduced dilator effect. In response to changes in blood flow, venous concentration is a better index of interstitial adenosine concentration than is adenosine release.

EFFECT OF CAPILLARY PERMEABILITY-SURFACE AREA (PS) ON ADENOSINE MOVEMENT ACROSS THE CAPILLARY

In Figure 22, the effect of capillary PS on the relationship between interstitial adenosine concentration and adenosine release or venous concentration is shown. Figure 22A shows the influence of capillary PS on adenosine release in the absence of endothelial cell uptake. Since the influence of cellular uptake is absent, an increase in capillary PS under these conditions indicates an increase in cleft surface area for diffusion. For a given interstitial adenosine concentration, an increase in capillary PS increases adenosine release. The sensitivity of release to changes in capillary PS is reduced in the presence of endothelial cell uptake (Figure 22B). Venous concentration is greater in the absence of endothelial uptake (Figure 22C) than when cellular uptake is present (Figure 22D). An increase in capillary PS further increases venous concentration, because the surface area for diffusion from the interstitium is increased. However, capillary PS has a small effect on plasma venous concentration, since the increase in venous concentration from adenosine diffusing from the interstitium is minimized by an increase in surface area for cellular uptake of adenosine by endothelial cells (Figure 22D). Therefore, the effect of capillary PS on the relationship between interstitial and venous concentration is smaller than the effect on interstitial concentration and adenosine release.

The above relationships (Figures 20, 21, and 22) indicate that endothelial cell uptake significantly influences venous adenosine

Figure 22. Effect of capillary PS on adenosine release and VEN[ADO] in the presence and absence of endothelial cell uptake. The upper panels show the relationship between interstitial adenosine concentration (ISF[ADO]) and adenosine release. The bottom panels show the relationship between ISF[ADO] and venous plasma adenosine concentration (VEN[ADO]). ART[ADO]= arterial plasma adenosine concentration (uM); BF= blood flow (ml/min/100g); PS= permeability-surface area product (ml/min/100g); hematocrit =45%.

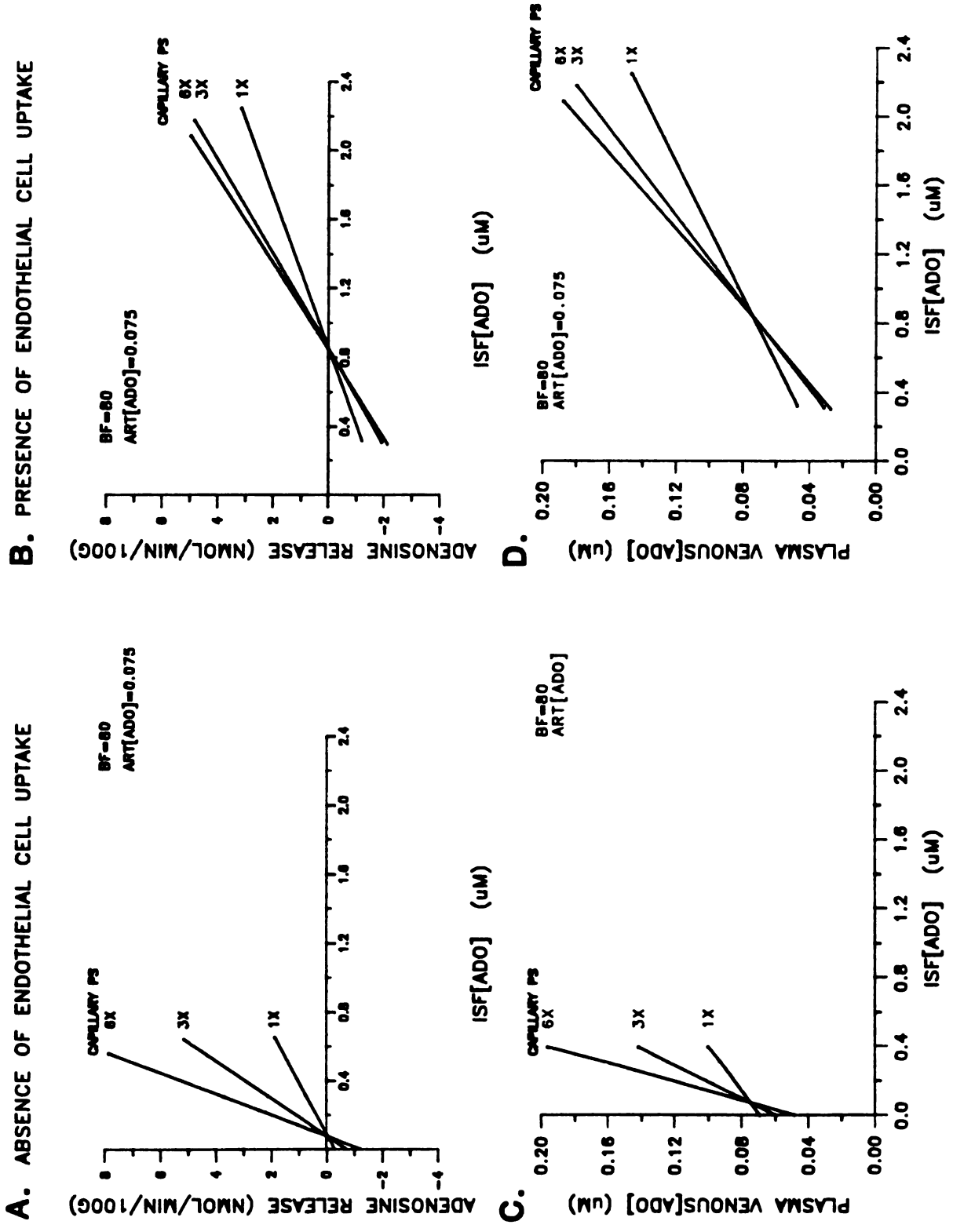
A. ABSENCE OF ENDOTHELIAL CELL UPTAKE
B. PRESENCE OF ENDOTHELIAL CELL UPTAKE
C.
D.
ADENOSINE RELEASE (NMOL/MIN/100G)
PLASMA VENOUS[ADO] (uM)
ISF[ADO] (uM)
CAPILLARY PS
6X
3X
1X
BF=80
ART[ADO]=0.075
ART[ADO]
8
6
4
2
0
-2
-4
0.20
0.16
0.12
0.08
0.04
0.00
0.0
0.4
0.8
1.2
1.6
2.0
2.4

Figure 22

concentration. Venous concentration is determined by the net effect of interstitial adenosine diffusing down its concentration gradient and the capacity for endothelial cells to take up adenosine. Adenosine release is determined by the product of venous-arterial concentration difference and plasma flow. Our model indicates that adenosine release is sensitive to changes in arterial adenosine concentration, blood flow, and capillary PS. Therefore, increases in adenosine release can be independent of increases in interstitial concentration. Venous concentration is insensitive to changes in these parameters. Therefore, we believe that plasma venous adenosine concentration may be a better index of changes in interstitial concentration.

SIGNIFICANCE OF INTERSTITIAL CONCENTRATION AND EXERCISE HYPEREMIA

When skeletal muscle increases its metabolic rate from rest to exercise there is a simultaneous change in flow, capillary PS (Casley-Smith et al., 1975), and arterial adenosine concentration. The model predicts that the magnitude of these changes may be sufficient to increase release measurements, independent of interstitial levels. Figure 12 shows a poor correlation ($r=0.567$, $P<0.005$) between interstitial concentration and adenosine release. The average adenosine release during 3 Hz exercise is 1.24 nmol/min/100g (Table 7). This is an increase of 1.49 nmol/min/100g above the resting value of -0.25 nmol/min/100g. At a given interstitial concentration the model predicts that a 4 fold increase in flow (20 to 80 ml/min/100g) results in an increased release of

0.41 nmol/min/100g. Since the experimental increase in flow was greater than 4 fold (i.e. 5.6 fold) the effect of flow on adenosine release would be expected to be greater than 0.41 nmol/min/100g. In addition, a 3 fold increase in capillary PS during exercise produces an increase in release of 0.26 nmol/min/100g, while a decrease in arterial concentration of 0.025 uM (0.075 to 0.05 uM) increases adenosine release by 0.97 nmol/min/100g.

When examined individually these variables cannot account for the magnitude of change in adenosine release from rest to exercise (1.49 nmol/min/100g). However, altering the three variables simultaneously could have an additive effect on release. Thus, simultaneous changes in arterial adenosine, flow, and capillary PS could explain the increase in release. Although an increase in adenosine release is measured during exercise it may occur without an increase in interstitial concentration.

Our model indicates that venous adenosine concentration may be a better index of interstitial concentration than release. The relationship between interstitial adenosine concentration and venous concentration is minimally affected by changes within a physiologic range in arterial adenosine concentration, flow, and capillary PS. There is a strong correlation (Figure 17, $r=0.997$, $P<0.001$) between interstitial and venous concentration during rest and exercise. Since there were no significant differences between the venous concentrations during rest and exercise, interstitial levels may not increase in response to 3 Hz exercise. These results suggest that sustained hyperemia during a continuous twitch rate of 3 Hz was not maintained by elevations in interstitial adenosine. The model

predicts that small increases in interstitial concentration during exercise would be measurable using venous concentration. Therefore, the 20 fold increase in the interstitial concentration necessary to overcome aminophylline blockade would be easily detected by measuring venous concentration. Since release can increase in the absence of an increase in interstitial concentration, while venous concentration remains constant, we conclude that venous concentration is a better estimate of interstitial concentration. Since the increase in metabolic rate may not be sufficient to produce elevated interstitial levels, we also conclude that adenosine does not mediate sustained hyperemia during 3 Hz exercise. Therefore, in the absence of elevated interstitial adenosine concentration, receptor blockade by aminophylline has no effect on exercise hyperemia.

In this study we were unable to detect a role for adenosine in mediating exercise hyperemia. However, other investigators have concluded that adenosine is a mediator of vasodilation during free flow exercise (Steffen et al., 1983; Proctor and Duling, 1982; Sparks and Fuchs, 1983). A possible explanation for the divergent results between our study and the results of others, is the possibility of an oxygen supply/demand imbalance in the latter studies. Although the preparations from the above studies have the ability to increase flow, flow to all areas may not be uniform, and hypoxic areas that produce adenosine may exist.

In canine gracilis muscle preparations (Steffen et al., 1983) hypoxic areas may be formed at the origin of the muscle where it is ligated to prevent collateral flow. These hypoxic areas may be

responsible for the increase in adenosine content measured during exercise.

In hamster cremaster muscle preparations (Proctor and Duling, 1982), adenosine deaminase reduced vasodilation by 30% in response to 1 Hz stimulation (1.5 min). Because adenosine is deaminated to vasoinactive inosine by adenosine deaminase, it was suggested that the reduction in exercise vasodilatation was due to removal of endogenous adenosine. However, it is unknown whether oxygen consumption is altered in the presence of adenosine deaminase, since blood gas values of the animal and arterial and venous oxygen tensions of the preparation were not determined. The contribution of adenosine in mediating vascular relaxation may be more important in a preparation when interstitital adenosine levels are initially elevated due to hypoxic perfusion.

Sparks and Fuchs (1983), using blood perfused canine skeletal muscle, demonstrated an increase in adenosine release and venous concentration during free flow exercise. Their preparation was identical to that used in the present study, except muscles were stimulated to contract at 6 Hz with no evidence of fatique. However, exercising muscles had the same venous PO_2 values during 6 Hz exercise (26 ± 5 mmHg) as measured in the present study during 3 Hz exercise (26 ± 1 mmHg). At 3 Hz exercise oxygen extraction is maximal, therefore, increased oxygen demand at 6 Hz exercise is matched solely by an increase in oxygen delivery. Hypoxic areas may occur at exercise rates that approach the metabolic capacity of muscles, if delivery of oxygen is inadequate. Therefore, the contribution of

adenosine as a vasoregulator of blood flow may be more important when there is an imbalance between oxygen supply and oxygen demand.

When oxygen demand and oxygen delivery to skeletal muscle are in balance, the stimulus for adenosine formation may be insufficient to elevate interstitial adenosine levels. In the presence of hypoxia, the imbalance between the rate of oxygen delivery and oxidative phosphorylation may lead to accelerated ATP breakdown producing increases in interstitial adenosine (Berne, 1980). An increase in adenosine monophosphate (AMP) through the action of adenylate kinase may stimulate adenosine formation by 5'nucleotidase. This enzyme is inhibited by ATP (Baer and Drummond, 1968; Baer et al., 1966), ADP (Burger and Lowenstein, 1970; Sullivan and Alpers, 1971) and phosphocreatine (PCr)(Rubio et al., 1979). When ATP breakdown is accelerated, magnesium chelated to ATP is released, leading to an increase in free magnesium levels (Sullivan and Alpers, 1971). With increased ATP synthesis, phosphorylation of ADP by PCr causes a reduction in PCr levels (McGilvery and Murray, 1974). Since increased magnesium (Sullivan and Alpers, 1971) and decreased PCr (Rubio et al., 1979) disinhibit 5'nucleotidase, accelerated ATP utilization could lead to increased adenosine formation. Increased ATP breakdown could also provide elevated AMP levels and enhance adenosine formation by 5'nucleotidase. However, in the presence of a balanced oxygen consumption/delivery, ATP and ADP content may not be reduced to levels which stimulate adenosine formation, since little change in the total content of ADP and ATP occurs with increased work. Lack of changes in ADP and ATP would be prevented by the high

creatine kinase activity (Goodman and Lowenstein, 1977; Lowenstein and Goodman, 1978).

During 3 Hz exercise, when oxygen delivery is sufficient to meet the oxygen demand of contracting muscles, adenosine may not accumulate in the interstitium. Consequently, interstitial adenosine may not increase. The present study does not support the view that increases in flow concomitant with increases in oxygen consumption are related to increases in interstitial adenosine concentration. However, if there are tissue areas inadequately perfused, such that tissue PO_2 is compromised, ATP hydrolysis may exceed ATP synthesis resulting in nucleotide breakdown and adenosine formation. Therefore, despite sufficient oxygen supply to the total tissue, particular areas that are underperfused may be capable of accumulating adenosine. Adenosine may not accumulate if the rate of oxidative phosphorylation is sufficient to balance the rate of ATP hydrolysis during contraction, thus preventing nucleotide breakdown. The lack of increase in adenosine formation during exercise may indicate a balance between ATP synthesis (via oxidative phophorylation) and ATP breakdown (via hydrolysis).

SUMMARY AND CONCLUSIONS

In this study the role of adenosine in mediating sustained hyperemia during muscle contraction was examined. Adenosine receptor blockade was effective during rest and 3 Hz exercise. Aminophylline had no effect on flow, oxygen consumption, adenosine release, or venous concentration during rest or 3 Hz exercise. Using a mathematical model to describe capillary transport, the relationships between interstitial levels of adenosine, adenosine release, and venous adenosine concentration were determined for conditions of rest and exercise. The model indicates that despite significant increases in adenosine release the interstitial concentration does not change with increased oxygen consumption. The model also indicates that the relationship between interstitial concentration and adenosine release is much more dependent on flow, capillary permeability-surface area, and arterial adenosine concentration than is the relationship between interstitial and venous concentration. The sensitivity of adenosine release to physiologic changes in these variables could explain increased release in the absence of enhanced interstitial levels. Therefore, when all three variables change simultaneously with exercise, release may be a poor index to predict changes in interstitial concentration. Venous adenosine concentration more closely predicts changes in interstitial concentration. The relationship between interstitial adenosine concentration and venous concentration is relatively insensitive to changes in flow, capillary permeability-surface area, and arterial concentration. Since venous concentration did not increase with an increase in oxygen

consumption, interstitial concentration may not have changed. Based on these results we conclude the following:

1) Aminophylline is effective in reducing the flow response to adenosine infusion during rest and exercise.

2) Sustained hyperemia during 3 Hz exercise is not blocked by aminophylline.

3) The lack of effect of adenosine receptor blockade on sustained hyperemia is due to a lack of increase in interstitial adenosine concentration.

4) Interstitial adenosine concentration may increase only in response to an imbalance between the oxygen supply/demand relationship.

APPENDICES

APPENDIX A

MATHEMATICAL MODEL FOR ADENOSINE TRANSPORT

The method used in the determination of adenosine transport is shown in Figure 23. The capillary was approximated by ten segmented compartments. The concentration in each compartment is homogeneous. The water soluble substance of adenosine diffuses freely between the interstitium and capillary spaces through interendothelial clefts and radially between segmented interstitial compartments. Adenosine can be taken up by endothelial and parenchymal cells via high affinity carrier mediated mechanisms. Adenosine can enter the interstitial fluid space by parenchymal cell production and diffusion from the capillary space through interendothelial clefts. Adenosine can also be convected downstream to the next capillary segment by plasma flow. The net flux of adenosine in and out of each compartment determines its instantaneous mass. The concentration for each compartment is determined as the quotient of the mass and the compartment volume.

Given ten axial segments for both the capillary and interstitial space and one segment for the venous space, the system of 21 differential equations is solved using the fourth order Runge-Kutta algorithm. At t=0, the initial condition for adenosine concentration in all compartments is set at zero.

Figure 23. Model structure of adenosine (ADO) movement between tissue and vascular compartments. ISF= interstitial; ART= arterial; CAP= capillary; VEN= venous; F= plasma flow; Ca and C_v= arterial and venous plasma ado concentration, respectively.

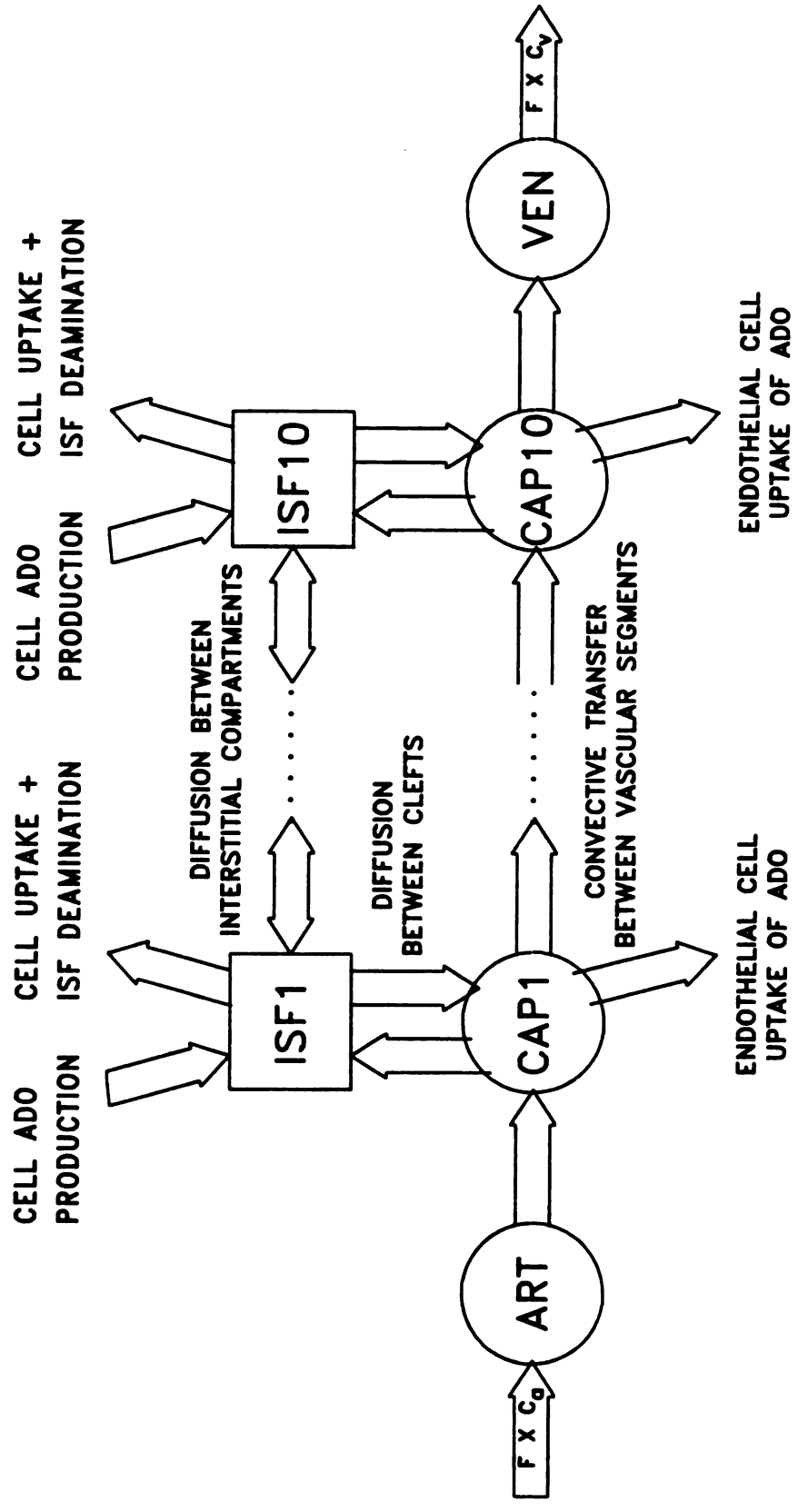

Figure 23. Model structure of adenosine (ADO) movement.

TRANSFER EQUATIONS FOR ADENOSINE TRANSPORT

(1) Conservation of Mass in Capillary:

CHANGE IN MASS IN CAPILLARY SPACE = FLUX OF ADO ENTERING ARTERIAL END OF CAPILLARY + FLUX OF ADO DIFFUSING BETWEEN CAP AND ISF - FLUX OF ADO INTO ENDOTHELIAL CELLS - FLUX OF ADO INTO BLOOD ELEMENTS - DISAPPEARANCE BY PLASMA ADA - FLUX OF ADO CONVECTIVELY TRANSFERRED

For Compartment 1:

$$Vc_1 \frac{dCc_1}{dt} = Fpl_a(Ca) - \frac{SA_{cleft}/10 \times D_{ado}}{L_{cleft}} (Cc_1 - Cisf_1)$$

$$- \frac{[V_{max(ec)}/SA_{ec} \times (SA_{endothelium}/10)] \times Cc_1}{Cc_1 + K_{m(ec)}} - K_{bld} (Cc_1 \times Vc_1) - Fpl_c(Cc_1)$$

Fpl_a = blood flow x (1 - HCT)
Fpl_c = blood flow x (1 - HCT_c)

For Compartments 2 through 10 (i = 2,3,4...10)

$$Vc_i \frac{dCc_i}{dt} = Fpl_c(Cc_{i-1}) - \frac{SA_{cleft}/10 \times D_{ado}}{L_{cleft}} (Cc_i - Cisf_i)$$

$$- \frac{[V_{max(ec)}/SA_{ec} \times (SA_{endothelium}/10)] \times Cc_i}{Cc_i + Km(ec)} - K_{bld} (Cc_i \times Vc_i) - Fpl_c(Cc_i)$$

$Vc_1 = Vc_i = Vc/10$

(2) Conservation of Mass in the Interstitium:

CHANGE IN MASS IN INTERSTITIUM = ADO PRODUCTION BY PARENCHYMAL CELLS − FLUX OF ADO INTO PARENCHYMAL CELLS + FLUX OF ADO DIFFUSING BETWEEN ISF AND CAP + FLUX OF ADO FROM ADJACENT ISF COMPARTMENT − FLUX OF ADO INTO ADJACENT ISF COMPARTMENT

For Compartment 1:

$$Visf_1 \frac{dCisf_1}{dt} = P_{ado} - \frac{V_{max(pc)} \times Cisf_1}{Cisf_1 + K_{m(pc)}} + \frac{SA_{cleft}/10 \times D_{ado}}{L_{cleft}} (Cisf_1 - Cc_1)$$

$$+ \frac{SA_{isf} \times D_{ado}}{L_{isf\ segment}} (Cisf_1 - Cisf_2)$$

For Compartments 2 through 9 (i = 2,3,4...9):

$$Visf_i \frac{dCisf_i}{dt} = P_{ado} - \frac{V_{max(pc)} \times Cisf_i}{Cisf_i + K_{m(pc)}} - \frac{SA_{cleft}/10 \times D_{ado}}{L_{cleft}} (Cisf_i - Cc_i)$$

$$+ \frac{SA_{isf} \times D_{ado}}{L_{isf\ segment}} (Cisf_{(i-1)} - Cisf_i) - \frac{SA_{isf} \times D_{ado}}{L_{isf\ segment}} (Cisf_i - Cisf_{(i+1)})$$

For Compartment 10:

$$Visf_{10} \frac{dCisf_{10}}{dt} = P_{ado} - \frac{Vmax(pc) \times Cisf_{10}}{Cisf_{10} + Km(pc)} - \frac{SA_{cleft}/10 \times D_{ado}}{L_{cleft}} (Cisf_{10} - Cc_{10})$$

$$- \frac{SA_{isf} \times D_{ado}}{L_{isf\ segment}} (Cisf_{10} - Cisf_9)$$

$Visf_1 = Visf_i = Visf_1 = Visf/10$

(3) Conservation of Mass in the Venous Compartment:

CHANGE IN MASS IN VENOUS COMPARTMENT	=	FLUX OF ADO CONVECTIVELY TRANSFERRED FROM CAP COMPARTMENT	-	FLUX OF ADO INTO BLOOD ELEMENTS	-	DISAPPEARANCE BY PLASMA ADA

For Venous Compartment:

$$V_v \frac{dC_v}{dt} = F_{pl}(Cc_{10} - Cv) - K_{bld}\,(C_v \times V_v)$$

DEFINITION OF MATHEMATICAL TERMS

Volumes and Concentrations

V_c	capillary plasma volume
V_{isf}	interstitial fluid volume
V_v	venous plasma volume
C_{isf}	interstitial adenosine (ADO) concentration
C_c	capillary plasma ADO concentration
C_a	arterial plasma ADO concentration
C_v	venous plasma ADO concentration
F_{pl}	plasma flow rate (assumed flow through exchanging vessels only)

Dimensional Parameters

SA_{cleft}	surface area of interendothelial clefts open to capillary space
$SA_{endothelium}$	surface area of capillary endothelium
SA_{ec}	surface area of single endothelial cell
SA_{isf}	surface area for diffusion between interstitial compartments (Visf/ unit capillary length)
$L_{isf\ segment}$	length of single interstitial compartment (unit capillary length/10)
L_{cleft}	average cleft length from capillary to interstitium
D_{ado}	diffusion coefficient for adenosine

Kinetic Parameters

$V_{max(ec)}$	maximal uptake rate of adenosine by single endothelial cell
$V_{max(ada)}$	maximal enzyme activity of adenosine deaminase
$V_{max(pc)}$	maximal uptake rate of adenosine by parenchymal cell (per 100g basis)
$K_{m(ec)}$	K_m of endothelial cells for adenosine uptake
$K_{m(ada)}$	K_m for adenosine deaminase
$K_{m(pc)}$	K_m of parenchymal cells for adenosine uptake
P_{ado}	rate of adenosine production by parenchymal cells
K_{bld}	rate of adenosine disappearance in canine blood (.18/min) (includes uptake rate by cellular elements of blood and deamination by plasma adenosine deaminase)

ASSUMPTIONS OF THE MODEL

1. Each of the assumed ten separate individual interstitial and capillary compartments are considered well mixed.

2. All capillaries are identical.

3. All skeletal muscle blood flow is through exchanging (nutrient) vessels only.

4. Adenosine crosses the capillary wall via simple diffusion through interendothelial clefts.

5. Interendothelial clefts are considered to be uniformally distributed along the length of the capillary.

6. Restricted diffusion for adenosine is considered insignificant.

7. Adenosine uptake and degradation in the interendothelial clefts is insignificant.

8. Cleft length from capillary to interstitium is twice the average thickness of capillary endothelium accounting for cleft tortuosity.

9. Diffusion coefficient for adenosine (M.W.= 267.2) and Ara-H (M.W.= 267.2) are equal to the diffusion coefficient of sucrose (M.W.=342.3) based on similar molecular weights.

10. Diffusion coefficient of sucrose in a ventricular muscle slice is equal to the diffusion coefficient of sucrose in interendothelial clefts in skeletal muscle.

11. Capillary endothelium acts as a metabolic sink for adenosine.

12. Kinetic parameters for adenosine uptake obtained from isolated pig aortic endothelial cell preparations are identical to those for dog skeletal muscle endothelial cells under in vivo conditions.

13. Capillary permeability does not change during exercise.

14. The increase in cleft surface area during exercise is directly proportional to the increase in capillary surface area.

15. Capillary volume is derived from total capillary inner circumference and total capillary length. Thus, capillaries are assumed perfect cylinders.

16. Interstitial fluid space is composed of a homogeneous polysaccharide fiber matrix.

17. The only source of endogenous venous effluent plasma adenosine is from the interstitium via parenchymal cell production. Therefore, there is no direct input from other cell types such as platelets, red and white blood cells, and capillary endothelial cells. Adenosine production is homogeneous throughout the interstitial fluid space.

18. Adenosine transport in the interstitium is via simple diffusion only.

APPENDIX B

LIST OF MODEL PARAMETERS

PARAMETERS	UNITS	REST	EXERCISE
Volumes			
V_a	ml/100g	0.06	0.06
V_c	ml/100g	0.74	2.31
V_{isf}	ml/100g	15.00	15.00
V_v	ml/100g	2.20	2.20
HCT_c	%	0.20(HCT)	0.80(HCT)
Cleft			
L_{cleft}	cm	4.00×10^{-5}	4.00×10^{-5}
$D_{ado}=D_{suc}$	cm^2/min	1.06×10^{-4}	1.06×10^{-4}
SA_{cleft}	$cm^2/100g$	1.32	4.1
Kinetic Parameters			
V_{max}(ec)	nmol/min/cell	250.0	250.0
K_m (ec)	um	3.0	3.0
SA (ec)	cm^2/cell	1.6×10^{-5}	1.6×10^{-5}
SA (endothelium)	$cm^2/100g$	7.0×10^{3}	2.2×10^{4}
V_{max} (ada)	nmol/min/ml	4.8	4.8
K_m (ada)	um	40.0	40.0
V_{max} (pc)	nmol/min/100g	320.0	320.0
K_m (pc)	um	9.9	9.9
K_{bld}	min^{-1}	0.18	0.18

Abbreviations: a, c, isf, v= arterial, capillary, interstitial, venous, respectively; HCT= large vessel hematocrit; D= diffusion coefficient; ado= adenosine; suc= sucrose; PS= permeability surface area product; SA= surface area; ec= endothelial cell; ada= adenosine deaminase; pc= parenchymal cell; L_{cleft}= length of cleft between intravascular and interstitial space; K_{bld}= ado disappearance rate.

REFERENCES FOR MODEL PARAMETERS

PARAMETERS	UNITS	REFERENCES
Volumes		
V_a	ml/100g	Assumption based on vascular volume
V_c	ml/100g	Casley-Smith et al., 1975
V_{isf}	ml/100g	Aukland and Nicolaysen, 1981
V_v	ml/100g	Kamiya et al., 1979
HCT_c	%	Klitzman and Duling, 1979
Cleft		
L_{cleft}	cm	Berne and Rubio, 1979
$D_{ado}=D_{suc}$	cm^2/min	Suenson et al., 1974
SA_{cleft}	$cm^2/100g$	Calculation on page 54. (PS_{ara-H}= 3.5 ml/min/100g= $-F_{pl}$ x $\ln(1-E_{ara-H})$; where F_{pl}= 7.8±1.6(SE) ml/min/100g and E_{ara-H}= extraction= 36±7(SE)(n=4); M.Gorman, personal commun.)
Kinetic Parameters		
V_{max}(ec)	nmol/min/cell	Pearson et al., 1978
K_m (ec)	um	Pearson et al., 1978
SA (ec)	cm^2/cell	Gimbrone, 1976
SA (endothelium)	$cm^2/100g$	Landis and Pappenheimer, 1963
V_{max}(ada)	nmol/min/ml	van Belle, 1969
K_m (ada)	um	Fox and Kelly, 1978
V_{max}(pc)	nmol/min/100g	Sixma et al., 1976
K_m (pc)	um	Sixma et al., 1976
K_{bld}	min^{-1}	Manfredi and Sparks, 1982

Abbreviations: HCT= large vessel hematocrit; D= diffusion coefficient; ado= adenosine; suc= sucrose; PS= permeability surface area product; SA= surface area; ec= endothelial cell; ada= adenosine deaminase; pc= parenchymal cell; Lcleft= length of cleft between intravascular and interstitial space; K_{bld}= ado disappearance rate.

APPENDIX C

CALCULATION FOR PERCENT RECOVERY OF ADENOSINE ASSAY*

$$\text{PERCENT RECOVERY (\%)} = \frac{\text{Measured Plasma [ADO]}}{\text{Expected Plasma [ADO]}} \times 100$$

Measured Plasma [ADO] = [ADO]spiked - [ADO]endogenous

[ADO]spiked (pmol/ul) = plasma [ADO] of sample tube containing collecting solution plus known amount of adenosine standard

[ADO]endogenous (pmol/ul) = plasma [ADO] of sample tube containing collecting solution without adenosine standard

$$\text{Expected Plasma [ADO] (pmol/ul)} = \frac{\text{ADO amount added to sample tube}}{\text{Total plasma sample volume}}$$

[ADO]= adenosine concentration

Recovery variation between animals (n=7) (MEAN ± SD)
85.3 ± 28.2%
(Samples obtained during rest (control).

Recovery variation within a single animal (n=3) (MEAN ± SD)
94.0 ± 3.2%
(Samples obtained during rest (before and after exercise) and exercise (postaminophylline). Samples were not obtained during exercise.)

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* Nucleotide (ATP, ADP, and AMP) degradation to adenosine via plasma 5'nucleotidase could contribute to the variability of plasma adenosine concentration. Previous results in our laboratory have indicated that plasma adenosine concentration is not different in the presence or absence of alpha, beta,-methylene adenosine 5'-diphosphate (AOPCP) (an inhibitor of 5'nucleotidase). In this study, recovery and experimental samples were obtained in the absence of AOPCP and it was assumed that nucleotide degradation contributes insignificantly to plasma adenosine concentration.

BIBLIOGRAPHY

BIBLIOGRAPHY

Afonso, S., and G.S. O'Brien. Enhancement of coronary vasodilator action of adenosine triphosphate by dipyridamole. Circulation Res. 20:403, 1967.

Afonso, S. Inhibition of coronary vasodilating action of dipyridamole and adenosine by aminophylline in the dog. Circulation Res. 26:743-752, 1970.

Agarwall, R.P., T. Spector, and R.E. Parks, Jr. Tight binding inhibitors. IV. Inhibition of adensoine deaminases by various inhibitors. Biochem. Pharm. 26:359-367, 1976.

Altura, B.M. and B.T. Altura. Magnesium and vascular tone and reactivity. Blood Vessels. 15:5-16, 1978.

Anrep, G.V., and E. von Saalfeld. The blood flow through skeletal muscle in relation to its contraction. J. Physiol. 85:375-399, 1935.

Aukland, K., and G. Nicolaysen. Interstitial fluid volume: local regulatory mechanisms. Physiol. Rev. 61:556-643, 1981.

Baer, H.P, G.I. Drummond, and E.L. Duncan. Formation and deamination of adenosine by cardiac muscle enzymes. Mol. Pharmacol. 2:67-76, 1966.

Baer, H.P., and G.I. Drummond. Catabolism of adenosine nucleotides by the isolated perfused rat heart. Proc. Soc. Exp. Biol. Med. 127:33-36, 1966.

Barcroft, H. Blood flow and metabolism in skeletal muscle. In: Circulation in Skeletal Muscle. Ed. by O. Hudlicka. Pergamon, Oxford. 1968.

Belardinelli, L., F. Belloni, R. Rubio, and R. Berne. Atrioventricular conduction disturbances during hypoxia. Possible role of adenosine in rabbit and guinea pig heart. Circulation Res. 47:684-691, 1980.

Belardinelli, L., E. Mattos, and R. Berne. Evidence for adenosine mediation of atrioventricular block in the ischemic canine myocardium. J. Clin. Invest. 68:195-205, 1981.

Belardinelli, L., R. Fenton, A. West, J. Linden, J. Althaus, and R. Berne. Extracellular action of adenosine and the antagonism by aminophylline on the atrioventricular conduction of isolated perfused guinea pig and rat hearts. Circulation Res. 51:569-579, 1982.

Belloni, F.L., R.D. Phair, and H.V. Sparks. The role of adenosine in prolonged vasodilation following flow-restricted exercise of canine skeletal muscle. Circulation Res. 44:759-766, 1979.

Berne, R.M., J.R. Blackmon, and T.H. Gardner. Hypoxemia and coronary blood flow. J. Clin. Invest. 36:1101-1106, 1957.

Berne, R.M. Cardiac nucleotides in hypoxia: possible role in regulation of coronary blood flow. Am. J. Physiol. 204:317-322, 1963.

Berne, R.M., R. Rubio, J.G. Dobson, and R.R. Curnish. Adenosine and adenosine nucleotides as possible mediators of cardiac and skeletal muscle blood flow regulation. Circulation Res. 28:(Suppl. I)115-119, 1971.

Berne, R.M., and R. Rubio. Coronary Circulation. Chap. 25. In: Handbook of Physiology. Section 2. The Cardiovascular System. Ed. by R.M. Berne, N. Sperelakis, and S.R. Geiger. Bethesda, Md: Am. Physiol. Soc., 1979.

Berne, R.M. The role of adenosine in the regulation of coronary blood flow. Circulation Res. 47:807-813, 1980.

Bockman, E.L., R.M. Berne, and R. Rubio. Release of adenosine and lack of release of ATP from contracting skeletal muscle. Pflügers Arch. 355:229-241, 1975.

Bockman, E.L., R.M. Berne, and R. Rubio. Adenosine and active hyperemia in dog skeletal muscle. Am. J. Physiol. 230:1531-1537, 1976.

Bockman, E.L., J.E. McKenzie, and J.L. Ferguson. Resting blood flow and oxygen consumption in soleus and gracilis muscles in cats. Am. J. Physiol. 239(8):H516-H524, 1980.

Bockman, E.L. and J.E. McKenzie. Tissue adenosine content in active soleus and gracilis muscles of cats. Am. J. Physiol. 244:H552-H559, 1983.

Bockman, E.L. Possible role of K^+ as a mediator of active hyperemia in gracilis (white) muscles of cats. Proc. Int. Union Physiol. Sciences. Sidney, Australia. XXIXth Congress. XV:572.09, 1983.

Bonaccorsi, A., K. Hermsmeyer, O. Aprighano, C.B. Smith, and D.F. Bohr. Mechanism of potassium relaxation in arterial muscle. Blood Vessels. 14:261-276, 1977.

Borgers, M., J. Schaper, and W. Schaper. Adenosine-producing sites in the mammalian heart: a cytochemical study. J. Mol. Cellular Cardiol. 3:287-296, 1971.

Bruns, R.R., and G.E. Palade. Studies on blood capillaries. I. General organization of blood capillaries in muscle. J. Cell Biol. 37:244-276, 1968.

Bünger, R., F. Haddy, and E. Gerlach. Coronary responses to dilating substances and competitive inhibition by theophylline in the isolated perfused guinea pig heart. Pflügers Arch. 358:213-224, 1975.

Burger, R., and J.M. Lowenstein. Preparation and properties of 5'-nucleotidase from smooth muscle of small intestine. J. Biol. Chem. 245:6274-6280, 1970.

Casley-Smith, J.R., H.S. Green, J.L. Harris, and P.J. Wadey. The quantitative morphology of skeletal muscle capillaries in relation to permeability. Microvascular Res. 10:43-64, 1975.

Chang, A.E., and R. Detar. Oxygen and vascular smooth muscle revisited. Am. J. Physiol. 238:H716-H728, 1980.

Chaudry, I.H., and A.E. Baue. Further evidence for ATP uptake by rat tissues. Biochim. Biophys. Acta 628:336-342, 1980.

Chaudry, I.H., and M.G. Clemens. Dissociation between ATP and adenosine uptake by hepatocytes. Fed. Proc. 41:1156, 1982.

Chaudry, I. Does ATP cross the cell plasma membrane? Yale J. Biol. Med. 55:1-10, 1982.

Chen, W.J., B.H. Selleck, and J.B. Scott. Evidence for participation of adenine nucleotides in exercise hyperemia. Fed. Proc. 31:379, 1972.

Conway, J., and R. Hutton. Effects of prostaglandins E_1, E_2, A_1, and A_2 on the resistance and capacitance vessels in the hindlimb of the dog. Cardiovasc. Res. 9:229-235, 1979.

Curnish, R., R. Berne, and R. Rubio. Effect of aminophylline on myocardial reactive hyperemia. Proc. Soc. Exp. Biol. Med. 141:593-598, 1972.

Dawes, G.S. Vasodilator action of potassium. J. Physiol. (London) 99:224, 1941.

DeMey, J.G., and P.M. Vanhoutte. Heterogenous behavior of the canine arterial and venous wall. Importance of the endothelium. Circulation Res. 51:439-447, 1982.

Dierterle, Y., C. Doy, A. Ehrensberger, H. Stalder, and A. Junod. Metabolism and uptake of adenosine triphosphate and adenosine by porcine aortic and pulmonary endothelial cells and fibroblasts in culture. Circulation Res. 42:869-876, 1978.

Dobson, J.G., R. Rubio, and R.M. Berne. Role of adenine nucleotides, adenosine, and inorganic phosphate in the regulation of skeletal muscle blood flow. Circulation Res. 29:375-384, 1971.

Drury, A.N., and A. Szent-Gyorgyi. The physiological activity of adenine compounds. J. Physiol. 68:213-237, 1929.

Duling, B.R. Oxygen sensitivity of vascular smooth muscle. II. In vivo studies. Am. J. Physiol. 227:42-49, 1974.

Duling, B.R. and R.N. Pittman. Oxygen tension: dependent or independent variable in local control of blood flow? Federation Proc. 34:2020-2024, 1975.

Duran, W.N. Effects of muscle contraction and of adenosine on capillary transport and microvascular flow in dog skeletal muscle. Circulation Res. 41:642-647, 1977.

Dusting, G.J., and J.R. Vane. Some cardiovascular properties of prostacyclin (PGI_2) which are not shared by PGE_2. Circulation Res. 46:(Suppl. I)I183-I187, 1980.

Feigl, E.O., and L.G. D'Alecy. Normal arterial blood pH, oxygen, and carbon dioxide tensions in unanesthetized dogs. J. Appl. Physiol. 32:152-153, 1972.

Folkow, B. and H.D. Halicka. A comparison between "red" and "white" muscle with respect to blood supply, capillary surface area and oxygen uptake during rest and exercise. Microvasc. Res. 1:1-14, 1968.

Forrester, T. and A.R. Lind. Identification of adenosine triphosphate in human plasma and the concentration in venous effluent before, during, and after sustained contractions. J. Physiol.(London) 204:347-364, 1969.

Fox, I.H., and W.N. Kelley. The role of adenosine and 2'-deoxyadenosine in mammalian cells. Annu. Rev. Biochem. 47:655-686, 1978.

Fritzon, P. Regulation of nucleotidase activities in animal tissues. Adv. in Enzyme Regulation. 16:43-61, 1978.

Gaskell, W.H. On the changes of the blood stream in muscle through stimulation of their nerves. J. Anat. 11:360, 1877.

Gimbrone, M.A. Culture of vascular endothelium. Prog. Hemostasis Thromb. 3:1-28, 1976.

Glynn, I.M. Membrane adenosine triphosphate and cation transport. Br. Med. Bull. 24:165-169, 1968.

Gorczynski, R.J., and B.R. Duling. The role of oxygen in contraction induced arteriolar vasodialtion in hamster striated muscle. Fed. Proc. 35:448, 1976.

Goldstein, A. Biostatistics. An introductory test. Macmillan Company, New York. 1964.

Goodman, M.N. and J.M. Lowenstein. The purine nucleotide cycle: studies of ammonia production by skeletal muscle in situ and in perfused preparations. J. Biol. Chem. 252:5054-5060, 1978.

Greenberg, R.A., and H.V. Sparks. Prostaglandins and consecutive vascular segments of the canine hindlimb. Am. J. Physiol. 216:567-571, 1969.

Haddy, F.J. Local effects of sodium, calcium, and magnesium upon small and large blood vessesl of the dog forelimb. Circulation Res. 8:57-70, 1960

Haddy, F.J. and J.B. Scott. Metabolically linked vasoactive chemicals in local regulation of blood flow. Physiol. Rev. 48:688-707, 1968.

Haddy, F.J. and J.B. Scott. Metabolic factors in peripheral circulatory regulation. Fed. Proc. 34:2006-2011, 1975.

Hershfield, M.S. and N.M. Kredich. S-adenosylhomocysteine hydrolase is an adenosine binding protein: a target for adenosine toxicity. Science 202:757-760, 1978.

Hester, R.L., A.C. Guyton, and B.J. Barber. Reactive and exercise hyperemia during high levels of adenosine infusion. Am. J. Physiol. 243:H181-H186, 1982.

Hilton, S.M., M.G. Jeffries, and G. Vrbova. Functional specialization of the vascular bed of soleus. J. Physiol. (London). 206:543-562, 1970.

Hintze, T.H., and S.F. Vatner. Reactive dilation of large coronary arteries in conscious dogs. Circulation Res. 54:50-57, 1984.

Honig, C.R. and J.L. Frierson. Role of adenosine in exercise vasodilation of dog gracilis muscle. Am. J. Physiol. 238:H703-H715, 1980.

Hudlicka, O. Resting and postcontraction blood flow in slow and fast muscles of the chick during development. Microvasc. Res. 1:390-402, 1969.

Hudlicka, O., D. Pette, and H. Staudte. The relation between blood flow and enzymatic activities in slow and fast muscles during development. Pflügers Arch. 343:341-356, 1973.

Imai, S., A.L. Riley, and R.M. Berne. Effect of ischemia on adenine nucleotides in cardiac and skeletal muscles. Circulation Res. 15:443-450, 1964.

Itoh, R., C. Usami, T. Nishino, and K. Tsushima. Kinetic properties of cytosol 5'-nucleotidase from chicken liver. Biochim. Biophys. Acta 526:154-162, 1978.

Jackson, W.F., and B.R. Duling. The oxygen sensitivity of hamster cheek pouch arterioles. In vitro and in situ studies. Circulation Res. 53:515-525, 1983.

Jacques, J.A. Compartmental analysis in biology and medicine. Kinetics of distribution of tracer-labeled materials. Elsevier Publishing Co. Amsterdam/London/NewYork. 1972.

Jöbsis, F.F. What is a molecular oxygen sensor? What is a transduction process? In: Tissue hypoxia and ischemia. Ed. by M. Reivich, R. Coburn, S. Lahiri, and B. Chance. New York, Plenum. 1977.

Kadowitz, P.J. Effect of prostaglandins E_1, E_2, and A_2 on vascular resistance and response to noradrenaline, nerve stimulation and angiotensin in the dog hindlimb. Br. J. Pharmacol. 46:395-400, 1972.

Kamiya, A., B. Rippe, and B. Folkow. Volume of the capillary exchange vessels measured with a gravimetric method of rat hindquarters. Microvasc. Res. 17:142-159, 1979.

Karlsson, J., S. Rosell, and B. Saltin. Carbohydrate and fat metabolism in contracting canine skeletal muscle. Pflügers Arch. 331:57-69, 1972.

Kille, J.M., and R.E. Klabunde. Adenosine as a mediator of postcontraction hyperemia in dog gracilis muscle. Am. J. Physiol. 246:H274-H282, 1984.

Kjellmer, I. The effect of exercise on the vascular bed of skeletal muscle. Acta Physiol. Scand. 62:18-30, 1964.

Kjellmer, I. The potassium ion as a vasodilator during muscular exercise. Acta Physiol. Scand. 63:460, 1965.

Kjellmer, I., and H. Odelram. The effect of physiological vasodilators on the vascular bed of skeletal muscle. Acta Physiol. Scand. 63:94-102, 1965.

Klabunde, R.E., and D.G. Althouse. Adenosine metabolism in dog whole blood: effect of dipyridamole. Life Sciences. 28:2631-2641, 1981.

Klabunde, R.E. Effects of dipyridamole on postischemic vasodilation and extracellular adenosine. Am. J. Physiol. 244:H273-H280, 1983.

Klitzman, B., and B. Duling. Microvascular hematocrit and red cell flow in resting and contracting striated muscle. Am. J. Physiol. 237(4): H481-H490, 1979.

Kramer, K., F. Obal, and W. Quensel. Untersuchungen über den Muskelstoffwechseldes warmeblüters. III. Mittilung. Die saverstoffaufnahmedes Muskels während ryhthmischer Tatigkeit. Pflueger Arch. Ges. Physiol. 241:717-729, 1939.

Kuroda, Y. Physiological roles of adenosine derivates which are released during neuro-transmission in mammalian brain. J. Physiol (Paris). 74:463-470, 1978.

Landis, E.M., and J.R. Pappenheimer. Exchange of substances through the capillary walls. Chap 29. In: Handbook of Physiology. Circulation. Section 2, Vol. II. Washington, D.C. Am. Physiol. Soc. 1963.

Laurent, T.C. The structure and function of the intercellular polysaccharides in connective tissue. In:Capillary Permeability. C. Crone and N.A. Lassen (eds.). New York, Academic, pp.261-277, 1970.

Linton, M., and P. Gallo. The practical statistician: simplified handbook of statistics. Brooks/Cole Publishing Company. Monterrey, Ca. 1975.

Lowenstein, J.M., and M.N. Goodman. The purine nucleotide cycle in skeletal muscle. Fed. Proc. 37:2308-2312, 1978.

Manfredi, J.P., and H.V. Sparks. Adenosine's role in coronary vasodilation induced by atrial pacing and norepinephrine. Am. J. Physiol. 243(12): H536-H545, 1982.

Maxwell, L.C., J.R. Barclay, D.E. Mohrman, and J.A. Faulkner. Physiological characteristics of skeletal muscles of dogs and cats. Am. J. Physiol. 233(1):C14-C18, 1977.

McGilvery, R.W., and T.W. Murray. Calculated equilibria of phosphocreatine and adenosine phosphates during utilization of high energy phosphate by muscle. J. Biol. Chem. 249:5845-5850, 1974.

McKenzie, J.E., R.P. Steffen, R.B. Price, and F.J. Haddy. Effects of theophylline on adenosine and coronary vascular resistance during increased cardiac work. Physiologist. 24:123, 1981.

Mellander, S., and J. Lundvall. Role of tissue hyperosmolality in exercise hyperemia. Circulation Res. 28:I130-I145, 1971.

Merrill, G.F., F.J. Haddy, and J.M. Dabney. Adenosine, theophylline and perfusate pH in the isolated, perfused guinea pig heart. Circulation Res. 42:225-229, 1978.

Mohrman, D.E., and H.V. Sparks. Resistance and venous oxygen dynamics during sinusoidal exercise of dog skeletal muscle. Circulation Res. 33:337-345, 1973.

Mohrman, D.E. Lack of influence of potassium or osmolality on steady-state exercise hyperemia. Am. J. Physiol. 242:H949-H954, 1982.

Mohrman, D.E., and L.J. Heller. Effect of systemic aminophylline on the microvascular response to topically applied adenosine. Fed. Proc. 41(5):1681, 1982.

Morganroth, M.L., D.E. Mohrman, and H.V. Sparks. Prolonged vasodilation following fatiguing exercise of dog skeletal muscle. Am. J. Physiol. 229(1):38-43, 1975.

Mustafa, S. Effects of coronary vasodilator drugs on the uptake and release of adenosine in cardiac cells. Biochem. Pharm. 28:2617-2624, 1979.

Nakatsu, K. and G.I. Drummond. Adenylate metabolism and adenosine formation in the heart. Am. J. Physiol. 223:1119-1127, 1972.

Olsson, R.A., J.A. Snow, M.K. Gentry, and G.P. Frick. Adenosine uptake by canine heart. Circulation Res. 31:767-778, 1972.

Pearson, J.D., J.S. Carleton, A. Hutchings, J.C. Gordon. Uptake and metabolism of adensoine by pig aortic endothelial and smooth muscle cells in culture. Biochem. J. 170:265-271, 1978.

Pearson, J.D., J.S. Carelton, and J.L. Gordon. Metabolism of adenine nucleotides by ectoenzymes of vascular endothelial and smooth muscle cells in culture. Biochem. J. 190:421-429, 1980.

Pearson, J.D., P.G. Hellewell, and J.L. Gordon. Adenosine uptake and adenine nucleotide metabolism by vascular endothelium. In: Regulatory Function of Adenosine. Ed. by R.M. Berne, T.W. Rall, and R. Rubio. Martinus Nijhoff Publishers, The Hague/ Boston/London. 1983.

Phair, R.D. and H.V. Sparks. Adenosine content of skeletal muscle during active hyeremia and ischemic contraction. Am. J. Physiol. 237(1):H1-H9, 1979.

Pittman, R.N. and Duling B.R. Oxygen senstivity of vascular smooth muscle. I. In vitro studies. Microvascular Res. 6:202-211, 1973.

Proctor, K.G. and B.R. Duling. Adenosine and free flow functional hyperemia in striated muscle. Am. J. Physiol. 242:H688-H697, 1982.

Prothero, J., and A.C. Burton. The physics of blood flow in capillaries. I. The nature of the motion. Biophys. J. 1:565, 1961.

Renkin, E.M., O. Hudlicka, and R.M. Sheehan. Influence of metabolic vasodilatation on blood-tissue diffusion in skeletal muscle. Am. J. Physiol. 211(1):87-98, 1966.

Roos, H., and K. Pfleger. Kinetics of adenosine uptake by erythrocytes and influence of dipyridamole. Molec. Pharm. 8(4):417, 1972.

Rubio, R., R.M. Berne, and J.G. Dobson. Sites of adenosine production in cardiac and skeletal muscle. Am. J. Physiol. 225:938-953, 1973.

Rubio, R., L. Belardinelli, C. Thompson, and R. Berne. Cardiac adenosine: electrophysiological effects, possible significance in cell function and mechanisms controlling its release. In: Physiological and Regulatory Functions of Adenosine and Adenine Nucleotides. Ed. by H.P. Baer and G.I. Drummond. Raven Press, New York. 1979.

Samuelsson, B., M. Goldyne, E. Granström, M. Hamberg, S. Hammarström, and C. Malmsten. Prostaglandins and Thromboxanes. Ann. Rev. Biochem. 47:997-1029, 1978.

Schrader, J., W. Schütz, and H. Bardenheuer. Role of S-adenosylhomocysteine hydrolase in adenosine metabolism in mammalian heart. Biochem. J. 196:65-70, 1981.

Schütz, W., J. Schrader, and E. Gerlach. Different sites of adenosine formation in the heart. Am. J. Physiol. 240:H963-H970, 1981.

Scott J.B., R.M. Daugherty, J.M. Dabney, and F.J. Haddy. Role of chemical factors in regulation of flow through kidney, hindlimb, and heart. Am. J. Physiol. 208: 813-824, 1965.

Scott, J.B., M. Rudko, D. Radawski, and F.J. Haddy. Role of osmolarity, K^+, H^+, Mg^{++}, and O_2 in local blood flow regulation. Am. J. Physiol. 218:338-345, 1970.

Scott, J.B., W.T. Chen, B.T. Swindal, J.M. Dabney, and F.J. Haddy. Evidence from bioassay studies indicating a role for adenosine in cardiac ischemic and hypoxic dilation in the dog. Circ. Res. 45:451-459, 1979.

Sixma, J.J., J.P. Lips, A.M. Trieschnigg, and H. Holmsen. Transport and metabolism of adenosine in human blood platelets. Biochim. Biophys. Acta. 443:33-48, 1976.

Skinner, N.S., and J.C. Costin. Interactions of vasoactive substances in exercise hyperemia: O_2, K^+, and osmolality. Am. J. Physiol. 219:1386-1392, 1970.

Sokal, R.R., and F.J. Rohlf. Biometry. The principles and practice of statistics in biological research. (2nd ed.) W.H. Freeman and Company. San Francisco, Ca., 1981.

Sparks, H.V. Skin and Muscle. In: Peripheral Circulation. Ed. by P. Johnson. John Wiley and Sons, NewYork/Chichester/Brisbane/ Toronto. pp.193-230, 1978.

Sparks, H.V. and F.L. Belloni. The peripheral circulation: local regulation. Ann. Rev. Physiol. 40:59-81, 1978.

Sparks, H.V. Effect of local metabolic factors on vascular smooth muscle. Chap. 17 In: Handbook of Physiology; The Cardiovascular System Vol. II. Ed. by D.F. Bohr, A.P. Somlyo, and H.V. Sparks. Am. Physiol. Soc., Washington, D.C. 1980.

Sparks, H.V. and B.D. Fuchs. Adenosine as a sustained mediator of exercise hyperemia. In: Future Issues in Exercise Biology. Ed. by K.T. Borer, D.W. Edington and T.P. White. Human Kinetics Press, Inc., Champaign, Ill. 1983.

Steffen, R.P., J.E. McKenzie, and F.J. Haddy. The possible role of acetate in exercise hyperemia in dog skeletal muscle. Pflügers Arch. 392:315-321, 1982.

Steffen, R.P., J.E. McKenzie, E.L. Bockman, and F.J. Haddy. Changes in dog gracilis muscle adenosine during exercise and acetate infusion. Am. J. Physiol. 244:H387-H395, 1983.

Stowe, D.F., T.L. Owen, D.K. Anderson, F.J. Haddy, and J.B.Scott. Interaction of O2 and CO2 in sustained exercise hyperemia of canine skeletal muscle. Am. J. Physiol. 229:28-33, 1975.

Suenson, M., D.R. Richmond, and J.B. Bassingthwaighte. Diffusion of sucrose, sodium, and water in ventricular myocardium. Am. J. Physiol. 227:1116-1123, 1974.

Sullivan, J.M., and J.B. Alpers. In vitro regulation of rat heart 5'nucleotidase by adenine nucleotides and magnesium. J. Biol. Chem. 246:3057-3063, 1971.

Tabaie H.M., J.B. Scott, and F.J. Haddy. Reduction of exercise dilation by theophylline. Proc. Soc. Exp. Biol. Med. 154:93-97, 1977.

Thompson, L.P., and D.E. Mohrman. Blood flow and oxygen consumption in skeletal muscle during sympathetic stimulation. Am. J. Physiol. 245:H66-H71, 1983.

Thompson, L.P., F. Belloni, B. Fuchs, D. Steindler, and H. Sparks. Endothelial cells sequester infused adenosine in canine skeletal muscle. Fed. Proc. 42:1097, 1983.

Ueland, P.M. Interaction of adenosine with adenosine-binding protein S-adenosylhomocysteine hydrolase. In: Regulatory Function of Adenosine. Ed. by R.M. Berne, T.W. Rall, and R. Rubio. Martinus Nijhoff Publishers, The Hague/Boston/London. 1983.

van Belle, H. Uptake and deamination of adenosine by blood; species differences, effect of pH, ions, temperature and metabolic inhibitors. Biochim. Biophys. Acta. 192:124-132, 1971.

Weissel, M., G. Raberger, and O. Kraupp. The effects of intra-arterial adenosine infusion on substrate levels and blood flow in skeletal muscle of the dog. Naunyn-Schmiedeberg's Arch. Pharmacol. 277:239-252, 1973.

Winder, W.W., R.L. Terjung, K.M. Baldwin and J.O. Holloszy. Effect of exercise on AMP deaminase and adenylosuccinase in rat skeletal muscle. Am. J. Physiol. 227(6):1411-1414, 1974.

Woo, Y., J. Manery, and E.E. Dryden. Theophylline inhibition of nucleoside transport and its relation to cyclic AMP in skeletal muscle. Can J. Physiol. Pharmacol. 52:1063-1073, 1974.

Young, E.W. and H.V. Sparks. Prostaglandin E release from dog skeletal muscle during restricted flow exercise. Am. J. Physiol. 236(4):H596-H599, 1979.

Young, E.W. and H.V. Sparks. Prostaglandins and exercise hyperemia of dog skeletal muscle. Am. J. Physiol. 238:H190-H195, 1980.