THE REGULATION OF SKELETAL MUSCLE ACTIVE HYPEREMIA: THE DIFFERENTIAL ROLE OF ADENOSINE IN MUSCLES OF VARIED FIBER TYPES

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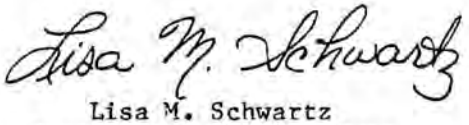

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ABSTRACT

Title of Dissertation: The Regulation of Skeletal Muscle Active Hyperemia: the differential role of adenosine in muscles of varied fiber types

Lisa M. Schwartz, Doctor of Philosophy, 1986

Dissertation Directed by: Jack E. McKenzie, Associate Professor;
Emma L. Bockman, Associate Professor;
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Adenosine is one of several metabolites which have been proposed as metabolic regulators of the increased blood flow to exercising skeletal muscle. It has been shown that adenosine production with exercise varies in muscles having different oxidative capacities. Initial experiments suggested that adenosine may be produced under physiological conditions in oxidative muscles but not in glycolytic muscles. The purpose of these studies was to more clearly define the conditions under which a cause-and-effect relationship might exist between adenosine and skeletal muscle active hyperemia. In addition, these studies were designed to determine whether or not the intrinsic metabolic profile of the muscle involved, as indicated by fiber type, influences the extent to which adenosine modulates blood flow. Cat soleus (slow-twitch, oxidative) and cat gracilis (fast-twitch, glycolytic) muscles were stimulated to contract isometrically in the presence of adenosine deaminase (ADA), an enzyme which specifically degrades adenosine, or ADA which had been deactivated by boiling. Stimulation parameters were chosen to provide similar high and low blood flow responses in the two muscle types. ADA did not affect resting blood flow or vascular resistance. In the soleus muscle, ADA attenuated both the increase in blood flow and oxygen consumption, and the decrease in vascular resistance at the high level of muscle stimulation. In addition, muscle performance

decreased to 60% of its initial level in the presence of ADA, although the same initial performance level was maintained over the stimulation period during boiled ADA infusion. We infer that oxygen supply to the soleus did not increase sufficiently to meet its oxygen demand, resulting in a decreased mechanical function. However, ADA had no effect on active hyperemia in either muscle at the low stimulation level. Additionally, ADA did not attenuate active hyperemia in the gracilis when stimulated at a level which normally produced muscle fatigue. Therefore, these data support a role for adenosine in mediating vasodilation in skeletal muscle composed of high-oxidative fibers at high levels of muscle performance, but do not support a role for adenosine in skeletal muscle having low-oxidative fibers, even at levels of exercise which produce fatigue.

THE REGULATION OF SKELETAL MUSCLE ACTIVE HYPEREMIA:

the differential role of adenosine in muscles of varied fiber types

by

Lisa M. Schwartz

Dissertation submitted to the Faculty of the Department of Physiology
Graduate Program of the Uniformed Services University of the
Health Sciences in partial fulfillment of the
requirements for the degree of
Doctor of Philosophy 1986

This work is dedicated to the memory of

Dr. Emma L. Bockman

my teacher and friend

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BACKGROUND

The theory of the metabolic control of blood flow maintains that blood flow to a tissue is regulated so that the oxygen supply can meet its oxygen demand. An increased need for oxygen in exercising skeletal muscle can be met by an increase in oxygen extraction with little change in blood flow until extraction becomes maximal (Bockman, 1983; Granger et al., 1976). Further increases in oxygen demand result in an increase in blood flow to the exercising muscle in proportion to its oxygen consumption (Bockman, 1983). The oxygen balance is thereby restored.

If, as Gaskell first suggested (1877), this increase in blood flow is due to the local release of a dilatory substance, it would seem likely that such a factor would have a rate of production that is regulated by the oxygen balance of the tissue. Several substances, including adenosine, have been proposed as mediators of the increased blood flow, although no single substance has yet been found to account for the entire response. It seems more likely that the vasodilation mechanism involves more than one metabolite, and that the particular combination involved depends on the type of contraction, and the duration and conditions of the exercise. In addition, it is possible that the metabolic pattern of the muscle, as indicated by its fiber type, influences the specific mediators involved.

SKELETAL MUSCLE METABOLISM

Skeletal muscle is a highly organized tissue with the specialized ability to generate force and motion. The interaction of myosin

with actin, and the recycling of cross-bridges between these filaments in the presence of ATP, represent the fundamental molecular basis of muscle contraction. ATP is hydrolyzed in a sequence of chemical reactions occurring during this interaction, allowing conversion of the chemical energy of ATP into mechanical energy and the generation of force and work. However, the amount of ATP contained in muscle is capable of sustaining contractile activity for only a fraction of a second. In order to maintain contractile activity, molecules of ATP must be synthesized as rapidly as they are broken down. As illustrated in Figure 1, the three sources for this ATP production in skeletal muscle are creatine phosphate, mitochondrial oxidative phosphorylation, and substrate phosphorylation during glycolysis.

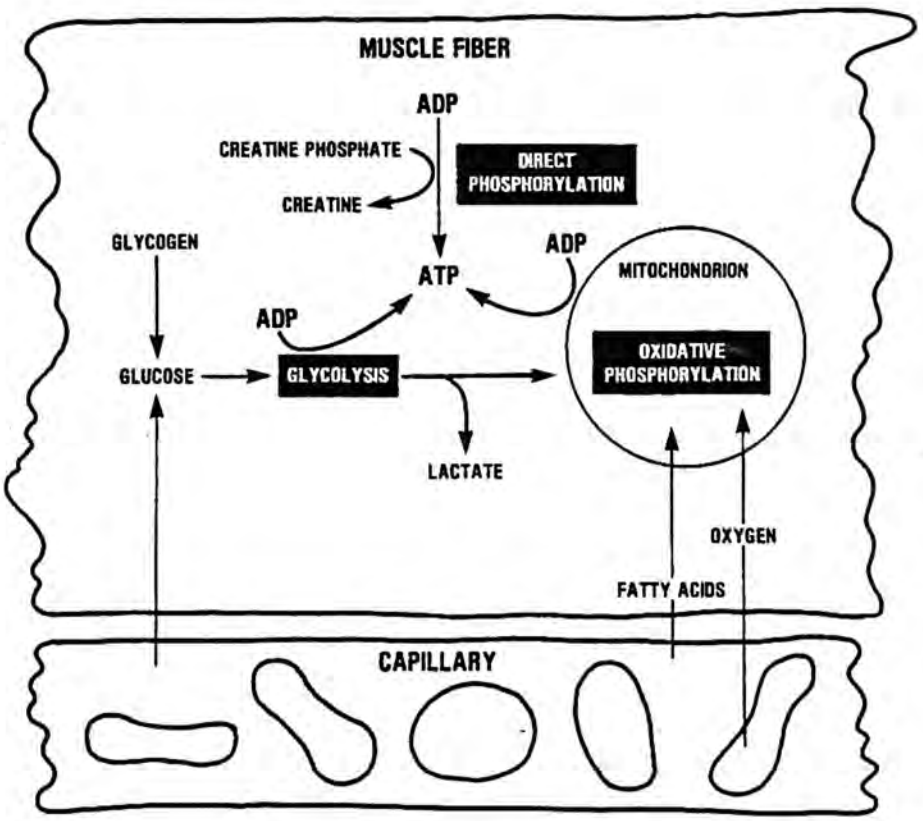
Synthesis of ATP

Creatine phosphate is a highly unstable organic compound and serves as a reservoir of high-potential phosphoryl groups in skeletal muscles. Creatine kinase catalyzes the very rapid transfer of a phosphoryl group from creatine phosphate to ADP to form ATP:

$$\text{creatine phosphate} + \text{ADP} \xrightarrow{\text{creatine kinase}} \text{ATP} + \text{creatine}$$

As contractions begin and ATP levels fall, mass action favors the formation of creatine from creatine phosphate. During muscle activity, the supply of creatine phosphate is rapidly depleted, decreasing the amount of ATP which is formed. If contractile activity is to be continued, the muscle must be able to derive ATP from sources other than the limited supply of creatine phosphate.

FIGURE 1. Biochemical pathways producing ATP utilized during muscle contraction. Creatine phosphate serves as the immediate source of phosphoryl groups for ATP production in all muscles. Cat soleus muscle has numerous mitochondria and a high capacity for aerobic metabolism, producing ATP primarily through oxidative phosphorylation. Its glycogen stores and glycolytic capacity are low. Cat gracilis muscle has few mitochondria and a low capacity for oxidative phosphorylation. High levels of glycogen in the gracilis muscle enable it to synthesize most of its ATP through glycolytic pathways.


MUSCLE FIBER
ADP
CREATINE PHOSPHATE
DIRECT PHOSPHORYLATION
CREATINE
GLYCOGEN
ADP
ATP
MITOCHONDRION
ADP
OXIDATIVE PHOSPHORYLATION
GLUCOSE
GLYCOLYSIS
LACTATE
OXYGEN
FATTY ACIDS
CAPILLARY

Oxidative phosphorylation is the process in which ATP is formed as electrons are transferred from NADH or $FADH_2$ to O_2 by a series of electron carriers. During moderate levels of muscle activity and rates of ATP breakdown, most of the ATP can be formed by the process of oxidative phosphorylation, using fatty acids as the predominant source of fuel and carbohydrates to a lesser extent. Under aerobic conditions, pyruvate formed through glycolysis enters mitochondria where it is completely oxidized to CO_2 and H_2O through the citric acid cycle and the electron-transport chain. However, during very intense exercise accompanied by a very rapid breakdown of ATP, the oxygen demand exceeds supply to the muscle. This imbalance, coupled with the decreased availability of substrates and the limited rates at which the enzymes in the metabolic pathways can process these substrates, restricts the cell's ability to replace ATP by oxidative phosphorylation.

Glycolysis is the cytosolic sequence of reactions that converts glucose into pyruvate, generating the production of two molecules of ATP. During intense muscular activity when the amount of oxygen is limiting, pyruvate is reduced by NADH to form lactate in a reaction catalyzed by lactate dehydrogenase. The regeneration of NAD^+ in this reaction permits the continuation of glycolysis under anaerobic conditions and subsequent production of ATP. Although producing only small quantities of ATP from each molecule of glucose metabolized, glycolysis can proceed at a very high rate. Therefore, the rate of ATP formation by glycolysis is greater than the rate of ATP formation by oxidative phosphorylation.

Large amounts of glucose must be consumed to produce relatively small amounts of ATP through glycolysis. When the glycogen stores are

depleted, the muscle becomes fatigued because the ATP supply cannot meet the demand. At extreme levels of exercise intensity, fatigue may occur prior to depletion of glycogen stores because the rate of ATP breakdown is even faster than the rate of ATP formation through glycolysis.

Fiber Types and Metabolic Pattern

Skeletal muscle is a heterogeneous tissue, composed of muscle fibers possessing inherently different metabolic profiles which are reflected in varied enzyme activities, substrate levels, and macroscopic appearance. Although all muscle fibers are capable of generating ATP through both oxidative and glycolytic pathways, the degree to which these processes occur is not the same among all muscle fibers. Some fibers contain numerous mitochondria and thus have a high capacity for oxidative phosphorylation. The activity of the glycolytic enzymes in these fibers is relatively low, so that most of the ATP produced is dependent upon a supply of oxygen. These fibers are generally surrounded by numerous capillaries and contain large amounts of myoglobin which provide their distinctive red coloration. In contrast, so-called "white" muscle fibers have little myoglobin and capillarization, few mitochondria, a large store of glycogen, and a very high glycolytic capacity. These fibers are specialized for the production of ATP by glycolysis in the absence of oxygen.

Another feature which differentiates fiber types is their contractile property. The speed of contraction of a skeletal muscle fiber is dependent upon the rate at which its myosin splits ATP, thereby determining the rate of cross-bridge recycling. Slow-contracting fibers exhibit a relatively long time to peak tension during twitch contractions

and low myosin ATPase activity. Fast-contracting fibers contain myosin with a high myosin ATPase activity and display a relatively short time to peak tension.

A widely-used scheme combining the reaction of myofibrillar ATPase with physiological and metabolic properties distinguishes three types of muscle fibers: Type I, Type IIA, and Type IIB (Buller et al., 1960; Burke et al., 1973; Burke et al., 1974; Peter et al., 1972). Although most muscles are mixtures of two or more fiber types, some muscles may contain predominately one type of fiber. The contractile and metabolic properties that combine to produce the physiological characteristics of particular skeletal muscles enable them to engage in a wide variety of activities.

Type I. These fibers combine low myosin ATPase activity with a high oxidative capacity. Also known as "slow-twitch" or "fatigue-resistant" fibers, they manifest a low speed of contraction and an ATP production through oxidative phosphorylation which readily matches ATP breakdown. Their rich capillary network further ensures that these fibers do not undergo fatigue. These fibers are well-suited for prolonged activity, where ATP can be produced by directing substrate flux through the oxidative pathways of the mitochondria.

Type IIA. These fibers combine a high myosin ATPase activity with both a high oxidative and a high glycolytic capacity. Also called "fast-twitch, fatigue-resistant" or "super-red" fibers, they manifest a rapid speed of contraction and will undergo fatigue only if maintained in a contracted state for long periods of time. These fibers are well-suited for prolonged, high-intensity activity, providing energy for contraction from both oxidative phosphorylation and glycolysis.

Type IIB. These "fast-twitch" or "fatigable" fibers combine high myosin ATPase activity with low oxidative capacity, a high glycolytic capacity, and a limited blood supply. Although their speed of contraction is fast, they fatigue rapidly as their glycogen stores are depleted. These fibers are particularly well-suited for high-intensity, short-duration activity, where a glycolytic degradation of glycogen to lactate is the primary source of ATP production.

SKELETAL MUSCLE BLOOD FLOW

Numerous studies have shown that blood flow through a vascularly isolated muscle or limb increases with the frequency of muscle contraction until some functional limit is attained (Barclay et al., 1974; Folkow and Halicka, 1968; Kjellmer, 1965). This phenomenon, referred to as active hyperemia, is necessary in order for oxygen delivery to meet oxygen requirements, thus maintaining a favorable energy balance without loss of muscle performance.

Blood Flow During Resting Conditions

Reported resting blood flow values for skeletal muscle have varied considerably both within and among studies. Several studies have also noted differences in blood flow and oxygen consumption measurements taken during resting conditions in muscles of one or another fiber type (Folkow and Halicka, 1968; Hilton, 1977; Hilton et al., 1970; Hudlicka, 1969; Hudlicka, 1975; Hudlicka et al., 1973).

Cat gracilis muscle is composed largely of fast-twitch fibers, approximately 75% of which exhibit a glycolytic, low-oxidative metabolic profile (Bockman and McKenzie, 1983). Whalen et al. (1973) found resting

blood flow to vascularly isolated gracilis muscle ranges from 0.3 to 8.5 $ml \cdot min^{-1} \cdot 100g^{-1}$. Oxygen consumption by these muscles reportedly ranged from 0.50 to 0.55 ml $O_2 \cdot min^{-1} \cdot 100g^{-1}$. Similar values were reported by Bockman et al. (1980). Cat gastrocnemius muscle is composed primarily of fast-twitch fibers of which 40-50% have a high-glycolytic and low-oxidative capacity (Close, 1972; Maxwell et al., 1977). Values similar to those of the gracilis for resting blood flow and oxygen consumption have been reported in the cat gastrocnemius (Hilton, 1977; Hilton et al., 1970; Hudlicka, 1969; Hudlicka, 1975; Hudlicka et al., 1973).

Cat soleus muscle is composed almost entirely of slow-twitch, high-oxidative fibers. Reported values for blood flow to these muscles in isolated preparations vary considerably, ranging from 7.5 $ml \cdot min^{-1} \cdot 100g^{-1}$ (Bockman et al., 1980) to 65 $ml \cdot min^{-1} \cdot 100g^{-1}$ (Hudlicka, 1969). Resting oxygen consumption values have also varied greatly. Bockman et al. (1980), reported resting soleus oxygen consumption values of about 0.25 ml $O_2 \cdot min^{-1} \cdot 100g^{-1}$. Values reported by Hudlicka were ten-fold higher (1969, 1975). Differences in isolation techniques may account for some of the between-study variation. Bockman et al. (1980), whose isolated preparation produced blood flows which were similar to those obtained with microspheres, noted that numerous branches to extraneous tissue may have been missed in the isolation techniques used by Hudlicka. Higher blood flows would be expected if these branches remained intact. More recent studies confirmed the lower resting soleus blood flow values (Bonde-Peterson and Robertson, 1981; Petrofsky et al., 1981). They are similar to those in the gracilis muscle (Bockman et al., 1980).

Blood Flow During Exercise

Several studies have indicated that the vascular response to contraction varies among muscles of different fiber types. Blood flow to gracilis muscle increases with exercise (Bockman, 1983). Blood flow to the gastrocnemius muscle also increases severalfold when the muscle is stimulated to contract (Hudlicka, 1975). However, Hilton et al., (1970) and Hudlicka (1975) observed no change in blood flow to contracting soleus muscle. Oxygen consumption by the muscle doubled, but did not correlate with an increase in blood flow. It appeared that the soleus muscle's blood supply at rest was sufficiently high to meet the increased demand during exercise. Based on these observations, it appeared that the circulation and its control are fundamentally different in muscles of different fiber types.

Several other studies reported that soleus muscle does exhibit active hyperemia and increases in oxygen consumption during muscle contraction (Bockman, 1983; Bonde-Peterson and Robertson, 1981; Folkow and Halicka, 1968; Petrofsky et al., 1981). In general, the preparations which had the lower resting blood flows exhibited active hyperemia. Those having the higher blood flows did not exhibit active hyperemia.

Although there did not appear to be any fundamental, qualitative differences in the vascular and metabolic response to muscle contraction between slow-twitch and fast-twitch muscles in the cat, several quantitative differences were found (Bockman, 1983). A threefold greater oxygen cost for contraction was found in the gracilis compared to the soleus muscle, possibly related to its greater velocity of shortening. This agrees with the observations of Goldspink et al. (1970) that in various hamster muscles the efficiency of muscle contraction is inversely

related to the rate of tension development.

In addition, the apparent maximal oxygen consumption in gracilis was found to be only one-half that of soleus muscle (Bockman, 1983). After obtaining a maximal oxygen consumption of about 3 ml $O_2 \cdot min^{-1} \cdot 100g^{-1}$, the gracilis muscle fatigued to a level at which oxygen consumption was linearly related to muscle performance. In contrast, soleus muscle was able to attain oxygen consumption values of 5-6 ml $O_2 \cdot min^{-1} \cdot 100g^{-1}$, enabling it to maintain higher levels of muscle performance. However, measurements of blood flow in the soleus muscle were only 20-30% higher than those of the gracilis. This indicates that the majority of the difference in oxygen consumption resulted from greater oxygen extraction by the soleus compared to the gracilis, possibly due to the higher oxidative capacity of the muscle. In the soleus muscle, blood flow may eventually be the factor limiting oxygen consumption and muscle performance.

ADENOSINE AND METABOLIC REGULATION OF BLOOD FLOW

In 1929, Drury and Szent-Gyorgyi reported that adenosine injected intravenously into mammals increased coronary blood flow. Adenosine has since received considerable attention as a possible mediator of metabolically-linked blood flow regulation, particularly in the coronary circulation. The first comprehensive hypothesis to explain the action of adenosine was formulated by Berne (1963). He proposed that the formation of adenosine from a net ATP breakdown occurred in myocardial cells under conditions of ischemia, hypoxia, or increased workload when the demand for ATP exceeded supply. The adenosine, so formed, then diffused to the coronary resistance vessels, mediated vasodilation, and thus increased the substrate and oxygen availability.

There are currently two hypotheses explaining adenosine's initiation of coronary relaxation. Adenosine is proposed to act as a Ca^{2+} entry blocker, and has been shown to inhibit the slow inward current in coronary myocytes (Harder et al., 1979) via cell surface receptors. This hypothesis is also supported by data which demonstrated that adenosine inhibits ^{45}Ca uptake by both intact and cultured vascular smooth muscle cells (Fenton et al., 1982). A second hypothesis suggests that adenylate cyclase mediates the coronary relaxation induced by adenosine. Adenosine-induced relaxation is accompanied by cyclic AMP accumulation in bovine epicardial rings (Kukovetz et al, 1979). These two hypotheses may be connected. One possibility is that cyclic AMP may activate a protein kinase which phosphorylates the plasma membrane proteins of the Ca^{2+} channels (Katz et al., 1982; Walsh et al., 1979). This is supported by evidence of phosphorylation of sarcolemmal proteins by intramembranous cyclic AMP-dependent protein kinase (Walsh et al., 1979). Adenosine is also a potent vasodilator in skeletal muscle. A similar role for adenosine may exist in blood flow regulation to this tissue (Dobson et al., 1971; Olsson, 1983).

Mechanism of Adenosine Formation

During conditions of anoxia or increased work load, the rates of ATP synthesis and utilization by an organ are changed. These changes are accompanied by alterations in the concentrations of several intermediates of energy metabolism, including ATP, ADP, AMP, creatine phosphate, and creatine. However, the concentration of free ATP remains relatively stable. This is due to the equilibrium of the creatine kinase reaction which, as written, lies far to the right:

$$\text{creatine phosphate} + \text{ADP} + H^+ \xrightarrow[\text{kinase}]{\text{creatine}} \text{creatine} + \text{ATP}$$

$$K_{eq} = 1.51 \times 10^{8} M^{-1}$$

If the activity of creatine kinase is sufficient to maintain equilibrium, an assumption justified by the very high levels of these enzymes in skeletal muscle (McGilvery and Murray, 1974), an increase in ATP utilization will result in a decrease in creatine phosphate and a relatively small decrease in ATP. This decrease in ATP will be reflected in an increase in ADP, which produces a rise in AMP through the action of adenylate kinase:

$$2\ \text{ADP} \xrightarrow[\text{kinase}]{\text{adenylate}} \text{ATP} + \text{AMP}$$

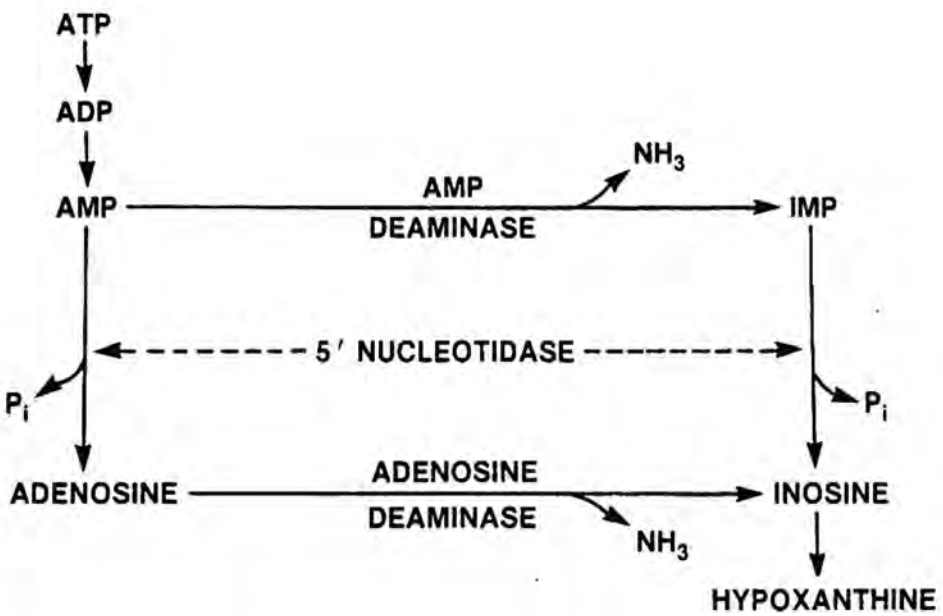
If the adenylate kinase reaction remains in equilibrium, the AMP concentration rises in proportion to the square of the ADP concentration (Lowenstein et al., 1983). ADP is a signal of energy deficit, and a rise in ADP increases the rate of oxidative phosphorylation and oxygen consumption. The rise in AMP also signals an energy deficit, increasing the rate of glycogen mobilization and glycolysis.

Many organs are capable of hydrolyzing some of the AMP to adenosine through a reaction catalyzed by 5'-nucleotidase. Alternatively, AMP can be deaminated to inosine monophosphate (IMP) by the action of AMP deaminase. 5'-Nucleotidase also catalyzes the conversion of IMP to inosine, as shown in Figure 2. An increase in high energy phosphate utilization by the tissue increases the production of AMP and the potential for adenosine production.

Evidence presented by Rubio et al. (1979) indicates that the production of adenosine may be coupled to the oxygen demand of the

Figure 2. Pathways of AMP degradation. AMP formed during the breakdown of ATP can be degraded by two routes. AMP can be deaminated to IMP by the action of AMP deaminase or dephosphorylated to adenosine by the action of 5'-nucleotidase. 5'-nucleotidase also catalyzes the conversion of IMP to inosine. Inosine is also formed from adenosine by adenosine deaminase.

AMP DEGRADATION



tissue relative to its oxygen supply. The activity of 5'-nucleotidase is stimulated by a decreased energy status of the cell as measured by the energy charge [energy charge = (ATP + 0.5ADP)/(ATP + ADP + AMP)] of the adenylate pool.

Conversely, ATP, ADP, and creatine phosphate inhibit 5'-nucleotidase (Olsson et al., 1973). This inhibition is reversed by Mg^{++}, whose intracellular concentration would be expected to increase as adenine nucleotides are degraded and ATP-chelated Mg^{++} is released (Rubio et al., 1979). An imbalance between oxygen demand and supply, resulting in a decrease in energy charge and creatine phosphate, would produce an increased substrate concentration and stimulation of 5'-nucleotidase. Adenosine production would be expected to increase.

Production of Adenosine by Skeletal Muscle

Early studies of skeletal muscle using simple paper chromatography revealed the presence of only the degradation products of adenosine, namely inosine and hypoxanthine (Deuticke et al., 1966). Subsequent studies (Imai et al., 1964) using chromatographic methods and enzymatic analysis also failed to show the existence of adenosine in skeletal muscle, even after periods of ischemia which produced significant reductions in creatine phosphate and adenine nucleotides. Using improved methodologies with even greater sensitivity, adenosine was found in the effluents of isolated rat hindquarters perfused with Krebs-Henseleit solution (Bockman et al., 1975). In these experiments, there was a progressive release of adenosine into the perfusate, which was accelerated when the muscles were stimulated to contract. In dog hindlimb preparations perfused at constant flow (Belloni et al., 1979; Bockman et al., 1975, 1976), tissue adenosine

levels doubled when the leg was stimulated to contract and returned to control levels after contraction was stopped. A decrease in vascular resistance was associated with the increased adenosine concentration (Bockman et al., 1976). The presence of adenosine in high concentrations while the vasculature was dilated suggested that adenosine may have a role in sustained hyperemia associated with skeletal muscle activity.

Results from studies using free-flow perfusion techniques have been less clear. Using a canine anterior calf muscle preparation, Phair and Sparks (1979) found no increase in tissue adenosine during induced contractions if normal hyperemia were allowed to occur. They concluded that adenosine was found in skeletal muscle only when the oxygen supply was limited by a restricted arterial inflow. These results conflicted with a later study which used a vascularly-isolated dog gracilis muscle under conditions of free-flow (Steffen et al., 1983). It was demonstrated that tissue adenosine content increased progressively with increases in stimulation frequency and correlated with an increase in vascular conductance.

One possible source for the discrepancy between these studies is the finding that 5'-nucleotidase activity has been found to be highest in skeletal muscle membranes near blood vessels, with lesser amounts present in avascular areas (Rubio et al., 1973). Thus, adenosine may be formed and released only in the vicinity of arterioles. An increase in adenosine concentration from AMP near arterioles might be diluted in whole-tissue assays by the large portion of tissue not near blood vessels. Conversely, all the adenosine may be produced and degraded intracellularly by adenosine deaminase without reaching the interstitial space where it would affect vascular smooth muscle. In other words,

there may be a vasodilator pool that is not detected in whole tissue measurements, or the detectable pool may be ineffective. A subsequent study by Fuchs et al. (1981) indicated that exercise of the anterior calf muscles of the dog perfused under free-flow conditions increased release of adenosine into the venous blood.

A second possible source of the discrepancy may be related to the muscles studied. Although both Steffen et al. and Phair and Sparks stimulated their muscles at a frequency of 2 Hz, almost twofold differences in oxygen consumption existed between the preparations, indicating possible differences in oxidative capacities. The muscle having the higher exercising oxygen consumption produced increases in tissue adenosine levels.

Differences Due to Fiber Type

The potential for adenosine production depends on the relative activities of the two enzymes which degrade AMP: AMP deaminase and 5'-nucleotidase. Some evidence indicates that the relative activities of these two enzymes is dependent upon the fiber type of which the muscle is composed.

Bockman and McKenzie (1983) examined the relative activities of these enzymes in a number of tissues and found that those with higher oxidative capacities had greater potential for adenosine production from a particular concentration of AMP than those with lower oxidative capacities. Heart muscle, which had the highest oxidative capacity, had the greatest 5'-nucleotidase/AMP deaminase ratio. Although significantly less than heart tissue, cat soleus muscle had a higher ratio than cat gracilis. It would be predicted from these studies that,

for a particular concentration of AMP, the potential for adenosine production is greater in soleus than in gracilis muscle.

There are a number of problems in directly transferring this difference in potential for adenosine production to the intact muscle. The absolute activities of the two enzymes are different. AMP deaminase activity in muscle measures in $\mu moles \cdot min^{-1} \cdot 100g^{-1}$, whereas 5'-nucleotidase activity ranges in $nmoles \cdot min^{-1} \cdot 100g^{-1}$. In addition, the K_m of each of these enzymes is different:

AMP Deaminase	$K_m = 5 \times 10^{-4} M$
5'-Nucleotidase	$K_m = 2 \times 10^{-5} M$

Therefore, 5'-nucleotidase will be nearer its maximal activity at physiological AMP concentrations of 0.1 mM or less (Meyer and Terjung, 1979). Also, ATP inhibits 5'-nucleotidase activity and stimulates AMP deaminase activity.

The best test of the expression of the potential for adenosine production is the change in interstitial adenosine content in response to various stimuli. The 5'-nucleotidase activity may be sufficient to produce adenosine concentrations which are physiologically effective in regulating vascular resistance. All studies to date which have examined differences in adenosine production by muscles composed of varied fiber types have used tissue adenosine content as an index of this concentration. Bockman and McKenzie (1983) found no correlation between blood flow and adenosine content in cat soleus and gracilis muscle. Adenosine content of the gracilis muscle changed little even in the presence of large increases in lactate content and fatigue as high as 60%. In the soleus muscle, there appeared to be a relationship

between increased muscle lactate and muscle adenosine content, suggesting that adenosine may be produced during hypoxia in the soleus muscle.

Whether such conditions occur during exercise, particularly in oxidative skeletal muscle, is unclear. Jobsis and Stainsby (1968) reported that the oxygen supply did not limit activity of the respiratory chain under conditions of normal flow. However, a recent study by Idstrom et al. (1985), using both the ^{31}P-nuclear magnetic resonance technique and conventional biochemical methods, indicates that a causal relationship exists between the oxygen supply and energy state in contracting oxidative and glycolytic muscles of the rat. They concluded that the energy production of contracting muscles can be oxygen-limited under physiological conditions. Furthermore, the energy state is directly influenced by the availability of oxygen during exercise.

More recently, pharmacologic methods have been undertaken in order to provide direct evidence of adenosine's role in active hyperemia. These approaches have so far proven inconclusive.

Kille and Klabunde (1984) reported that blocking uptake or degradation of adenosine increased the blood flow response to exercise in the highly oxidative dog gracilis muscle, whereas blocking adenosine production decreased the hyperemic response. Proctor (1984) found theophylline, a competitive antagonist of adenosine, attenuated the response in the low-oxidative hamster cremaster muscle. However, Mohrman and Heller (1984) found that aminophylline, two theophylline molecules joined by ethylenediamine, blocked the effects of exogenously applied adenosine in hamster cremaster muscle but did not affect the vascular response to muscle stimulation. Differences in the exact drugs used, dosages, and stimulation frequencies make it difficult to

resolve the differences found. In addition, as noted by Proctor (1984), the effects of theophylline in blocking adenosine were irreversible and theophylline often damaged the preparation.

A more direct approach to assessing the role of adenosine is the use of adenosine deaminase to specifically degrade adenosine to nonvasoactive inosine. Using this method, Proctor (1984) found that the addition of adenosine deaminase to the suffusion solution adjacent to the arterioles of the transilluminated hamster cremaster muscle significantly reduced the exercise-induced dilation of the muscle arterioles, regardless of the PO_2 of the suffusion solution. The effects of adenosine deaminase were reversible, indicating that it did not damage the tissue. This provided direct evidence that adenosine had at least a partial role in mediating the response. However, it is likely that the stimulation frequencies at which the greatest effect was seen caused muscle fatigue. Even if adenosine is important only during fatiguing exercise in the glycolytic hamster cremaster muscle, that does not preclude a role for adenosine in mediating active hyperemia in other muscle.

Future studies must define more clearly the conditions under which adenosine plays a role in skeletal muscle active hyperemia. In addition, it must be determined whether or not the fiber type and intrinsic metabolic profile of the muscle cells involved influences the extent to which adenosine mediates blood flow.

RATIONALE

Blood flow to exercising skeletal muscle increases during exercise in proportion to its oxygen consumption. Control of this active hyperemia is considered to be due primarily to vasoactive metabolites. Tissue levels of adenosine, a potent vasodilator, have been found to increase in certain muscles with exercise, particularly in muscles having a high capacity for oxidative metabolism. Adenosine content in glycolytic muscles, composed of fibers with a low oxidative capacity, does not appear to change with stimulation.

These studies were designed to explore the hypothesis that adenosine plays a role in the mediation of skeletal muscle active hyperemia. In addition, differences in the inherent metabolic profile of the muscle, as indicated by fiber type, influence the extent to which adenosine is involved. The role is greater in muscles having a high oxidative capacity and less in muscles having a low oxidative capacity.

Preliminary experiments were performed in order to determine the pattern of lactate release in cat soleus (slow-twitch, oxidative) and gracilis (fast-twitch, glycolytic) muscles during exercise. Because adenosine production has been found to correlate with lactate content in the cat soleus muscle, this provided an indication of the exercise conditions that might be necessary for adenosine release. Additionally, ammonia release by the muscle was studied over a range of muscle performance levels as an indication of IMP formation. In vitro activity of AMP deaminase relative to 5'-nucleotidase has been found to be higher in glycolytic muscle compared to oxidative muscle. Differences

in the formation of IMP in muscles of different fiber types consistent with the finding of a differential production of adenosine by the muscles could indicate that differences also exist in the in vivo activity of these enzymes in muscles of different fiber types.

In order to study a cause-and-effect relationship between adenosine and active hyperemia, adenosine deaminase was infused in order to specifically degrade adenosine and alter its vascular actions. Stimulation levels were chosen to provide similar "high" and "low" blood flow responses in the cat soleus and gracilis muscles. Specific parameters were determined from the results of the initial experimental series. One stimulation level was chosen in which little lactate production by the soleus muscle was expected. The other level was chosen as one in which the soleus muscle does produce lactate. In addition, the gracilis muscle was stimulated to contract at one frequency in which muscle performance was expected to be maintained. Another frequency was chosen in which loss of muscle performance was expected to occur in order to determine whether adenosine is important only during fatiguing exercise in glycolytic muscle. Muscle levels of creatine phosphate, inorganic phosphate, creatine, and lactate were measured to determine if inactivating adenosine has an effect on resting or exercising metabolism. If the muscle was not receiving an adequate supply of oxygen through its blood flow, tissue concentrations of these metabolites would be expected to change. The results from these studies indicated whether adenosine is a mediator of the vascular events in exercising skeletal muscle and provided evidence of the exercise conditions necessary for its involvement.

A third series of experiments using a vascularly-isolated muscle preparation was performed to investigate whether the presence of adenosine deaminase affects muscle oxygen consumption. An effect of adenosine deaminase on oxygen consumption at rest would indicate an effect on muscle metabolism, rather than an effect due to its action on adenosine. Exercising muscle oxygen consumption would be expected to be attenuated with adenosine deaminase, if adenosine is a mediator of the vasodilation during active hyperemia, due to an attenuation of blood flow. Muscle metabolite contents were again measured to determine if its oxygen supply was adequate. In addition, the duration of the hyperemic response was studied in this preparation, allowing determination of the time course of adenosine's involvement in mediating the hyperemic response.

MATERIALS AND METHODS

SURGICAL PREPARATION

Mongrel cats (2.5 - 3.5 kg) of either sex were anesthetized with α-chloralose (80 mg/kg; i.v.). A tracheal cannula was inserted to maintain an open airway and allow spontaneous ventilation. Blood gases and pH were maintained within physiological limits by room-air ventilation with a Harvard positive pressure respirator and/or intravenous administration of sodium bicarbonate. A cannula in an external jugular vein allowed administration of drugs. Blood pressure was monitored and arterial blood samples drawn through a cannula in one carotid artery (venous outflow preparations) or the left brachial artery (microsphere preparations). In preparations requiring injection of radioactive microspheres, a 3F SONES cardiac catheter was introduced through the right carotid artery and advanced into the left ventricle. Correct placement of the catheter was determined by ventricular pressure recording and confirmation upon autopsy. In these animals, an additional cannula was placed in the right brachial artery for withdrawal of blood. Left ventricular pressure and phasic and mean arterial blood pressures were measured by means of a Statham pressure transducer (P23Db) and recorded on a Gould recorder (model 2400). Rectal temperature was maintained at 37-38°C by a servo-controlled heating pad (Yellow Springs Instruments) beneath the cat.

In all animals in the adenosine deaminase study, abdominal aortic flow was monitored via an electromagnetic flow probe (Carolina Medical Electronics, model 501 Electromagnetic Blood Flowmeter) placed at a level just proximal to the inferior mesenteric artery. A 24-gauge

angiocath (Deseret) was introduced into the inferior mesenteric artery and advanced to the level of the abdominal aorta. This was connected to an infusion line which allowed administration of adenosine deaminase or the boiled control infusate.

Venous Outflow Preparations

Ammonia and Lactate Study. The circulation to either a soleus or gracilis muscle was isolated as described by Bockman et al. (1980). As reported, the distribution of the gracilis artery and vein, which constitute the major blood vessels of the gracilis muscle, has two basic patterns. In one of these, the blood vessels first penetrate the semimembranosus muscle before entering the gracilis muscle. Isolation of this type of vasculature was relatively difficult and frequently unsuccessful, usually requiring isolation of the soleus muscle in cats displaying this particular type of anatomy. Assignment to muscle preparation group in this series was, therefore, not always random.

In the gracilis muscle preparation, all branches of the gracilis artery and vein not supplying the gracilis muscle were ligated. All branches of the femoral artery and vein, other than the gracilis artery and vein, from the inguinal region to about 3 cm distal to the gracilis vessels were also ligated. Arterial as well as venous branches were doubly ligated and cut to reduce fluid accumulation in the tissue supplied, and to reduce the possibility of perfusion of distal arteries through collateral blood vessels. Sodium heparin (1000 U/kg; i.v.) was then administered and supplemented every thirty minutes (250 U/kg; i.v.) for adequate prevention of coagulation. The femoral artery and vein were tied distal to the gracilis branches, and a cannula was

advanced in the femoral vein to the level of the gracilis vein. Blood was allowed to flow through this cannula to a chamber where the drops were detected by a photoelectric cell (Grass PTTI). The output was displayed on the recorder for calculation of blood flow. The system was calibrated by collecting the blood into a graduated cylinder during a known time interval. Blood flow from the chamber was returned to the femoral vein proximal to the gracilis branches. The cannula leading to the drop counter was fitted with a sampling port in order to obtain muscle venous samples. A small screw-clamp was then placed around the tendon of insertion, maintaining the in situ conformation of the muscle. The tendon was then cut, allowing attachment to a force displacement transducer (Grass, model FT 10C) at its in situ length through a ligature tied to the clamp. The nerve supplying the muscle was severed, and isometric contractions were elicited by stimulation of the nerve with square wave pulses. Stimulation parameters were set to obtain maximum force development (10 V, 10 msec). The muscle was covered with warm, saline-soaked, gauze sponges and Saran Wrap, and the surface temperature maintained at 36-37°C by a servo-controlled heat lamp.

In the soleus muscle preparations, the gracilis and semimembranosus muscles were severed at their insertions. The medial head of the gastrocnemius muscle was severed at its origin to expose the femoral vessels. The biceps femoris muscle was cut in order to completely expose the soleus muscle. All branches off the femoral artery and vein proximal to the soleus muscle were ligated. The soleus muscle was freed from surrounding connective tissue except at its attachment to the tibia. After heparinization of the cat, the cannulae for the drop counter circuit were placed in the vein. A ligature placed around the

severed tendon of insertion was attached to the force displacement transducer, maintaining the muscle at its resting, in situ length. The nerve supplying the muscle was severed, allowing stimulation of the muscle as previously described. The muscle was then covered with gauze sponges and Saran Wrap, and the surface temperature maintained at 36-37°C.

This technique often produced visible constriction of the femoral artery and vein as proximal as the inguinal region. In an effort to prevent this, lidocaine sponges were placed directly over the vessels.

Adenosine Deaminase Study. Isolation of the left soleus muscle in these experiments was similar to the previous soleus isolation with the following exceptions. The artery and vein supplying the soleus muscle were freed of extraneous branches to a point where the vessels could be easily distinguished, just distal to the knee. The arterial supply proximal to this remained untouched and in its original conformation. All branches of the vein were ligated to a point just proximal to the knee. Following heparinization, an 18- or 20-gauge angiocath (Deseret) was placed in the isolated femoral vein and connected to the drop-counter line. Outflow from the chamber was returned to the cat through a cannula inserted into the contralateral femoral vein. This technique did not appear to produce the constriction of vessels often seen in the ammonia and lactate study, so that lidocaine sponges were unnecessary. Verification of complete isolation of the soleus blood supply was confirmed at the end of the experiment by clamping the artery and observing outflow. Attachment to the force transducer and stimulator was the same as previously described.

The right soleus muscle was exposed and a suture placed around the tendon of insertion for rapid removal. The nerve supplying the muscle was cut, and the muscle wrapped in saline sponges and Saran Wrap.

Microsphere Preparations

Both soleus and both gracilis muscles were exposed and freed from surrounding connective tissue with minimal trauma. Care was taken not to disturb the vasculature of the hindlimb. The tendon of insertion of one soleus and the contralateral gracilis muscle were attached to force displacement transducers and stabilized at resting length as previously described and illustrated in Figure 3. The opposite soleus and gracilis muscles served as controls and remained *in situ* with a suture placed around the tendon of insertion for rapid removal. The designation of experimental versus control was random. The nerves supplying the four muscles were severed, and those to muscles designated as experimental were stimulated as described above. All four muscles were wrapped in saline-soaked gauze and Saran Wrap, and maintained at 36-37°C at resting length.

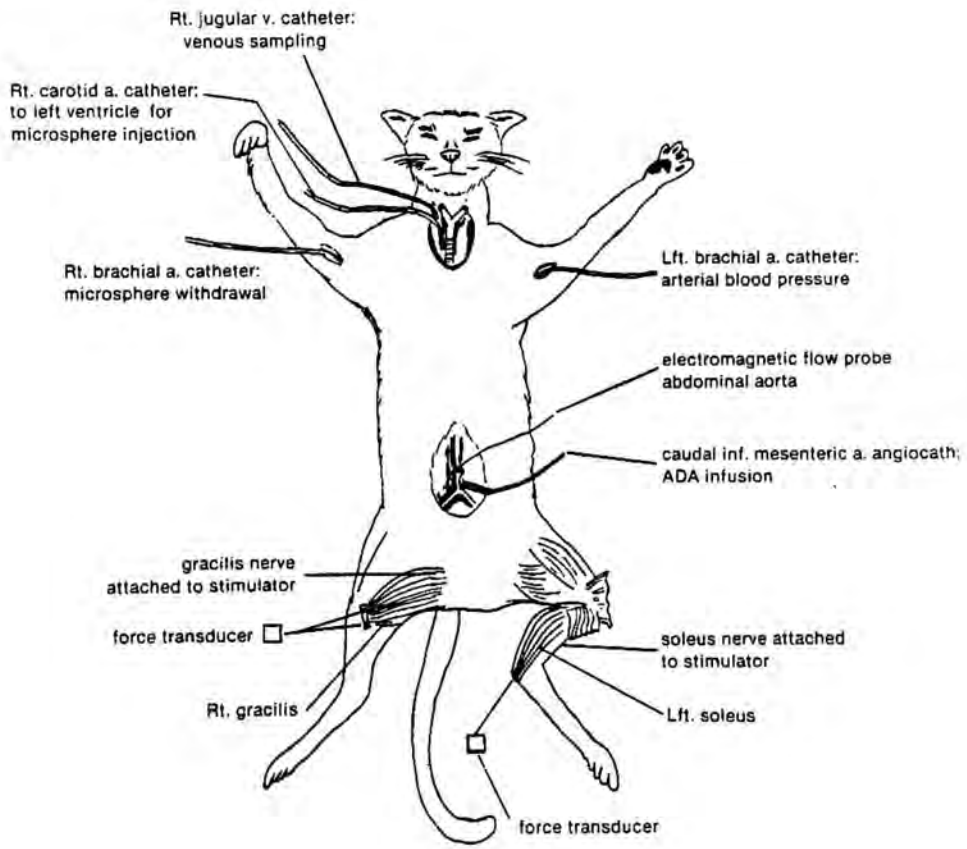
EXPERIMENTAL DESIGN

Following surgical preparation, the tissue temperature and blood flow were allowed to equilibrate for 30 minutes. The animals were then subjected to one of the protocols described below.

Ammonia and Lactate Study (n=17)

Soleus (n=10) and gracilis (n=7) muscles were vascularly isolated, perfused under free-flow conditions, and stimulated to contract isomet-

Figure 3. Preparation of muscles for use of radioactive microspheres in adenosine deaminase study. Experimental soleus and gracilis muscles were carefully freed and attached to force displacement transducers. Contralateral muscles served as controls for blood flow and metabolite measurements (artwork courtesy of E. Warner).


Rt. jugular v. catheter:
venous sampling
Rt. carotid a. catheter:
to left ventricle for
microsphere injection
Rt. brachial a. catheter:
microsphere withdrawal
Lft. brachial a. catheter:
arterial blood pressure
electromagnetic flow probe
abdominal aorta
caudal inf. mesenteric a. angiocath:
ADA infusion
gracilis nerve
attached to stimulator
force transducer
Rt. gracilis
soleus nerve attached
to stimulator
Lft. soleus
force transducer

rically for 15 minutes. Carotid arterial and muscle venous samples were obtained during rest and 15 minutes exercise. Muscles were stimulated at one of several frequencies (soleus: 0.5, 1.0, 2.0 Hz; gracilis: 0.2, 0.4 Hz) in order to produce a range of performance levels. Arterial and muscle venous blood was sampled (0.5-1.0 ml) anaerobically for the determination of oxygen content (Lex-O_2-Con, Lexington Instruments), pH, PO_2, PCO_2 (ABL-3, Radiometer) and hematocrit. Muscle oxygen consumption was calculated as the product of the arterial-venous oxygen content difference and the blood flow at the time of sampling. Arterial and venous blood (1.0 ml) was collected into glass test tubes packed in ice, centrifuged at 3000 rpm (model J-21B, Beckman Instruments), and the plasma decanted and stored for later analysis of ammonia and lactate as described below.

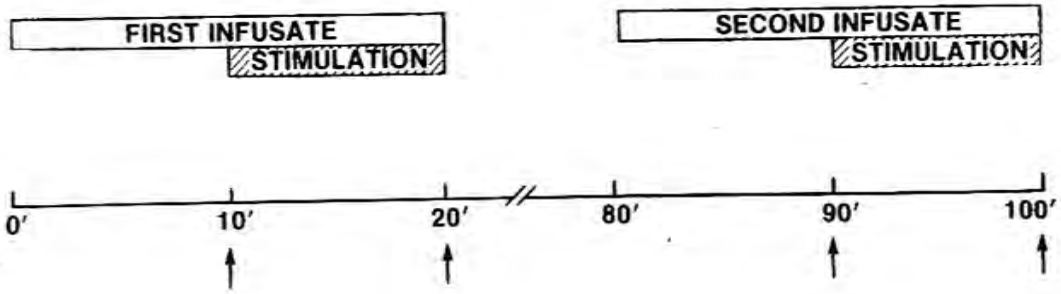
Muscle blood flow at rest and after 15 minutes of exercise was calculated from drop counter signals. Following the experiment, the total muscle was excised and muscle weight was obtained. Initial and final force developed (g force•g tissue^{-1}) during the exercise period was calculated. The product of developed force times frequency of stimulation (Fxf) was used as a relative index of muscle performance.

Adenosine Deaminase Study

Microsphere series (n=12). Following surgical preparation and a 30-minute equilibration period, a 20-minute close-arterial infusion (0.2 ml•min^{-1}) of adenosine deaminase (ADA) or ADA denatured by boiling (BADA) was begun. At 10 minutes infusion, resting muscle blood flow to both soleus and both gracilis muscles was determined using radioactive microspheres (Figure 4). Arterial samples were drawn for blood gas (ABL-3, Radiometer) and hematocrit analysis. Muscles were stimulated

Figure 4. Time course of the microsphere series in the adenosine deaminase study. A 20-minute infusion of either ADA or BADA was administered through the abdominal aorta. Stimulation of the experimental muscles was begun after 10 minutes of infusion. Blood flow was measured with radioactive microspheres at 10 minutes of infusion (rest) and 20 minutes of infusion (exercise). This process was repeated with the second infusate after a one-hour re-equilibration period. The order of infusates was random. Arrows indicate the times of blood flow and blood gas measurements.

ADENOSINE DEAMINASE STUDY: MICROSPHERE SERIES



↑ ARTERIAL BLOOD SAMPLE AND INJECTION OF RADIOACTIVE MICROSPHERE

to contract isometrically for 10 minutes at varied frequencies (soleus: 0.5 or 2.0 Hz; gracilis: 0.2 or 0.4 Hz) in order to study the effect of adenosine deaminase at different levels of muscle performance. These levels of muscle performance were expected to produce comparable high and low flow levels in the muscles (Bockman, 1983). At 20 minutes infusion with exercise, a second blood flow and blood gas measurement was taken. Following this, infusion and stimulation were terminated and a 60-minute re-equilibration time was begun to allow sufficient time for washout of the first infusate. The above procedure was then repeated with the second infusate. The order of infusates and muscle frequencies used were chosen randomly, although the same animal received only one level of stimulation for both infusions.

Following the fourth series of measurements, the stimulated muscles, as well as the contralateral soleus and gracilis muscles serving as controls for blood flow and metabolite measurements, were frozen *in situ* by clamping with aluminum tongs pre-cooled to the temperature of liquid nitrogen (-196°C). The frozen tissue was rapidly excised and remained at the temperature of liquid nitrogen until the tissue was weighed and processed for analysis of tissue adenosine, ADA activity, creatine, creatine phosphate, ATP, inorganic phosphate, and lactate as described below. Approximately 0.75 g tissue was used for these assays. Radioactivity of all frozen tissue not used for metabolite analysis was counted for blood flow calculation as described below. Remaining, unclamped muscle tissue was removed, weighed, and its radioactivity was also counted for blood flow calculation. Developed force (g force•g tissue^{-1}) and muscle performance (developed force x stimulation frequency) were calculated.

In order to verify that the re-equilibration period was sufficiently long to allow washout of adenosine deaminase, unstimulated cat soleus (n=6) and gracilis (n=7) muscle was obtained from cats which did not receive infusion of either ADA or BADA. Muscles were frozen *in situ* and processed for determination of ADA activity.

Venous outflow series (n=5). Following vascular isolation of the left soleus muscle and a 30-minute equilibration period, resting muscle blood flow was determined through drop counter measurements. A 20-minute infusion (0.2 $ml \cdot min^{-1}$) of BADA was begun (Figure 5). At 10 minutes of infusion, arterial and muscle venous samples were taken for blood gas (ABL-3, Radiometer) and hematocrit analyses (0.5-1.0 ml). Muscles were then stimulated to contract isometrically for 10 minutes at 2.0 Hz. At 20 minutes infusion with exercise, a second set of measurements and samples were taken. Following a one-hour re-equilibration period, arterial and muscle venous blood gas samples were taken. A 20-minute infusion of ADA followed (0.2 $ml \cdot min^{-1}$), and samples were obtained at rest (10 minutes infusion) and 2.0 Hz stimulation (20 minutes infusion). Both soleus muscles were then freeze-clamped *in situ* for ADA and metabolite analysis. All remaining, unclamped soleus muscle tissue was excised and weighed for total muscle weight calculation.

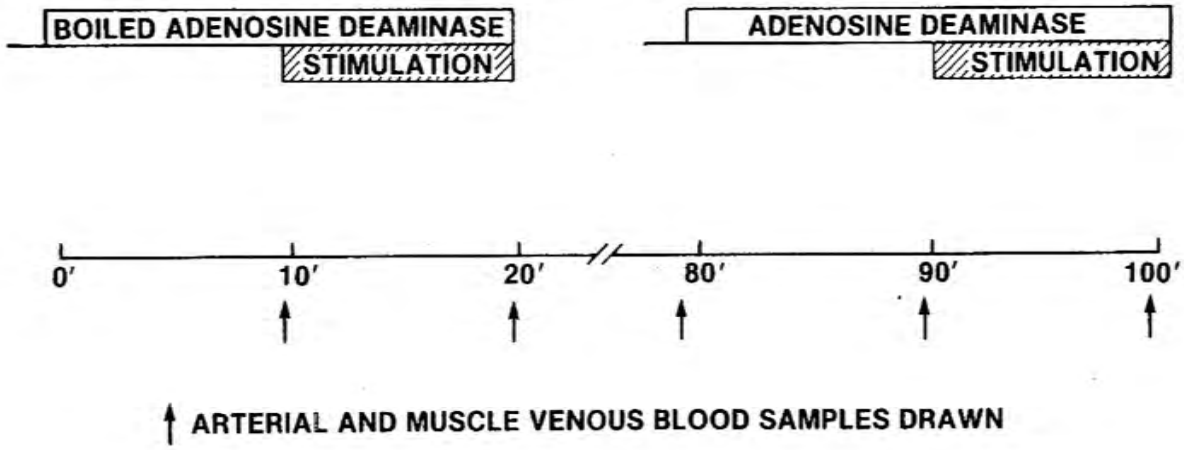
CHEMICAL ANALYSES

Adenosine

Tissues were stored in liquid nitrogen and processed within 24 hours. Pulverized frozen tissue was homogenized (Polytron) in ice-cold perchloric acid (0.5 N; 20 $ml \cdot g^{-1}$) in order to denature the tissue proteins, centrifuged for 10 minutes at 10,000 x g (Beckman, model J21-B), and

Figure 5. Time course of the venous outflow series in the adenosine deaminase study. A 20-minute infusion of BADA was administered through the abdominal aorta. At 10 minutes of infusion, stimulation of experimental muscles was begun. This process was repeated with ADA after a one-hour re-equilibration period. Blood flow was measured continuously using a drop counter. Arrows indicate the times of sampling of arterial and muscle venous blood.

ADENOSINE DEAMINASE STUDY: VENOUS OUTFLOW SERIES



↑ ARTERIAL AND MUSCLE VENOUS BLOOD SAMPLES DRAWN

the supernatant removed. The pellet was washed with 1.0 ml perchloric acid and recentrifuged. The total supernatant obtained was neutralized with potassium hydroxide (pH 8-8.5). After standing on ice for 30 minutes to ensure precipitation of potassium perchlorate salts, samples were centrifuged again for 10 minutes at 10,000 x g. The anion exchange resin Dowex AG1-X8-formate analytical grade resin (200 to 400 mesh; BIO-RAD Laboratories) was added to the supernatant (25 $mg \cdot ml^{-1}$) and vortexed for 2 minutes. The mixture was centrifuged at 10,000 x g for 10 minutes and the supernatant was air-dried and reconstituted in distilled water to give two-fold concentrations. Samples were filtered before analysis.

The adenosine in the partially purified samples was separated and quantitated with high pressure liquid chromatography using a reverse phase C_{18} analytical column (microsorb, Rainin Instr. Co.) by a modification of the method described by Klabunde (1979). The column length and internal diameters were 25 cm and 4.6 cm, respectively. Column temperature was ambient. Solvents were pumped through the column at a constant flow rate using Waters Model 6000A and Waters Model M-45 chromatography pumps. Column inlet pressure was 1600 psi when equilibrated with 20 mM KH_2PO_4 buffer at 0.8 $ml \cdot min^{-1}$. Samples were injected onto the column using a Waters Wisp 710B injector. Samples were eluted from the column with a nonlinear gradient of decreasing solvent polarity. The gradient was produced by two solvent reservoirs, beginning with a 90% Solution A (20 mM KH_2PO_4, pH 5.6) and 10% Solution B (25% aqueous methanol) and going to 100% Solution B over 65 minutes. The column gradient was controlled with a Waters system (controller). The flow rate was 0.8 $ml \cdot min^{-1}$.

The column eluate was directed into a flow-through cell on a Waters Lambda-Max Model 480 LC Spectrophotometer. U.V. absorbance at 260 nm was recorded on a Perkin-Elmer Model 023 recorder. The presence and quantitation of adenosine in samples was determined by comparison of retention times, peak height, and area on sample chromatograms with those obtained from adenosine standards. Retention time was defined as the time from sample injection to peak absorbance. Peak height was determined by measuring change from baseline absorbance. Peak area was determined using a Hewlett-Packard Model 3390A Integrator in-line with the spectrophotometer.

Selected samples were divided and one aliquot treated with adenosine deaminase before processing. Disappearance of the peak identified as adenosine after treatment with adenosine deaminase was taken as evidence that no substance co-elutes with adenosine. Recovery of adenosine was calculated by adding known adenosine standards to aliquots of samples before processing, averaging 84%. In addition, adenosine standard was added to aliqouts of the partially purified sample to assure that the retention time, peak height, and area was not affected by constituents of the sample. Sensitivity of this method for adenosine was in the range of 10 pmoles. Tissue adenosine content is expressed as nmoles•g tissue^{-1}.

Adenosine Deaminase

Tissue samples were pulverized and homogenized in ice-cold 50 mM phosphate buffer (pH 6.5; 9 ml•g^{-1}), centrifuged at 20,000 x g for 20 minutes, and the supernatants decanted. The supernatants were assayed immediately on a Perkin-Elmer spectrophotometer (model 556) at a wavelength of 265 nm. A 100 µl aliquot of supernatant was added to a cuvette

containing 2.86 ml (50 mM) phosphate buffer (pH 7.5) and 40 µl adenosine (3 mM). Output was registered on a Perkin-Elmer Recorder (model 56). The rate of change in absorbance as adenosine was converted to inosine was calculated to determine the tissue adenosine deaminase activity as follows:

$$\text{ADA Activity } (\text{U} \cdot \text{g tissue}^{-1}) = \frac{\text{change in absorbance per min}}{\text{extinction coefficient (8.1)}} \times \frac{3.0 \text{ ml}}{0.1 \text{ ml sample}} \times \frac{\text{sample volume}}{\text{sample weight}}$$

Activity was expressed as µmoles adenosine converted per minute (Units) per gram of tissue. Recovery of adenosine deaminase was estimated by adding adenosine deaminase standard to aliquots of samples before processing, and averaged greater than 95%. Activity of infusates was determined similarly, and expressed as Units•ml solution^{-1}.

Ammonia

Ammonia was measured enzymatically in plasma using an adaptation of the method developed by Sigma Chemical Company. This method is based on the reductive amination of α-ketoglutarate, catalyzed by L-glutamate dehydrogenase (GLDH), and occurs as follows:

$$\alpha\text{-ketoglutarate} + NH_4^+ + \text{NADH} \xrightarrow{\text{GLDH}} \text{glutamate} + \text{NAD}$$

The decreases in absorbance observed at 340 nm due to oxidation of NADH is proportional to the plasma ammonium ion concentration.

The assay solution contained 0.1 ml plasma and 0.9 ml ammonia reagent (2 mM α-ketoglutarate, 0.12 mM NADH, with buffer salts and stabilizers; Sigma Chem. Co.). The blank cuvette contained 0.1 ml water and 0.9 ml ammonia reagent. The initial absorbance at 340 nm versus

water as a reference was determined, and the reaction was started by adding 0.01 ml L-glutamic dehydrogenase (bovine liver; 1200 $U \cdot ml^{-1}$ in 50% glycerol and phosphate buffer; pH 7.4; Sigma Chem Co.). The solution was mixed, and the final absorbance after 5 minutes was recorded. Plasma ammonia concentration was calculated from the change (Δ) in absorbance units as follows:

$$\text{Ammonia } (\mu moles \cdot ml^{-1}) = \frac{(\Delta \text{ absorbance of sample}) - (\Delta \text{ absorbance of blank})}{\text{extinction coefficient (6.22)}} \times \frac{1.01 \text{ ml}}{0.1 \text{ ml sample}}$$

Ammonia release ($\mu moles \cdot min^{-1} \cdot 100g^{-1}$) was calculated as the product of plasma flow [blood flow x (1-hematocrit)] and the venous-arterial ammonia difference.

Metabolites

Tissue frozen in liquid nitrogen was assayed for creatine (Bernt et al., 1974), creatine phosphate (Lamprecht et al., 1974), ATP (Lamprecht and Trautschold, 1974), and inorganic phosphate (Gawehn, 1974). Lactate was determined by the spectrophotometric method of Hohorst (1965).

Tissue was homogenized in perchloric acid (5 $ml \cdot g^{-1}$), centrifuged for 10 minutes at 10,000 x g, and the supernatant neutralized with potassium hydroxide to pH 8-8.5. Following a 30-minute period allowing complete precipitation on ice, the samples were recentrifuged and refrigerated until assayed. Samples for creatine (0.5 ml) were stored at 0°C until assayed. A protein-free plasma filtrate was prepared for lactate analysis by adding 0.4 ml 1M PCA to 0.2 ml plasma. Following centrifugation at 3000 RPM for 15 minutes, 0.4 ml 1M K_2CO_3 was

used to neutralize 0.4 ml of the supernatant. This solution was then recentrifuged for 10 minutes. The supernatant was then assayed.

Lactate. Lactate analysis follows the increase in optical density at 340 nm due to the formation of NADH as diagrammed below:

$$\text{Lactate} \xrightarrow[\text{NAD}\quad\text{NADH}]{\text{LDH}} \text{Pyruvate}$$

The reagent was prepared using 2.0 ml glycine buffer, 0.1 ml lactate dehydrogenase, 10 mg NAD, and 4.0 ml distilled water. All chemicals were obtained from Sigma Chemical Company. Volumes were adjusted for the number of samples assayed. The assay solution for plasma contained 0.1 ml sample and 0.9 ml reagent. Water substituted for the sample in the reference solution. Standards of 0.044 and 0.088 μmoles lactate were used for each assay. The assay solution for tissue consisted of 0.7 ml buffer and 0.05 ml sample. For tissue, 0.89 and 0.44 μmole lactate standards were used. The assay solutions were incubated for 30 minutes at 37°C. The final absorbance at 340 nm (Beckman, model 26) against a water reference was recorded. Tissue lactate concentration was calculated from the absorbance units as follows:

$$\text{Lactate } (\mu\text{moles} \cdot g^{-1}) = \frac{\text{change in absorbance}}{\text{extinction coefficient (6.22)}} \times \frac{0.75 \text{ ml}}{0.05 \text{ ml sample}}$$

Plasma lactate concentration was calculated similarly, adjusting for a sample volume of 0.1 ml and an assay volume of 1.0 ml. Plasma lactate concentrations were expressed as $\mu\text{moles} \cdot ml^{-1}$. Lactate release ($\mu\text{moles} \cdot min^{-1} \cdot 100g^{-1}$) was calculated as the product of plasma flow [blood flow x (1-hematocrit)] and the venous-arterial plasma lactate difference.

Creatine. Creatine analysis of tissue samples is based on the conversion to creatine phosphate with ATP and creatine kinase. The ADP formed from this reaction can then be used to produce pyruvate, which subsequently forms lactate as follows:

$$\text{(1) Creatine + ATP} \xrightarrow[\text{(CK)}]{\text{creatine kinase}} \text{Creatine phosphate + ADP}$$

$$\text{(2) ADP + Phosphphoenolpyruvate} \xrightarrow[\text{(PK)}]{\text{pyruvate kinase}} \text{ATP + Pyruvate}$$
$$\text{(PEP)}$$

$$\text{(3) Pyruvate + NADH + } H^+ \xrightarrow[\text{(LDH)}]{\text{lactate dehydrogenase}} \text{Lactate + } NAD^+$$

The decrease of NADH, as measured by the change in extinction at 334 nm, is proportional to the amount of creatine present.

The reagent was prepared using 24 ml glycine buffer (0.2 M; pH 9.0), 0.9 ml ATP (1%; Sigma), 0.6 ml PEP (2%; BMC), 2.0 ml $MgCl_2$ (0.1 M), 1.5 ml KCl (1 M), 1.0 ml NADH (5 mM; Sigma), 0.1 ml LDH (BMC), and 0.1 ml PK (BMC). The assay solution contained 1.2 ml reagent and 0.1 ml sample. Water was substituted for the sample in the reference solution. A 1 mM standard was used for each assay. After an initial reading at 334 nm (Beckman, model 26), 25 µl CPK solution (25 mg of CPK dissolved in 0.5 ml of 0.2 M glycine buffer, pH 9) was added to each cuvette, including the reference, and absorbance readings were taken at 2-minute intervals. The reaction was completed within 20 minutes.

The amount of creatine present in the samples was calculated from the change in absorbance as follows:

$$\text{Creatine } (\mu\text{moles} \cdot g^{-1}) = \frac{\text{change in absorbance}}{\text{extinction coeff. (6.18)}} \times \frac{1.325 \text{ ml}}{0.1 \text{ ml sample}} \times \frac{\text{sample volume}}{\text{sample weight}}$$

ATP and creatine phosphate. ATP and creatine phosphate were measured sequentially using the hexokinase (HK), glucose-6-phosphate dehydrogenase (G-6-PDH) and creatine kinase (CK) reactions:

$$(1)\text{glucose} + \text{ATP} \xrightarrow{\text{HK}} \text{glucose-6-P} + \text{ADP}$$

$$(2)\text{glucose-6-P} + \text{NADP} \xrightarrow{\text{G6PDH}} \text{6-phosphogluconolactone} + \text{NADPH} + H^+$$

$$(3)\text{creatine phosphate} + H^+ + \text{ADP} \xrightarrow{\text{CK}} \text{creatine} + \text{ATP}$$

The increase in extinction is proprotional to the change in ATP and creatine phosphate.

The reagent was composed of 10 ml Tris/HCl (0.2 M; pH 7.8), 2.0 ml $MgCl_2$ (0.1 M), 1.0 ml NADP (1%; Sigma), 0.5 ml glucose (0.1 M), and 0.015 ml G-6-PDH (BMC). The assay solution consisted of 1.0 ml reagent, 0.9 ml water, and 0.1 ml sample. The blank contained water instead of sample. Standards of 1.0 mM creatine phosphate and ATP were used for each assay. After an initial reading at 334 nm (Perkin-Elmer, model 555), 5 µl HK (BMC) was added to each cuvette. Readings were taken at 2-minute intervals. ATP was completely reacted within 10 minutes. For analysis of creatine phosphate, 20 µl CK solution (20 mg CK (BMC) dissolved in 0.6 ml 50 mM Tris, 0.5 ml 10mM ADP (BMC); pH 7.8)

was then added to each cuvette. Absorbance readings were taken at 2-minute intervals. Complete reaction occurred by 12 minutes.

Creatine phosphate and ATP were calculated from the change in absorbance during each reaction as follows:

$$\text{ATP or creatine phosphate } (\mu\text{moles} \cdot g^{-1}) = \frac{\text{change in absorbance}}{\text{extinction coeff. (6.18) x cell light path (2)}} \times \frac{\text{total vol.}}{0.1 \text{ ml sample}} \times \frac{\text{sample vol.}}{\text{sample wt.}}$$

Inorganic phosphate. Analysis of inorganic phosphate is based on the phosphorylysis of glycogen by phosphorylase a as follows:

$$(1)\ P_i + \text{glycogen} \xrightarrow{\text{phosphorylase a}} \text{glucose-1-P}$$

$$(2)\ \text{glucose-1-P} \xrightarrow{\text{phosphoglucomutase}} \text{glucose-6-P}$$

$$(3)\ \text{glucose-6-P} + \text{NADP} \xrightarrow[\text{(G-6-PDH)}]{\text{glucose-6-P dehydrogenase}} \text{6-Phosphogluconolactone} + \text{NADPH} + H^+$$

The formation of NADPH is proportional to the amount of P_i present.

Buffer consisted of 10 ml imidazole buffer (50 mM; pH 7.0), 0.5 ml magnesium acetate (15 mM), 0.5 ml ethylenediaminetetra-acetate (EDTA, 30 mM; BMC), 1.0 ml NADP (10mM; BMC), 0.1 ml AMP (3 mM; Sigma), 1.0 ml glycogen (80 mg dissolved in 1.0 ml water, dialyzed overnight at 4°C against 50 mM imidazole buffer; pH 7.0; diluted to 1 ml with water), 0.05 ml phosphoglucomutase (BMC; 10 $mg \cdot ml^{-1}$ stock dialyzed overnight at 4°C against 20 mM acetate buffer; pH 5.3; diluted to twice volume with same buffer). The assay solution consisted of 1.0 ml buffer, 0.1 ml sample (or buffer as reference) and 0.2 ml water. Phosphate

standards of 0.2 and 0.5 mM were used for each assay. After reading the initial extinction at 334 nm (Beckman, model 26), 50 µl G-6-PDH (BMC; 5 $mg \cdot ml^{-1}$ stock dialyzed overnight at 4°C against 50 mM imidazole buffer; pH 7.0; diluted 1:2.5 with same buffer) was added to the assay solution, incubated for 45 minutes at room temperature, and final absorbance was read.

The amount of inorganic phosphate present was calculated from the absorbance change as follows:

$$\text{Phosphate } (\mu moles \cdot g^{-1}) = \frac{\text{change in absorbance}}{\text{extinction coeff.(6.18)}} \times \frac{1.350 \text{ ml}}{0.1 \text{ ml sample}} \times \frac{\text{sample volume}}{\text{sample weight}}$$

PREPARATION OF ADENOSINE DEAMINASE

Adenosine deaminase (Sigma Chemical Co., Type VIII) was diluted in Krebs buffer (pH 7.4, 200 $U \cdot ml^{-1}$) and dialyzed overnight against three changes of buffer at 4°C. The Krebs buffer consisted of 118.0 mM NaCl, 27.4 mM $NaHCO_3$, 4.8 mM KCl, 2.5 mM $CaCl_2$, 1.2 mM $MgSO_4$, and 1.0 mM KH_2PO_4. The dialysis tubing (Fisher Scientific) allowed the passage of compounds of molecular weight less than 12,000. The activity of the enzyme was tested after dialysis and averaged 154 $U \cdot ml^{-1}$ Krebs. Infusate for control measurements was prepared similarly and heat inactivated by fifteen minutes of boiling. The solution was then centrifuged to eliminate the precipitated protein.

MICROSPHERE TECHNIQUE FOR MEASUREMENT OF BLOOD FLOW

Blood flow to muscle was determined through the use of carbonized plastic tracer microspheres (3M Company, or New England Nuclear) as

described by Heymann et al. (1977). Microspheres are radiolabeled spheres having the same rheology as red blood cells which are too large to pass through the capillaries in perfused tissue. Essentially all microspheres are removed from the circulation in their first pass through the microcirculation. The measured radioactivity of the tissue, or counting rate, is proportional to the number of microspheres entrapped within the capillaries.

Microspheres measuring 15 ± 3 μm in diameter and labelled with ^{141}Ce, ^{113}Sn, ^{85}Sr, or ^{46}Sc were obtained in 1.0 mCi in 10% Dextran to which 0.05% polyoxyethylene 80 sorbitan monooleate (Tween-80), a detergent, had been added to minimize aggregation. Approximately 1.32×10^6 microspheres were diluted in saline providing an injectate with microspheres, which gave a final microsphere density of greater than 400 microspheres per sample. It has been determined that such an amount is the minimum necessary to achieve a precision of 10% (Heymann et al., 1977). Activity of the isotopes were determined to provide at least 400 cpm per sample. The mixture was sonicated for at least 30 minutes prior to injection to disperse the microspheres. Immediately before injection, the microspheres were removed from the sonicator and mechanically shaken (Vortex mixer) until the time of injection. Microspheres were injected in random order through the left ventricular catheter to ensure adequate mixing and even distribution within the blood stream, over a 30-second period. The cannula was then flushed with 3 ml of saline over 30 seconds. Five seconds prior to each microsphere injection, a constant withdrawal of a reference sample ($1.04\ ml \cdot min^{-1}$) from the right brachial artery was begun, terminating one minute after completion of microsphere injection and flush. This time period allowed

all microspheres to be cleared from the circulation during the period the reference sample was obtained. Arterial blood pressure was monitored throughout the injection and withdrawal. Any changes in blood pressure greater than 10 mmHg during the measurement, indicating an effect of the microspheres on the general circulation, negated that determination.

Following tissue clamping for metabolite analysis, all remaining portions of the soleus and gracilis muscles, as well as all portions not used for metabolite analysis, were harvested, weighed, and the activity of each isotope determined in a gamma counting system (Searle, model 1185). In addition, both kidneys were harvested and similarly processed in order to assure there was minimal microsphere streaming, providing an internal check of the adequacy of mixing. Energy windows were adjusted with standards of pure isotope in order to maximize counts of the isotope of interest while minimizing the counts of other isotopes in that window.

True activity of each isotope in the tissue samples was calculated as described by Heymann et al. (1977). Because four different isotopes were used, it was necessary to adjust for Compton scattering of gamma rays from the higher energy isotope or isotopes into the counts measured in the range of lower energy isotopes. The shape of the gamma spectrum of any isotope is constant within a detection system. Therefore, the counts measured between any energy limits are proportional to the total number of counts measured for that isotope. This means that the counts of one nuclide in any two windows are proportional.

A pure sample of each nuclide was counted in each energy window along with the experimental samples and these proportionality factors were evaluated. From this, radioactive emissions of other isotopes present

in the samples could be subtracted out to obtain the counts due solely to the isotope of interest. Calculation of true activity for each isotope was calculated as follows from total counts after subtracting background counts:

$$Ct(Sc) = Tcpm(Sc)$$

$$Ct(Sr) = Tcpm(Sr) - (Ct(Sc) \times Sr/Sc)$$

$$Ct(Sn) = Tcpm(Sn) - (Ct(Sc) \times Sn/Sc) - (Ct(Sr) \times Sn/Sr)$$

$$Ct(Ce) = Tcpm(Ce) - (Ct(Sc) \times Ce/Sc) - (Ct(Sr) \times Ce/Sr) - (Ct(Sn) \times Ce/Sn)$$

where Ct = counts$\cdot$min^{-1} due solely to that isotope, Tcpm = total counts$\cdot$min^{-1} in that energy window, and ratios (e.g. Sr/Sc) = proportionality factors for the windows obtained from pure standards.

Tissue blood flows were calculated from the formula:

$$\text{Organ flow } (ml \cdot min^{-1}) = \frac{\text{reference withdrawal rate } (ml \cdot min^{-1}) \times \text{counts} \cdot min^{-1} \text{ in tissue}}{\text{counts} \cdot min^{-1} \text{ in reference sample}}$$

Buckberg et al. (1971) found this method of measuring blood flow to be accurate provided at least 400 microspheres were present in the reference sample. Tissue flows were expressed as ml$\cdot$min$^{-1}\cdot$100g^{-1}, using the weights of the counted tissues.

Cardiac output at the time of injection of each isotope was calculated by the following formula:

$$\text{Cardiac output } (ml \cdot min^{-1}) = \frac{\text{Total cpm injected} \times \text{Withdrawal rate}}{\text{Withdrawal cpm}}$$

Because the radioactivity of the total injectate was beyond the linear limit of the gamma counting system, a diluted standard of each isotope was used. The total counts•min^{-1} of the injectate was determined from the counts•min^{-1} of the standard, the dilution factor, and the volume of the injectate. From this, the counts remaining in the syringe after injection were subtracted in order to determine the total counts•min^{-1} actually injected.

DATA ANALYSIS

Statistical analysis of differences between rest and exercise in the same muscles or between ADA and BADA in the same muscle was made with Student's T-test adapted for paired comparisons. Unpaired T-tests allowed comparisons between soleus and gracilis muscle preparations in the ammonia and lactate study. Linear regression was used to analyze linear relationships between muscle performance and ammonia or lactate release. Significance of linear regression was determined by analysis of variance. Differences in slopes of regression lines for the soleus and gracilis muscles were determined by analysis of covariance. One-way analysis of variance was used to analyze changes in hemodynamics over the course of the experiment in the adenosine deaminase study, with Duncan's test for comparison among samples. In the ADA venous outflow series, multiple samples were compared using two-way analysis of variance for repeated measures, with the Duncan's test for comparison among samples (Armitage, 1971). Differences between data were considered to be significant when $p<0.05$.

RESULTS

AMMONIA AND LACTATE STUDY

Blood Data and Arterial Blood Pressure

Arterial and muscle venous blood gas and hematocrit values are presented in Table I. Resting values for these parameters were not different between the soleus and gracilis muscle preparations. Arterial blood gases and hematocrit were not different between resting and exercising measurements. Muscle venous PCO_2 increased, and pH and PO_2 decreased with stimulation in both muscle preparations.

Mean arterial blood pressure averaged 106 ± 7 mm Hg in the soleus muscle preparations at rest, and did not change significantly during exercise. Mean arterial blood pressure averaged 121 ± 4 mm Hg in the gracilis preparations at rest and also was not different during exercise. Blood pressure was not different between muscle preparations during rest and exercise measurements.

Resting Muscle Blood Flow, Vascular Resistance, and Oxygen Consumption

Table II summarizes resting muscle blood flow, vascular resistance, and oxygen consumption in the soleus and gracilis muscles. Resting blood flow and vascular resistance were not significantly different between the soleus and gracilis muscle preparations. Oxygen consumption at rest also did not differ between the oxidative and glycolytic muscles. Resting oxygen consumption in all muscles was less than 1.0 ml $O_2 \cdot min^{-1} \cdot 100g^{-1}$.

Hemodynamics and Vascular Response to Exercise

As shown in Table III, a wide range of muscle performance levels

TABLE I

BLOOD DATA DURING REST AND EXERCISE:
AMMONIA AND LACTATE STUDY

SAMPLE	pH		PCO_2 (mmHg)		PO_2 (mmHg)		Hct (%)	
	Art	Ven	Art	Ven	Art	Ven	Art	Ven
I. Soleus (n=10)								
Rest	7.36 ±0.01	7.33 ±0.02	31 ±1	34 ±2	103 ±3	35 ±2	42 ±1	43 ±1
Exercise	7.37 ±0.01	7.27* ±0.02	30 ±1	47* ±1	106 ±5	20* ±2	41 ±1	42 ±1
II. Gracilis (n=7)								
Rest	7.34 ±0.01	7.33 ±0.01	35 ±2	34 ±2	107 ±3	37 ±4	41 ±1	42 ±2
Exercise	7.35 ±0.01	7.17* ±0.01	32 ±1	58* ±2	111 ±2	20* ±3	40 ±1	42 ±1

Values are mean ± S.E.M. Art = arterial
Hct = hematocrit Ven = venous
Rest = sample taken prior to stimulation
Exercise = sample taken at 15 minutes of stimulation (soleus: 0.5, 1.0, or 2.0 Hz; gracilis: 0.2 or 0.4 Hz)
* significantly different from rest, $p<0.05$

TABLE II

RESTING BLOOD FLOW, VASCULAR RESISTANCE AND OXYGEN CONSUMPTION: AMMONIA AND LACTATE STUDY

	Blood Flow ($ml \cdot min^{-1} \cdot 100g^{-1}$)	Vascular Resistance ($mmHg \cdot min \cdot 100g \cdot ml^{-1}$)	Oxygen Consumption ($ml\ O_2 \cdot min^{-1} \cdot 100g^{-1}$)
Soleus (n=10)			
mean ± S.E.M.	5.1±0.8	23.6±2.7	0.42±0.05
range	2.3-10.3	11.2-40.6	0.15-0.63
Gracilis (n=7)			
mean ± S.E.M.	4.0±0.4	32.5±4.0	0.30±0.05
range	2.3-5.9	22.9-55.6	0.15-0.45

Values are pooled from all muscles in the study and represent levels prior to stimulation.

TABLE III

MUSCLE PERFORMANCE AND VASCULAR RESPONSE TO EXERCISE:
AMMONIA AND LACTATE STUDY

	Initial Muscle Performance ($g \cdot g^{-1} \cdot sec^{-1}$)	Blood Flow ($ml \cdot min^{-1} \cdot 100g^{-1}$)	Vascular Resistance ($mmHg \cdot min \cdot 100g \cdot ml^{-1}$)	Oxygen Consumption ($ml \cdot min^{-1} \cdot 100g^{-1}$)
Soleus (n=10)				
range	66-272	5.3-52.2	1.7-17.9	0.82-9.08
Gracilis (n=7)				
range	19-69	4.2-29.1	3.8-26.4	0.66-2.85

Values were obtained from all muscles in the study at 15 minutes of stimulation. Frequency of stimulation was varied (soleus: 0.5, 1.0, or 2.0 Hz; gracilis: 0.2 or 0.4 Hz) in order to obtain a wide range of performance levels and blood flow responses.

was elicited by varying the frequency of stimulation. In the soleus muscle preparations, performance was maintained throughout the stimulation period. Final force x frequency in the soleus muscles was 91 ± 6% of the initial value. The amount of force initially developed by the soleus muscle was 127 ± 9 g force•g muscle^{-1}.

The initial amount of force developed by the gracilis muscle was not different from that of the soleus muscle, averaging 113 ± 19 g force•g muscle^{-1}. The initial performance levels achieved in the gracilis muscle with the stimulation parameters that were used were generally lower than in the soleus muscle. However, unlike the soleus muscle, the gracilis muscle fatigued to a final level of performance which was 68 ± 7% of the initial value.

Muscle contraction was associated with active hyperemia and increased oxygen consumption in both preparations. Blood flow increased in all preparations immediately upon stimulation in all muscles, reaching a level within 2 minutes which did not differ from the blood flow at 12 minutes.

Release of Ammonia During Rest and Exercise

As shown in the left column of Table IV, the soleus and gracilis muscles released little, if any, ammonia at rest. During exercise, both the soleus and gracilis muscles released ammonia, although quantitative differences in its release existed between the two muscles. As illustrated in Figure 6, ammonia release correlated with muscle performance in the gracilis ($r = 0.95$; $p<0.05$) and in the soleus muscle ($r = 0.67$; $p<0.05$). However, the gracilis muscle produced high amounts of ammonia even at low levels of stimulation. In comparison, ammonia release from the soleus muscle changed little for a given change in muscle performance.

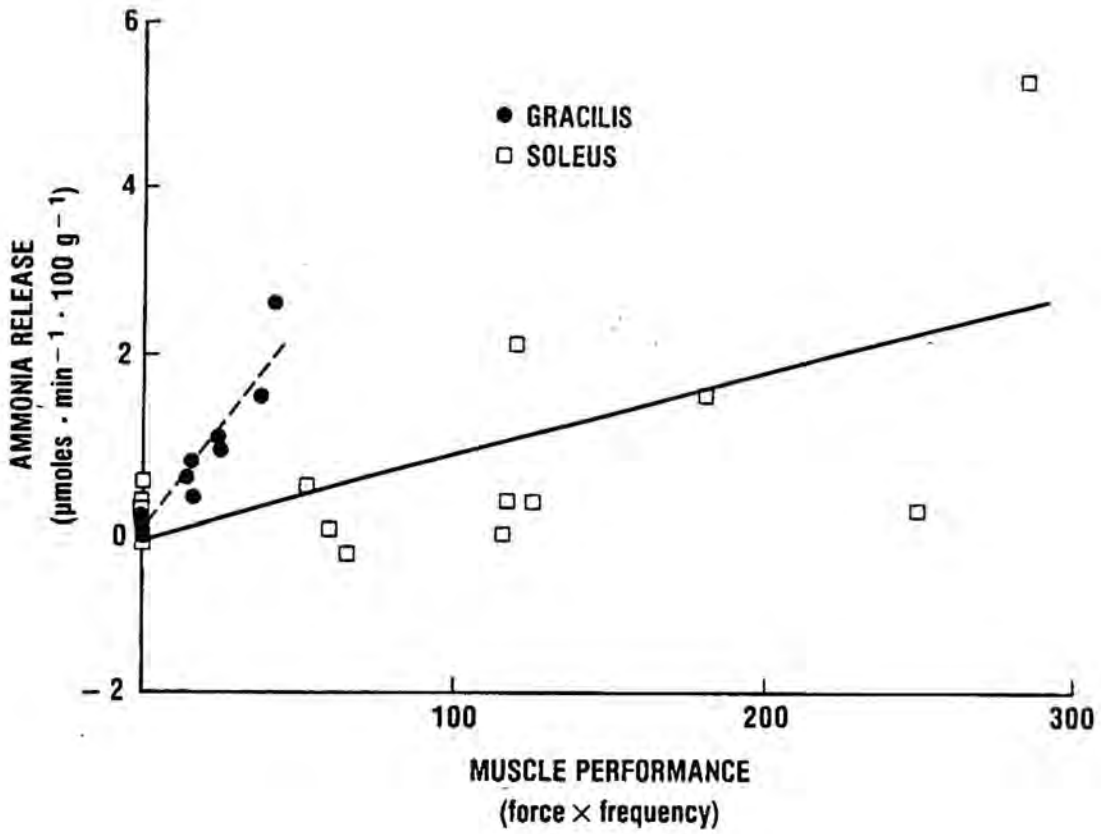
TABLE IV

AMMONIA AND LACTATE RELEASE DURING REST AND EXERCISE:
AMMONIA AND LACTATE STUDY

SAMPLE	NH_4^+ Release ($\mu moles \cdot min^{-1} \cdot 100g^{-1}$)	Lactate Release ($\mu moles \cdot min^{-1} \cdot 100g^{-1}$)
Soleus (n=10)		
Rest		
mean ±S.E.M.	0.05±0.06	8.02±1.22
range	-0.17-0.49	3.92-15.76
Exercise		
range	-0.37-5.34	13.06-36.75
Gracilis (n=7)		
Rest		
mean ±S.E.M.	-0.03±0.02	2.51±0.54*
range	-0.12-0.07	1.03-5.29
Exercise		
range	0.30-2.63	18.66-52.80

Rest = sample taken prior to stimulation
Exercise = sample taken at 15 minutes of stimulation (soleus: 0.5, 1.0, or 2.0 Hz; gracilis: 0.2 or 0.4 Hz)
* significantly different from soleus ($p<0.05$)

Figure 6. Ammonia release as a function of final muscle performance. Performance was varied by changing the frequency of stimulation (soleus: 0.5, 1.0, or 2.0 Hz; gracilis: 0.2 or 0.4 Hz). Each point represents one animal. Resting levels were plotted at zero performance. The solid line represents the regression relationship in the soleus muscle ($r = 0.67$; $p<0.05$). The dashed line represents the regression relationship in the gracilis muscle ($r = 0.95$; $p<0.05$).


6
4
2
0
– 2
100
200
300
● GRACILIS
□ SOLEUS
AMMONIA RELEASE
(μmoles · min⁻¹ · 100 g⁻¹)
MUSCLE PERFORMANCE
(force × frequency)

Release of Lactate During Rest and Exercise

As shown in the right column of Table IV, both the soleus and gracilis muscles released lactate at rest, although lactate release was significantly higher in the soleus than in the gracilis muscle. Both muscles also released lactate into the venous blood during exercise. As shown in Figure 7, lactate release in the soleus correlated closely with final muscle performance ($r=0.86$; $p<0.05$). In the gracilis muscle, large amounts of lactate were produced even at low levels of muscle performance. The release of lactate was also correlated with muscle performance in this muscle ($r=0.89$; $p<0.05$), although a greater change in lactate release occurred for a given change in performance than in the soleus muscle.

ADENOSINE DEAMINASE STUDY

Tissue Adenosine Deaminase Activity

The activity of adenosine deaminase in soleus and gracilis muscles was measured in all animals in the adenosine deaminase study. Due to the randomized design of the series in which microspheres were used, six animals received ADA as the last infusate. The stimulated soleus (n=6) and gracilis (n=6) muscles obtained from these animals represent the tissue ADA activity after 20 minutes infusion. Six other animals of the series received BADA as the final infusate. The exercising soleus (n=6) and gracilis (n=6) muscles obtained from these animals were assayed for ADA activity during BADA infusion. Tissue ADA was also measured in unstimulated, uninfused cat soleus (n=6) and gracilis (n=7) muscle. These data are shown in Figure 8.

Figure 7. Lactate release as a function of final muscle performance. Performance was varied by changing the frequency of stimulation (soleus: 0.5, 1.0, or 2.0 Hz; gracilis: 0.2 or 0.4 Hz). Each point represents one animal. Resting levels were plotted at zero performance. The solid line represents the regression relationship in the soleus muscle ($r= 0.86$; $p<0.05$). The dashed line represents the regression relationship in the gracilis muscle ($r=0.89$; $p<0.05$).

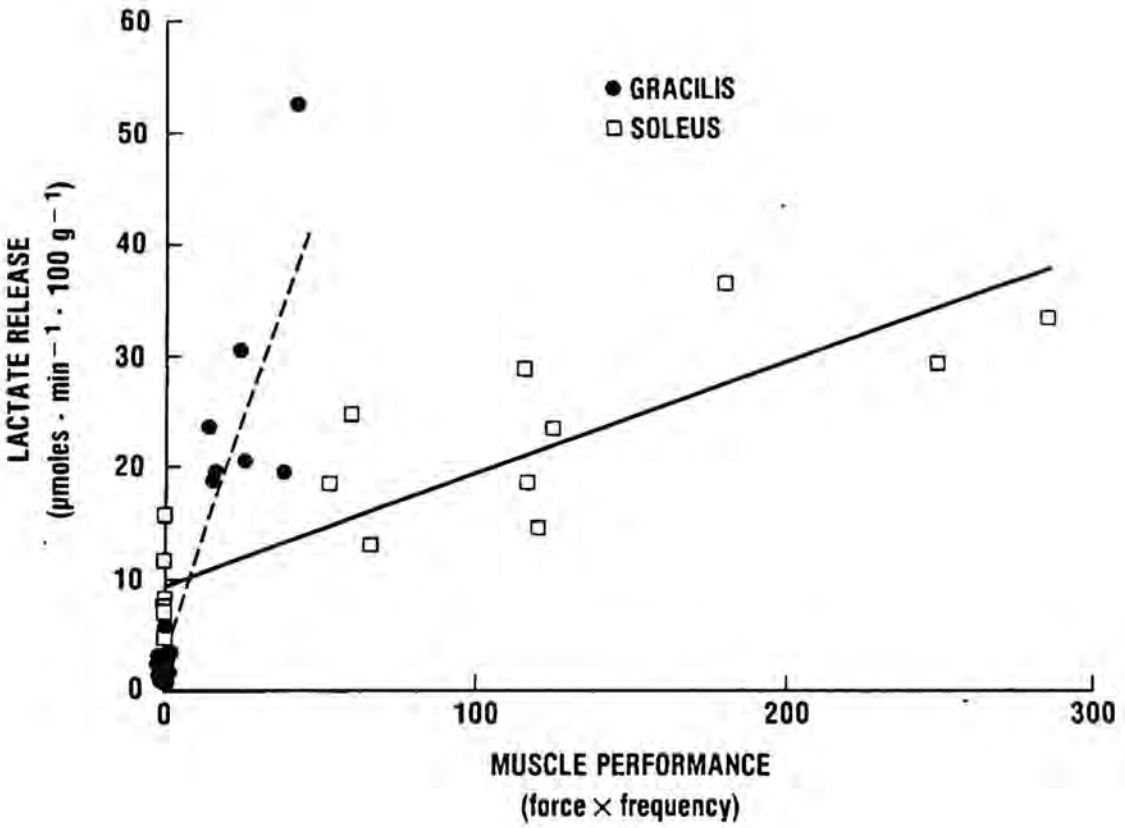
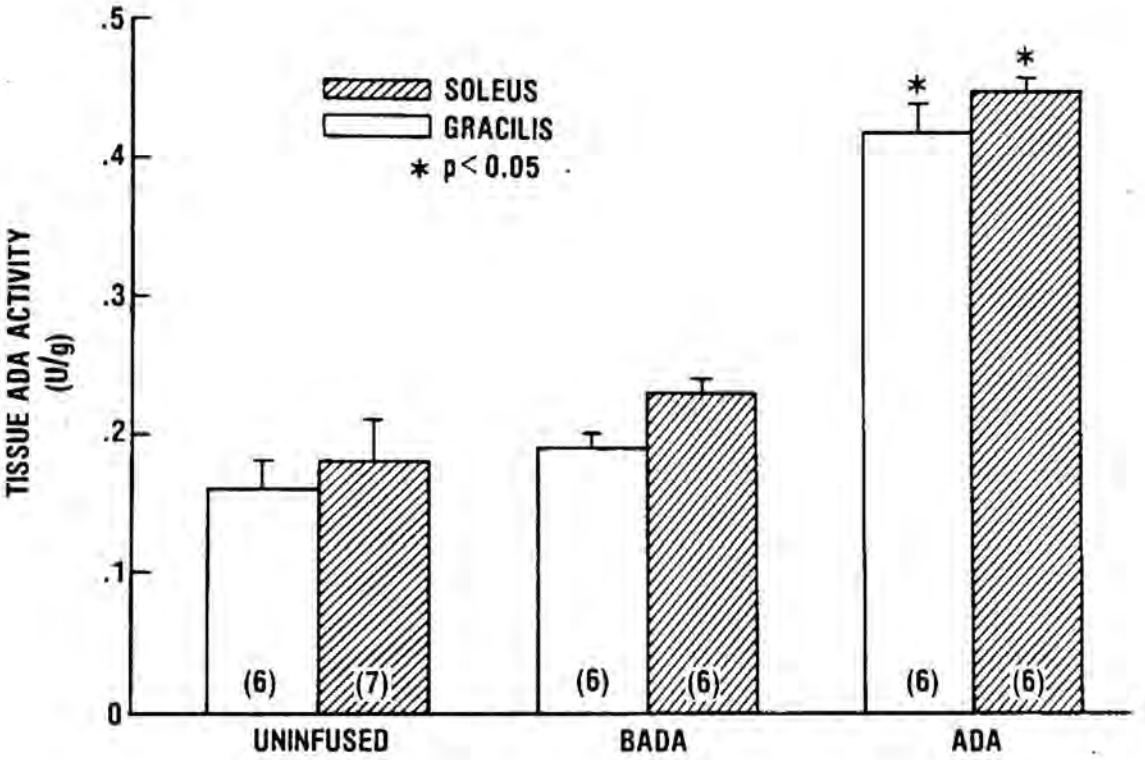

60
50
40
30
20
10
0
0
100
200
300
● GRACILIS
□ SOLEUS
LACTATE RELEASE
(μmoles · min⁻¹ · 100 g⁻¹)
MUSCLE PERFORMANCE
(force × frequency)

Figure 8. Activity of adenosine deaminase in skeletal muscle. Values are mean ± S.E.M. ( ) = number of muscles assayed for ADA activity. Uninfused = control muscles that had never been infused with boiled ADA at the time of tissue clamping. BADA = muscles which had been infused with boiled ADA at the time of tissue clamping. ADA = muscles which had been infused with ADA at the time of tissue clamping. * = significantly different from other groups for that muscle, $p<0.05$.


TISSUE ADA ACTIVITY (U/g)
.5
.4
.3
.2
.1
0
SOLEUS
GRACILIS
* p< 0.05
(6)
(7)
(6)
(6)
(6)
(6)
*
*
UNINFUSED
BADA
ADA

Mean ADA activity in stimulated muscles which had received ADA as the final infusate was twice as high as the concentration found in uninfused tissue and in tissue receiving BADA as the second infusate. The activity in muscles which had received BADA as the final infusate was not significantly different from that of uninfused tissue. ADA activity in soleus muscles obtained during ADA infusion ranged from 0.42 to 0.50 U/g tissue. Activity of the enzyme in ADA-infused gracilis preparations ranged from 0.38 to 0.48 U/g tissue. In experiments measuring venous outflow from the soleus muscle, all muscles received ADA as the second infusate. Enzyme activity in this series was not different from the ADA-infused soleus muscles of the microsphere series.

Microsphere Series

Blood Data and Hemodynamics. Arterial blood gas and hematocrit values of animals in the microsphere series are presented in Table V. Values did not change significantly over the course of the experiment. Resting and exercising parameters were not different between ADA and inactivated ADA infusions.

Arterial blood pressure, cardiac output, and total peripheral resistance were stable throughout the experimental period in both the high and low stimulation experiments. Mean values for these parameters are presented in Table VI. There were no significant differences in these indices among the treatments in groups subjected to either the high or low stimulation levels.

During infusion of BADA, aortic blood flow averaged 47 ± 8 $ml \cdot min^{-1}$ at rest. During low levels of muscle stimulation, aortic blood flow was 67 ± 17 $ml \cdot min^{-1}$. When the muscles were stimulated to contract at higher frequencies, aortic flow increased to 82 ± 12 $ml \cdot min^{-1}$. Aortic blood

TABLE V

BLOOD DATA DURING REST AND EXERCISE:
ADA MICROSPHERE SERIES

SAMPLE	Arterial pH	Arterial PCO_2 (mmHg)	Arterial PO_2 (mmHg)	Arterial Hct (%)
I. Low Stimulation (n=6)				
BADA-r	7.37 ± 0.02	29 ± 2	106 ± 4	40 ± 2
ADA-r	7.38 ± 0.01	30 ± 2	108 ± 4	40 ± 3
BADA-e	7.35 ± 0.02	32 ± 3	104 ± 3	40 ± 2
ADA-e	7.36 ± 0.02	31 ± 2	106 ± 4	39 ± 3
II. High Stimulation (n=6)				
BADA-r	7.39 ± 0.01	33 ± 3	104 ± 4	37 ± 3
ADA-r	7.41 ± 0.01	29 ± 3	109 ± 4	36 ± 2
BADA-e	7.34 ± 0.01	36 ± 4	92 ± 9	36 ± 3
ADA-e	7.37 ± 0.02	31 ± 4	106 ± 7	36 ± 3

Values are mean ± S.E.M.
Hct = hematocrit
Low stimulation = animals receiving 0.2 Hz (gracilis) and 0.5 Hz (soleus) stimulation.
High stimulation = animals receiving 0.4 Hz (gracilis) and 2.0 Hz (soleus) stimulation.
BADA = boiled adenosine deaminase
ADA = adenosine deaminase
r = rest
e = exercise

TABLE VI

ARTERIAL PRESSURE, CARDIAC OUTPUT, AND TOTAL PERIPHERAL RESISTANCE: ADA MICROSPHERE SERIES

SAMPLE	Arterial Blood Pressure (mm Hg)	Cardiac Output ($ml \cdot min^{-1}$)	Total Peripheral Resistance ($mmHg \cdot min \cdot 100g \cdot ml^{-1}$)
I. Low Stimulation (n=6)			
BADA-r	118 ± 11	243 ± 35	515 ± 60
ADA-r	130 ± 9	237 ± 29	598 ± 97
BADA-e	127 ± 11	263 ± 43	524 ± 57
ADA-e	118 ± 8	283 ± 32	446 ± 57
II. High Stimulation (n=6)			
BADA-r	112 ± 6	250 ± 34	490 ± 62
ADA-r	108 ± 5	266 ± 40	438 ± 52
BADA-e	112 ± 9	302 ± 33	396 ± 46
ADA-e	118 ± 6	264 ± 35	477 ± 52

Values above are mean ± S.E.M.
Low stimulation = animals receiving 0.2 Hz (gracilis) and 0.5 Hz (soleus) stimulation.
High stimulation = animals receiving 0.4 Hz (gracilis) and 2.0 Hz (soleus) stimulation.
BADA = boiled adenosine deaminase
ADA = adenosine deaminase
r = rest
e = exercise

flows measured during ADA infusion were not statistically different from those observed during infusion of BADA.

Effect of adenosine deaminase on the vascular response and muscle performance of the soleus muscle. Table VII summarizes the effect of adenosine deaminase on the blood flow, vascular resistance, and muscle performance of the stimulated muscles in experiments using microspheres. Blood flow to the resting soleus muscle ranged from 1.1 to 6.6 $ml \cdot min^{-1} \cdot 100g^{-1}$ during infusion of BADA and was not different during ADA infusion. Resting vascular resistance was not different between BADA and ADA infusions in groups receiving either high or low stimulation. Blood flow to the contralateral, unstimulated soleus muscles (n=12) was unaffected by infusion of ADA. Blood flow to these muscles was 2.5 ± 0.4 $ml \cdot min^{-1} \cdot 100g^{-1}$ at 10 minutes and 3.0 ± 0.5 $ml \cdot min^{-1} \cdot 100g^{-1}$ at 20 minutes of BADA infusion. Blood flow during 10 minutes of ADA was 2.8 ± 0.4 $ml \cdot min^{-1} \cdot 100g^{-1}$ and did not change significantly after 20 minutes of infusion, averaging 3.0 ± 0.4 $ml \cdot min^{-1} \cdot 100g^{-1}$. These values were not different from those obtained from the experimental muscles (n=12) at rest.

During the low level of stimulation (0.5 Hz), blood flow to the soleus muscle increased five-fold and was not altered by the presence of ADA (Figure 9). Vascular resistance decreased with exercise to 10.0 ± 0.5 $mmHg \cdot min \cdot 100g \cdot ml^{-1}$ during BADA infusion. This low level of stimulation resulted in a similar decrease in resistance in the presence of ADA. Initial muscle performance was determined at 30 seconds of stimulation. Final muscle performance was measured at 10 minutes of stimulation, during blood flow measurement. Initial muscle performance in the soleus at 0.5 Hz stimulation averaged 76 ± 11 $g\ force \cdot g\ tissue^{-1} \cdot sec^{-1}$ during BADA infusion and did not change significantly after 10 minutes.

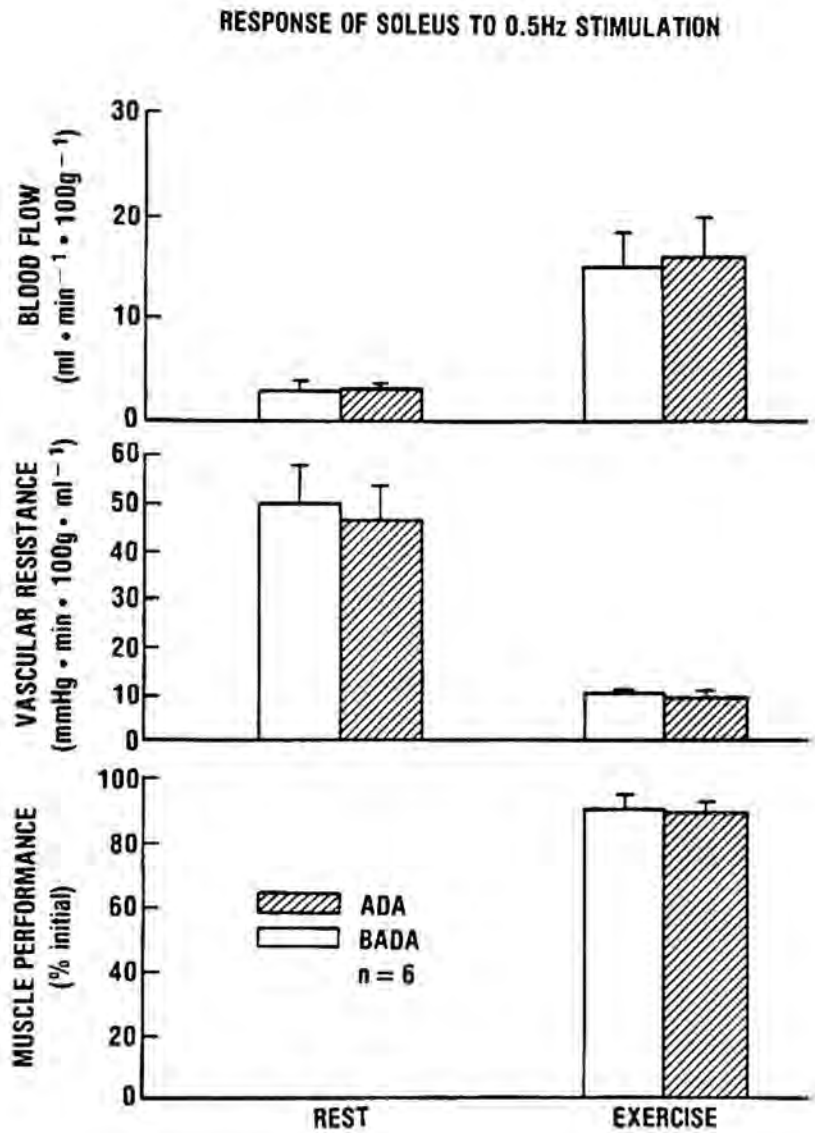
TABLE VII

BLOOD FLOW, VASCULAR RESISTANCE, AND PERFORMANCE OF STIMULATED MUSCLES: ADA MICROSPHERE SERIES

	SAMPLE	Q	R	$FXf_{(i)}$	$FXf_{(f)}$
I. Low Stimulation (n=6)					
SOLEUS:	BADA-r	2.9±0.8	50.1±8.1		
	ADA-r	3.2±0.5	46.5±7.3		
	BADA-e	15.1±3.3	10.0±0.5	76±11	69±11
	ADA-e	16.0±3.8	8.9±1.4	72±5	64±13
GRACILIS:	BADA-r	2.3±0.4	55.3±5.9		
	ADA-r	3.0±0.6	53.8±11.3		
	BADA-e	15.3±4.5	10.6±2.1	26±3	22±2
	ADA-e	15.8±3.3	8.7±1.5	26±3	22±2
II. High Stimulation (n=6)					
SOLEUS:	BADA-r	3.0±0.4	43.4±7.4		
	ADA-r	3.3±0.4	37.0±5.2		
	BADA-e	45.3±3.0	2.4±0.3	346±38	278±26
	ADA-e	29.4±5.2*	5.8±0.4*	336±41	198±28*
GRACILIS:	BADA-r	3.8±0.6	31.2±4.0		
	ADA-r	3.6±0.4	32.7±3.7		
	BADA-e	30.4±6.4	4.5±0.9	44±7	30±6
	ADA-e	26.3±2.2	4.7±0.6	43±8	27±4

Values are mean ± S.E.M.
Low stimulation = animals receiving 0.2 Hz (gracilis) and 0.5 Hz (soleus) stimulation.
High stimulation = animals receiving 0.4 Hz (gracilis) and 2.0 Hz (soleus) stimulation.
Q = muscle blood flow ($ml \cdot min^{-1} \cdot 100g^{-1}$)
R = muscle vascular resistance ($mmHg \cdot min \cdot 100g \cdot ml^{-1}$)
Fxf = initial or final muscle performance ($g \cdot g^{-1} \cdot sec^{-1}$)
BADA = boiled adenosine deaminase
ADA = adenosine deaminase
r = rest
e = exercise

Figure 9. Response of the soleus muscle to 0.5 Hz stimulation in the ADA microsphere series. Values are mean ± S.E.M. BADA = blood flow, vascular resistance, and percent of initial muscle performance during infusion of boiled adenosine deaminase. ADA = values during active ADA infusion. REST = 10 minutes of infusion. EXERCISE = 20 minutes of infusion with 10 minutes of stimulation.


RESPONSE OF SOLEUS TO 0.5Hz STIMULATION
BLOOD FLOW
(ml • min⁻¹ • 100g⁻¹)
30
20
10
0
VASCULAR RESISTANCE
(mmHg • min • 100g • ml⁻¹)
60
50
40
30
20
10
0
MUSCLE PERFORMANCE
(% initial)
100
80
60
40
20
0
ADA
BADA
n = 6
REST
EXERCISE

The initial level of muscle performance during ADA was similar, and the muscle showed no evidence of fatigue after 10 minutes of stimulation.

During the high level of stimulation (2 Hz), soleus muscle blood flow increased to 45.3 ± 3.0 $ml \cdot min^{-1} \cdot 100g^{-1}$ during BADA infusion. However, in the presence of ADA, this increase in blood flow was attenuated by 38% (Figure 10). Mean vascular resistance decreased 41 $mmHg \cdot min \cdot 100g \cdot ml^{-1}$ during BADA infusion, but only 32 $mmHg \cdot min \cdot 100g \cdot ml^{-1}$ during infusion of ADA. In addition, although essentially no muscle fatigue was observed in the soleus at 2.0 Hz stimulation during BADA infusion, the muscle fatigued to 60% of its initial level of muscle performance in the presence of ADA. This initial performance level, however, was unaltered by infusion of ADA.

Effect of adenosine deaminase on tissue metabolite and adenosine content of the soleus muscle. Creatine phosphate, creatine, inorganic phosphate, ATP, and lactate were measured in two soleus muscles stimulated to contract at 0.5 Hz and three muscles stimulated to contract at 2.0 Hz during BADA infusion. These metabolites were also measured in two soleus muscles contracting at 0.5 Hz and three contracting at 2.0 Hz in the presence of ADA. Metabolite content was assayed in six contralateral resting muscles during BADA and five during ADA infusion. These data are shown in Table VIII.

There was no significant difference in any of the measured metabolites in the resting muscle between BADA and ADA infusions. Due to the small number of muscles assayed for each stimulation frequency, there was a large amount of variation in the data. However, in each case, creatine phosphate concentrations fell and creatine and inorganic phosphate increased in the stimulated soleus muscles compared to values

Figure 10. Response of the soleus muscle to 2.0 Hz stimulation in the ADA microsphere series. Values are mean ± S.E.M. BADA = blood flow, vascular resistance, and percent of initial muscle performance during infusion of boiled adenosine deaminase. ADA = values during active ADA infusion. REST = 10 minutes of infusion. EXERCISE = 20 minutes of infusion with 10 minutes of stimulation.

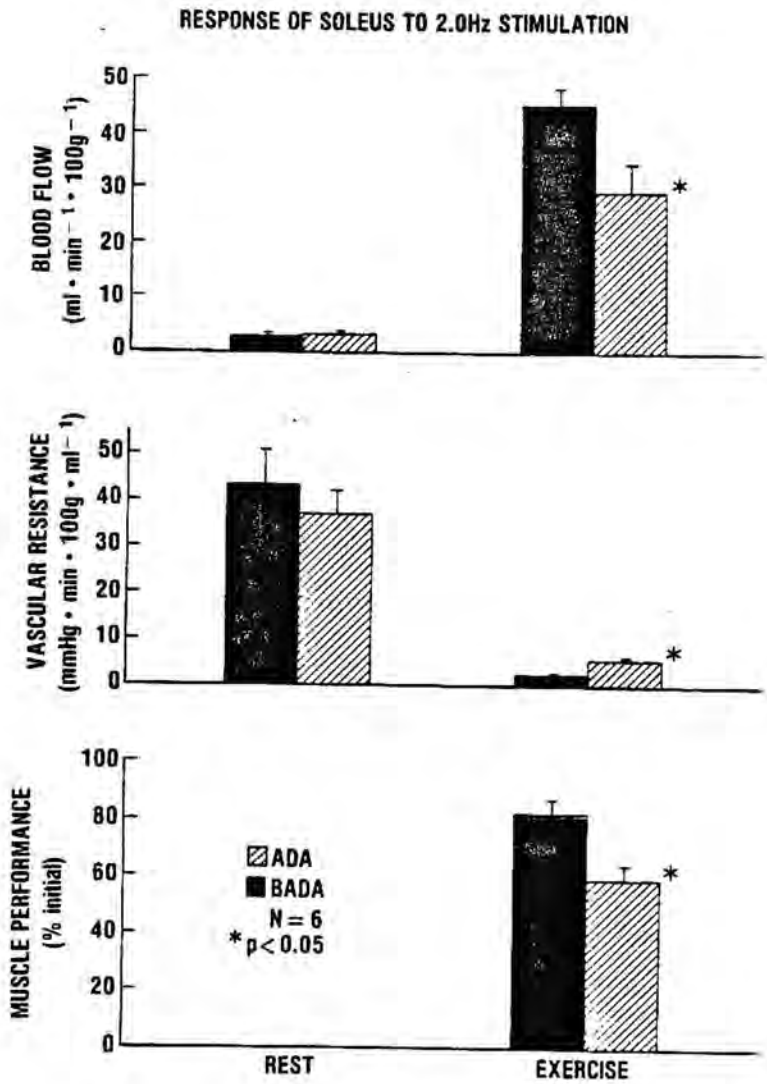

RESPONSE OF SOLEUS TO 2.0Hz STIMULATION
BLOOD FLOW
(ml • min⁻¹ • 100g⁻¹)
50
40
30
20
10
0
*
VASCULAR RESISTANCE
(mmHg • min • 100g • ml⁻¹)
50
40
30
20
10
0
*
MUSCLE PERFORMANCE
(% initial)
100
80
60
40
20
0
ADA
BADA
N = 6
* p < 0.05
*
REST
EXERCISE

TABLE VIII

TISSUE METABOLITE CONCENTRATIONS IN THE SOLEUS MUSCLE:
ADA MICROSPHERE SERIES

SAMPLE	Creatine Phosphate ($\mu moles \cdot g^{-1}$)	Creatine ($\mu moles \cdot g^{-1}$)	Inorganic Phosphate ($\mu moles \cdot g^{-1}$)	ATP ($\mu moles \cdot g^{-1}$)	Lactate ($\mu moles \cdot g^{-1}$)
BADA					
Rest (n=6)	21.7 ±8.9	10.0±1.9	9.2±2.5	6.4±1.4	3.4±0.3
Exercise 0.5 Hz (n=2)	18.6 ±4.2	25.0±0.2	10.8±6.7	6.7±1.5	3.4±0.1
Exercise 2.0 Hz (n=3)	16.0 ±5.8	27.3±2.4	13.5±3.4	6.3±2.0	5.4±0.4
ADA					
Rest (n=5)	23.6 ±6.5	11.2±3.3	7.5±1.0	6.6±1.9	3.3±0.4
Exercise 0.5 Hz (n=2)	19.9 ±3.0	27.7±5.4	9.5±4.2	6.8±1.3	3.8±0.3
Exercise 2.0 Hz (n=3)	9.1 ±3.4	32.9±4.8	16.2±1.9	5.8±1.0	11.5±1.9

Values are mean ± S.E.M.

obtained from the contralateral resting muscles. There were no other obvious tendencies in metabolite concentrations at the low level of stimulation, and similar values were obtained during both BADA and ADA infusion. Lactate increased during both ADA and BADA infusions with 2.0 Hz stimulation. However, the change in lactate concentration with this level of stimulation appeared to be greater in the presence of ADA. In addition, although creatine phosphate levels again tended to fall, along with an increase in creatine and inorganic phosphate concentrations, these changes appeared to be greater in the presence of ADA.

Tissue adenosine content was also measured in each of these muscles. Adenosine content in the contralateral soleus muscles during infusion of BADA was 1.64 ± 0.45 nmoles$\cdot g^{-1}$. During ADA infusion, adenosine content was 1.67 ± 0.48 nmoles$\cdot g^{-1}$. Adenosine content in the muscles stimulated at 0.5 Hz was 2.67 ± 0.76 nmoles$\cdot g^{-1}$ when BADA was infused, and 1.93 ± 1.02 nmoles$\cdot g^{-1}$ in the presence of ADA. Adenosine content in the soleus muscles stimulated to contract at 2.0 Hz during BADA infusion was 4.50 ± 0.19 nmoles$\cdot g^{-1}$ and 18.32 ± 8.6 nmoles$\cdot g^{-1}$ in the presence of ADA.

Effect of adenosine deaminase on the vascular response and muscle performance of the gracilis muscle. As summarized in Table VII, ADA had no effect on blood flow, vascular resistance, or muscle performance in the gracilis muscle. Blood flow to the resting gracilis muscle ranged from 2.0 to 5.9 ml$\cdot min^{-1}\cdot 100g^{-1}$ during infusion of BADA and was not different during ADA infusion. Vascular resistance at rest and exercise was not different between BADA and ADA infusions. Blood flow to the contralateral, quiescent gracilis muscle was 2.5 ± 0.3 ml$\cdot min^{-1}\cdot 100g^{-1}$ at 10 minutes and 2.7 ± 0.3 ml$\cdot min^{-1}\cdot 100g^{-1}$ at 20 minutes of BADA

infusion. Blood flow after 10 minutes of infusion of ADA was 2.6 ± 0.5 $ml \cdot min^{-1} \cdot 100g^{-1}$ and 3.0 ± 0.3 $ml \cdot min^{-1} \cdot 100g^{-1}$ after 20 minutes of ADA infusion. These values are similar to the resting values obtained from the experimental muscles at rest.

During the low level of stimulation (0.2 Hz), blood flow to the gracilis muscle increased six-fold and was not altered by the presence of ADA (Figure 11). Vascular resistance decreased to 10.6 ± 2.1 $mmHg \cdot min \cdot 100g \cdot ml^{-1}$ in response to 0.2 Hz stimulation during BADA infusion. Muscle vascular resistance decreased similarly in the presence of ADA. The gracilis achieved an initial level of muscle performance by 30 seconds of stimulation which averaged 26 ± 3 $g \cdot g \cdot sec^{-1}$ during BADA infusion. The gracilis muscle was able to maintain 88% of its initial level of performance throughout the 10-minute stimulation period. The initial level of muscle performance during ADA was similar, and the muscle showed no evidence of fatigue after 10 minutes of stimulation.

At the high level of stimulation (0.4 Hz), gracilis blood flow during BADA infusion was 30.4 ± 6.4 $ml \cdot min \cdot 100g^{-1}$ after 10 minutes. Exercising blood flow during ADA was similar (Figure 12). Vascular resistance decreased 27 $mmHg \cdot min \cdot 100g \cdot ml^{-1}$ with exercise during BADA infusion. Vascular resistance decreased similarly in the presence of ADA. During BADA infusion, the gracilis muscle achieved a mean initial performance level of 44 $g \cdot g \cdot sec^{-1}$. By 10 minutes of infusion, the muscle had fatigued to 68% of this initial value. These initial and final performance levels were unaffected by the presence of ADA.

Effect of adenosine deaminase on tissue metabolite and adenosine content of the gracilis muscle. Creatine phosphate, creatine, inorganic phosphate, ATP, and lactate were measured in three gracilis muscles

Figure 11. Response of the gracilis muscle to 0.2 Hz stimulation in the ADA microsphere series. Values are mean ± S.E.M. BADA = blood flow, vascular resistance, and percent of initial muscle performance during infusion of boiled adenosine deaminase. ADA = values during active ADA infusion. REST = 10 minutes of infusion. EXERCISE = 20 minutes of infusion with 10 minutes of stimulation.

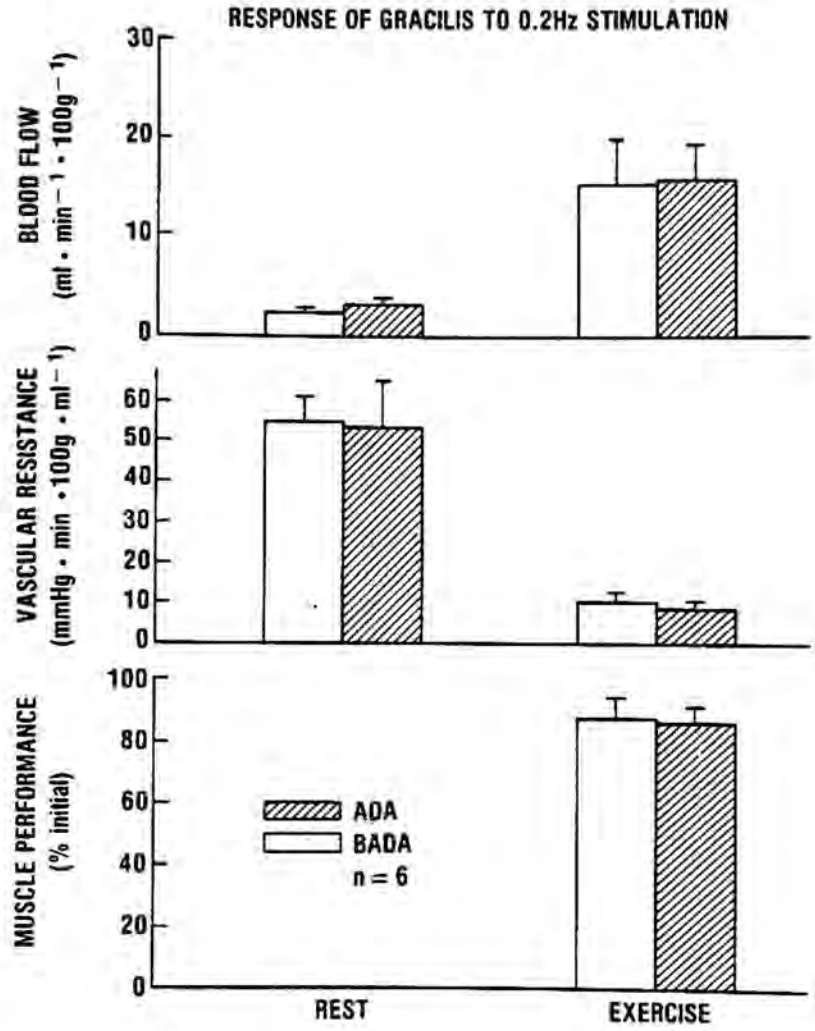
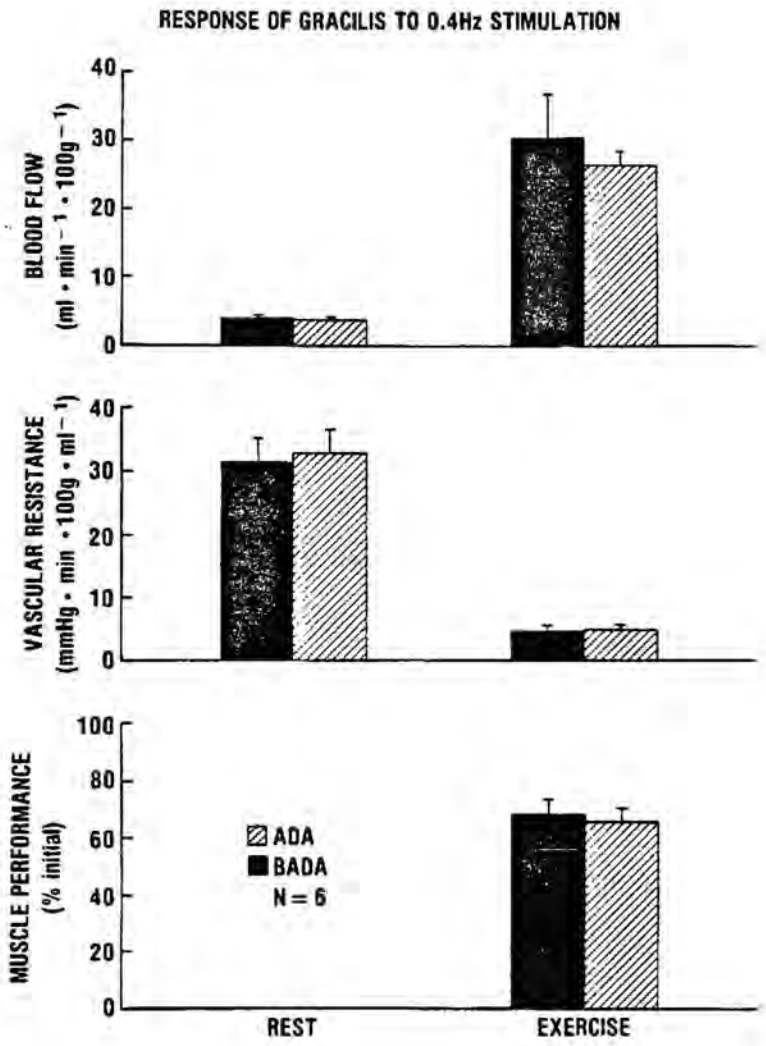

RESPONSE OF GRACILIS TO 0.2Hz STIMULATION
BLOOD FLOW
(ml • min⁻¹ • 100g⁻¹)
30
20
10
0
VASCULAR RESISTANCE
(mmHg • min • 100g • ml⁻¹)
60
50
40
30
20
10
0
MUSCLE PERFORMANCE
(% initial)
100
80
60
40
20
0
ADA
BADA
n = 6
REST
EXERCISE

Figure 12. Response of the gracilis muscle to 0.4 Hz stimulation in the ADA microsphere series. Values are mean ± S.E.M. BADA = blood flow, vascular resistance, and percent of initial muscle performance during infusion of boiled adenosine deaminase. ADA = values during active ADA infusion. REST = 10 minutes of infusion. EXERCISE = 20 minutes of infusion with 10 minutes of stimulation.


RESPONSE OF GRACILIS TO 0.4Hz STIMULATION
BLOOD FLOW
(ml • min⁻¹ • 100g⁻¹)
40
30
20
10
0
VASCULAR RESISTANCE
(mmHg • min • 100g • ml⁻¹)
40
30
20
10
0
MUSCLE PERFORMANCE
(% initial)
100
80
60
40
20
0
ADA
BADA
N = 6
REST
EXERCISE

stimulated to contract at 0.2 Hz and three muscles stimulated to contract at 0.4 Hz during BADA infusion. These metabolites were also measured in two muscles contracting at 0.2 Hz and three gracilis muscles contracting at 0.4 Hz in the presence of ADA. Metabolite content was assayed in six contralateral control muscles during BADA and five during ADA infusion. These data are shown in Table IX.

There were no significant differences in any of the measured metabolite concentrations between BADA and ADA infusions in the gracilis muscle at rest. Creatine phosphate levels fell and creatine and inorganic phosphate to increased in each stimulated muscle as compared to the contralateral resting muscle at both stimulation frequencies. Lactate was produced by the muscle even at 0.2 Hz stimulation. Although there were few muscles in each stimulation group, ADA infusion did not appear to affect the metabolic changes with exercise that occurred during infusion of BADA.

Tissue adenosine content was 2.00 ± 0.46 nmoles·g^{-1} in the six contralateral control muscles during BADA infusion. Adenosine content in the control gracilis muscles during ADA infusion was 1.50 ± 0.38 nmoles·g^{-1}. Adenosine content in muscles stimulated at 0.2 Hz was 2.42 ± 0.89 nmoles·g^{-1} when BADA was infused, and 1.16 ± 0.25 nmoles·g^{-1} when ADA was infused. Adenosine content in the gracilis muscles stimulated to contract at 0.4 Hz during BADA infusion was 1.74 ± 0.25 nmoles·g^{-1}, and 2.68 ± 0.80 nmoles·g^{-1} during infusion of ADA.

Venous Outflow Series

Blood data. Table X summarizes blood gas and pH values obtained during this series. Arterial pH, PCO_2, and PO_2 did not change significantly over the course of the experiment. However, there was a signif-

TABLE IX

TISSUE METABOLITE CONCENTRATIONS IN THE GRACILIS MUSCLE:
ADA MICROSPHERE SERIES

SAMPLE	Creatine Phosphate ($\mu moles \cdot g^{-1}$)	Creatine ($\mu moles \cdot g^{-1}$)	Inorganic Phosphate ($\mu moles \cdot g^{-1}$)	ATP ($\mu moles \cdot g^{-1}$)	Lactate ($\mu moles \cdot g^{-1}$
BADA					
Rest (n=6)	30.9 ±6.9	18.6 ±4.7	11.6 ±2.9	7.6 ±1.8	5.1 ±0.4
Exercise 0.2 Hz (n=3)	14.1 ±3.2	20.2 ±6.4	20.7 ±5.1	6.6 ±2.2	9.9 ±2.2
Exercise 0.4 Hz (n=3)	10.0 ±1.2	27.3 ±0.4	23.7 ±4.7	6.6 ±1.4	16.0 ±2.6
ADA					
Rest (n=5)	33.0 ±11.4	18.4 ±2.8	13.7 ±4.6	8.1 ±2.3	5.2 ±0.6
Exercise 0.2 Hz (n=2)	16.8 ±1.9	20.1 ±3.8	21.2 ±6.7	7.9 ±1.0	9.1 ±3.3
Exercise 0.4 Hz (n=3)	11.8 ±5.4	26.0 ±9.1	26.9 ±3.3	7.6 ±2.6	16.7 ±4.1

Values are mean ± S.E.M.

TABLE X

BLOOD DATA DURING REST AND EXERCISE:
ADA VENOUS OUTFLOW SERIES
(n=5)

SAMPLE	pH		PCO_2 (mmHg)		PO_2 (mmHg)		Hct (%)		O_2Ct (vol %)		$(A-V)O_2$ (vol %)
	Art	Ven	Art	Ven	Art	Ven	Art	Ven	Art	Ven	
BADA-r	7.42 ±0.01	7.33 ±0.02	31 ±2	45 ±2	101 ±2	35 ±5	37 ±2	38 ±2	18.4 ±1.0	10.5 ±1.5	7.9 ±2.1
BADA-e	7.38 ±0.03	7.17† ±0.05	36 ±5	71† ±5	101 ±9	15† ±1	34 ±2	35 ±2	16.6 ±0.8	1.8† ±0.3	14.7† ±0.6
PRE-ADA-r	7.44 ±0.01	7.28 ±0.06	29 ±4	50 ±5	103 ±4	25 ±4	31* ±3	34 ±3	15.1* ±0.7	7.0 ±1.6	8.3 ±1.2
ADA-r	7.44 ±0.02	7.29 ±0.04	29 ±3	46 ±4	106 ±5	27 ±5	31* ±3	33 ±3	15.0* ±0.06	7.3 ±1.6	7.7 ±0.6
ADA-e	7.44 ±0.01	7.18† ±0.07	29 ±2	60† ±7	111 ±3	16† ±3	29* ±2	31 ±2	15.0* ±0.05	1.5† ±0.3	13.5† ±0.6

Values are mean ± S.E.M
* = significantly different from BADA; $p<0.05$
† = significantly different from rest; $p<0.05$
O_2Ct = oxygen content
Hct = hematocrit
$(A-V)O_2$ = arterial-venous oxygen difference

BADA = boiled adenosine deaminase
PRE-ADA = following re-equilibration
ADA = adenosine deaminase
r = rest (10 min infusion)
e = 10 min 2.0 Hz exercise (20 min infusion)

icant decrease in arterial oxygen content in this series due to a decrease in hematocrit. This could be due to the extensive surgery involved in this series, combined with the length of the experimental protocol. Although hematocrit and arterial oxygen content were significantly different from the initial measurement during BADA infusion by the end of the re-equilibration period, oxygen extraction was not changed. This is reflected by a decrease in venous oxygen content compared to resting levels during BADA infusion. The venous oxygen reserve was sufficient to maintain the same exercising level of oxygen extraction during the second infusion. Arterial oxygen content during exercise was not different between the two infusions.

Venous PCO_2 increased, and pH, PO_2, and oxygen content decreased with exercise compared to resting levels during both infusions. All blood gas and hematocrit values were not different between the measurement immediately prior to infusion of ADA and after 10 minutes of infusion.

Resting aortic blood flow averaged 52 ± 9 $ml \cdot min^{-1}$ during infusion of BADA, and 60 ± 20 $ml \cdot min^{-1}$ during infusion of active deaminase. During muscle stimulation, aortic blood flow averaged 83 ± 18 $ml \cdot min^{-1}$ in the presence of BADA, and 58 ± 16 $ml \cdot min^{-1}$ during ADA infusion.

Effect of adenosine deaminase on vascular response and muscle performance. Table XI summarizes the vascular response to exercise in this series of experiments. Blood pressure did not change significantly over the experimental period. As in the microsphere series, blood flow increased to the soleus in response to stimulation during both infusions, indicating that the muscle was still capable of some degree of active hyperemia in the presence of ADA. However, the changes in blood flow and vascular resistance were attenuated with ADA. Muscle performance

TABLE XI

ARTERIAL PRESSURE, BLOOD FLOW, VASCULAR RESISTANCE, AND PERFORMANCE OF STIMULATED MUSCLES: ADA VENOUS OUTFLOW SERIES

SAMPLE	Pa	Q	R	$FXf_{(i)}$	$FXf_{(f)}$
BADA-r	108 ±1	9.0±1.9	14.4±3.5		
BADA-e	96 ±12	44.9±10.7†	2.5±0.4†	186 ±20	147 ±24
PRE-ADA-r	91 ±5	8.0±1.6	13.6±3.2		
ADA-r	86 ±8	7.3±1.4	13.7±3.4		
ADA-e	88 ±7	14.8±3.2*†	6.6±0.8*†	175±34	55±25*

Values are mean ± S.E.M.
† significantly different from rest
* significantly different from BADA
Pa = arterial blood pressure (mmHg)
Q = muscle blood flow ($ml \cdot min^{-1} \cdot 100g^{-1}$)
R = vascular resistance ($mmHg \cdot min \cdot 100g \cdot ml^{-1}$)
Fxf = initial or final muscle performance ($g \cdot g^{-1} \cdot sec^{-1}$)

BADA = boiled adenosine deaminase
ADA = adenosine deaminase
r = rest (10 min infusion)
e = 10 min 2.0 Hz exercise (20 min infusion)

was also affected by adenosine deaminase. A significant amount of muscle fatigue had occured after 10 minutes of stimulation in the presence of ADA. During infusion of BADA, the soleus was able to maintain the same initial level of performance throughout the stimulation period.

In this series of experiments, continuous measurement of blood flow with the drop counter method allowed analysis of transient changes in muscle blood flow, vascular resistance, and performance. This is illustrated in Figure 13. There were no significant differences in the vascular response to infusions of ADA and BADA in the first 10 minutes. In addition, resting muscle blood flow and vascular resistance were unaltered by either infusion. During exercise, in the presence of ADA, blood flow to the muscle never reached the level it did with BADA. Differences in the hyperemic response between BADA and ADA infusions occurred within the first minute of stimulation.

Effect of adenosine deaminase on oxygen consumption. Muscle oxygen consumption at rest and exercise during infusion of either BADA or ADA is illustrated in Figure 14. Also shown is the rate of oxygen consumed by the muscle after the one-hour re-equilibration period. Oxygen consumption by the muscle after this period was not different from the resting level during BADA infusion, indicating that the muscle had recovered from the first bout of exercise. Oxygen consumption after 10 minutes ADA infusion was not different from the level immediately prior to the start of infusion, indicating that adenosine deaminase had no direct effect on resting oxygen consumption in the soleus muscle. Oxygen consumption during exercise was attentuated with adenosine deaminase. Because oxygen extraction was not different

Figure 13. Response of the soleus muscle to ADA or BADA infusion over the experimental period in the ADA venous outflow series. Each point represents the mean response of 5 animals. No difference in blood flow, vascular resistance, or muscle performance exists between BADA and ADA treatments at rest. Blood flow, vascular resistance, and muscle performance in response to exercise differ between the two treatments starting from 1 min of stimulation ($p<0.05$).

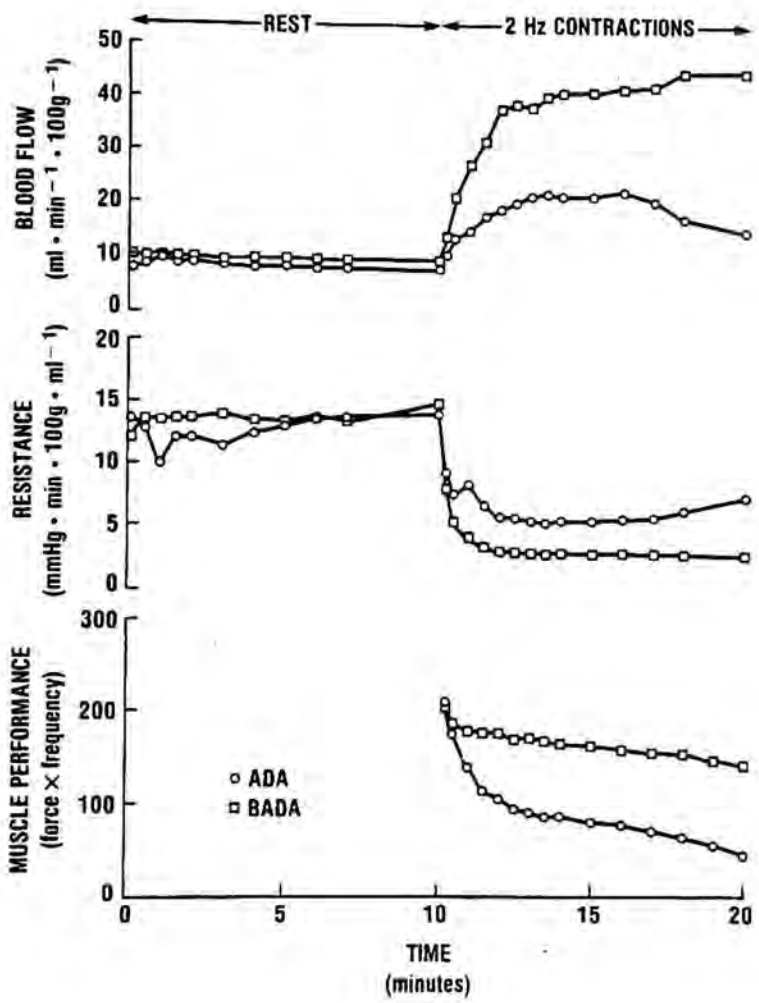
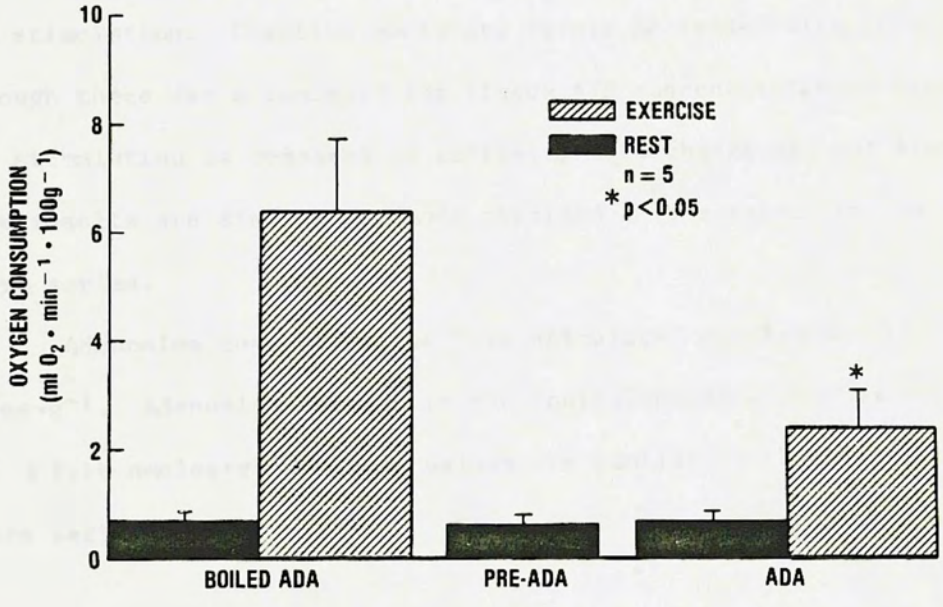

REST
2 Hz CONTRACTIONS
BLOOD FLOW
(ml • min⁻¹ • 100g⁻¹)
50
40
30
20
10
0
RESISTANCE
(mmHg • min • 100g • ml⁻¹)
20
15
10
5
0
MUSCLE PERFORMANCE
(force × frequency)
300
200
100
0
o ADA
□ BADA
0
5
10
15
20
TIME
(minutes)

Figure 14. Oxygen consumption in the soleus muscle. Values are mean ±S.E.M. BADA = during infusion of boiled adenosine deaminase. ADA = during infusion of active adenosine deaminase. PRE-ADA = following the one-hour re-equilibration period. * = significantly different from BADA exercising level ($p<0.05$).


OXYGEN CONSUMPTION
(ml O_2 • min^{-1} • $100g^{-1}$)
10
8
6
4
2
0
EXERCISE
REST
n = 5
*p<0.05
*
BOILED ADA
PRE-ADA
ADA

when measured during exercise with BADA and ADA infusions (Table X), this attenuated oxygen consumption results from the attenuated increase in blood flow.

Effect of adenosine deaminase on tissue metabolites and adenosine content. Lactate, creatine phosphate, ATP, creatine, and inorganic phosphate were measured in five soleus muscles which were stimulated to contract and five muscles serving as contralateral controls. All were being infused with ADA the time of sampling. These data are presented in Table XII.

Lactate, creatine, and inorganic phosphate increased significantly with stimulation. Creatine phosphate levels decreased with stimulation. Although there was a tendency for tissue ATP concentration to decrease with stimulation as compared to controls, this change was not significant. These results are similar to those obtained in the soleus in the microsphere series.

Adenosine content in the five stimulated muscles was 19.02 ± 2.50 $nmoles \cdot g^{-1}$. Adenosine content in the contralateral soleus muscles was 2.97 ± 1.16 $nmoles \cdot g^{-1}$. These values are similar to those of the microsphere series.

TABLE XII

TISSUE METABOLITE CONCENTRATIONS IN THE SOLEUS MUSCLE:
ADA VENOUS OUTFLOW SERIES

SAMPLE	Creatine Phosphate ($\mu moles \cdot g^{-1}$)	Creatine ($\mu moles \cdot g^{-1}$)	Inorganic Phosphate ($\mu moles \cdot g^{-1}$)	ATP ($\mu moles \cdot g^{-1}$)	Lactate ($\mu moles \cdot g^{-1}$)
Contralateral muscle (n=5)	24.7±4.0	11.7±3.4	5.7±1.1	7.7±2.2	4.1±0.1
Stimulated muscle (n=5)	6.3±2.1*	36.0±7.7*	14.3±1.4*	5.5±1.2	8.7±1.7*

Values are mean ± S.E.M.
*significantly different from contralateral control muscle, $p<0.05$

DISCUSSION

These studies were designed to evaluate the role of adenosine in the regulation of skeletal muscle blood flow during active hyperemia. The findings presented here indicate a role for adenosine as a mediator of active hyperemia in Type I, oxidative soleus muscle of cats during exercise. They do not indicate a similar role for adenosine in Type IIb, glycolytic gracilis muscle. These findings are consistent with the hypothesis that the intrinsic metabolic profile of a muscle determines the extent to which adenosine mediates its blood flow.

Several previous investigations have indicated that differences in the potential for adenosine production exist in muscles composed of different fiber types. Bockman and McKenzie (1983) demonstrated that the cat gracilis muscle does not produce adenosine during exercise even at stimulation levels which produced greatly elevated tissue concentrations of lactate and significant muscle fatigue. In contrast, adenosine production in the soleus muscle correlated well with muscle lactate content. Based on these observations, the authors concluded that adenosine might be important as a mediator of the hyperemic response in the soleus under conditions that elicit increases in lactate production.

Increases in lactate occur when the oxygen supply or the rates of the various enzymes in the metabolic pathways limit the entry of pyruvate into the mitochondria for oxidation through the citric acid cycle and the electron transport chain. Johsis and Stainsby (1968) reported that the oxygen supply never limited the respiratory chain activity during the contractions in oxidative muscle. However, in a perfused rat hindlimb preparation, Idstrom et al. (1985) reported a

causal relationship between the oxygen supply and energy state in contracting soleus (oxidative) and gastrocnemius (glycolytic) muscles. A linear relationship between oxygen delivery and oxygen consumption in the contracting hindlimb showed that mitochondrial respiration was directly dependent on the oxygen delivery. A significant negative correlation was found in both muscles between oxygen delivery and lactate release, indicating a compensatory increase in anaerobic energy. These authors concluded that the energy production of contracting muscles can be oxygen limited under physiological conditions.

In the present study, it was found that contraction of the gracilis resulted in a large amount of lactate released into the venous blood even at relatively low levels of muscle performance (Figure 7) and lactate release increased further with increases in muscle performance. Stimulation of the soleus muscle, however, resulted in little lactate release at low levels of performance, although lactate release from this muscle did increase in proportion to muscle performance. This lactate release occurred without loss of muscle performance in the soleus, indicating that oxygen delivery and availability of fuels was adequate to maintain the mechanical activity of the muscle. These findings indicate that lactate is produced by the soleus under physiological conditions. Because adenosine production was found to correlate with lactate in the cat soleus muscle (Bockman and McKenzie, 1983), this suggested that adenosine might also be produced under physiological conditions.

Additional indirect evidence of a possible differential involvement of adenosine in active hyperemia in the two muscle types was gained from the pattern of ammonia release in this preliminary

study. The major source of the ammonia produced by working muscle is the conversion of AMP to IMP by AMP deaminase (Lowenstein, 1972). By measuring ammonia release into the venous blood, we hoped to follow the conversion of AMP to IMP rather than to adenosine. For a given change in muscle performance, the gracilis muscle released more ammonia than the soleus muscle (Figure 6). Therefore, it appeared that more IMP is derived from AMP in the gracilis than in the soleus muscle. It is possible that AMP is instead degraded predominately to adenosine in the soleus muscle.

These data support the in vitro finding that 5'-nucleotidase and AMP deaminase activities vary between the soleus and gracilis muscles (Bockman and McKenzie, 1983). As AMP deaminase activity increases relative to 5'-nucleotidase activity, as in the cat gracilis muscle in comparison to the soleus, relatively more AMP would be expected to be converted to IMP than to adenosine. However, differences in enzyme kinetics (Meyer and Terjung, 1979; Rubio et al., 1973) and absolute activities (Bockman and McKenzie, 1983) complicate the extrapolation of these in vitro findings to the intact muscle. The present finding that differences exist in the pattern of ammonia production between the two muscles suggest that, at least in the case of AMP deaminase, both the in vivo and in vitro activity of the enzyme is different in the two muscles.

These findings are in agreement with a previous study by Meyer and Terjung (1979) who reported significant IMP and ammonia formation accompanying high rates of energy utilization in rat fast-twitch muscle. No evidence of AMP deaminase activity was found in slow-twitch red muscle of the rat even during intense, anoxic stimulation. Although a

depleted phosphocreatine content attested to the severely limited ATP supply, only slight accumulation of ammonia and lactate in the muscle was observed even during exercise associated with the loss of its aerobic supply to the point of complete loss of contractile function. These authors concluded that AMP deaminase was not appreciably activated in slow-twitch muscle during intense muscle performance, indicating a fundamental difference in adenine nucleotide metabolism associated with contractile performance between the different muscle types.

Although these studies taken together indicate that skeletal muscles vary in their potential to produce adenosine, direct evidence of a cause-and-effect relationship between adenosine and active hyperemia is lacking. In order to investigate more directly the role of adenosine in active hyperemia in muscles of different fiber types, adenosine deaminase was infused in an effort to inhibit the effect of adenosine on the resistance vessels. The use of adenosine deaminase to prevent the action of adenosine has several advantages over other nonspecific agents. For example, dipyridamole, which inhibits adenosine uptake by erythrocytes, or theophylline, a competitive adenosine antagonist, were found to be without effect on active hyperemia in exercising dog gracilis muscle (Honig and Frierson, 1980). These observations led to the conclusion that adenosine is not involved in the vascular control of this muscle. However, it is important to note that microvascular actions of these drugs may have complicated interpretation of the results. These actions include vasoactivity, inhibition of platelet aggregation, alteration of transcapillary exchange and pre- and postcapillary resistance ratios, altered intermediary metabolism, and interference with acetylcholine release in skeletal muscle (Goodman and Gilman, 1975). Further-

more, theophylline is reported to damage the microcirculation when applied topically to arterioles (Proctor, 1984). Since none of these effects are readily reversible, it is difficult to ascribe with certainty physiologic actions of adenosine based on the pharmacologic effects of dipyridamole or theophylline.

The effects of administered adenosine deaminase appear to be rapidly reversible. Proctor (1984) reported base-line estimates of blood flow in his hamster cremaster muscle preparation within 5 minutes after the deaminase had been washed out of the superfusate. He also reported that enzyme treatment did not appear to damage the microcirculation in the preparation. Treating the tissue with the enzyme seemed to cause no effect other than blocking the vasoactive effect of adenosine. The reversibility of adenosine deaminase is also evidenced by the work of Saito et al. (1981) who reported a return to a normal level of reactive hyperemia 10 minutes after termination of intracoronary administration of deaminase. Similarly, as measured by tissue adenosine deaminase activity, the one-hour re-equilibration period appeared to be sufficient to allow washout of the infused adenosine deaminase in our preparation (Figure 8).

Intravascularly-administered adenosine deaminase appears to have access to the intracellular space. Martin and Bockman (1986) found that intra-arterial infusion of adenosine deaminase into the subcutaneous adipose tissue of dogs produced an increase in interstitial concentration of the enzyme, as evidenced by its appearance in the lymph effluent of the tissue. Similarly, intracoronary infusion of adenosine deaminase results in increased cardiac lymph adenosine deaminase concentration (Dole et al., 1985, Saito et al., 1981). In the preparation used in the present study,

it was not feasible to measure changes in enzyme concentration in lymph vessels near the muscle. However, adenosine deaminase content doubled in the muscle during infusion (Figure 8). Adenosine deaminase is a large protein, approximately 40,000 molecular weight (Gewirtz et al., 1983) and is not expected to penetrate cell membranes and reach the intracellular space. Because adenosine deaminase is not thought to enter the muscle cell, this concentration in the muscle was assumed to reflect the increased adenosine deaminase content of the interstitial space.

In these studies, adenosine deaminase had no effect on active hyperemia in the low-oxidative gracilis even at levels of muscle performance which produced fatigue and tripled tissue lactate content (Figure 12). This agrees with the findings of Bockman and McKenzie (1983) in which tissue adenosine content in the gracilis did not change, even in the presence of large increases in lactate and muscle fatigue. In contrast, adenosine deaminase produced a 38% attenuation of active hyperemia in the high-oxidative soleus muscle at levels of muscle performance which normally increased tissue lactate content (Figure 10). This attenuation of blood flow and increased muscle vascular resistance was accompanied by a significant reduction in muscle performance over the 10-minute stimulation period which was not seen in the control group. Although the initial amount of tension production was unchanged from the level produced during boiled adenosine deaminase infusion, it appeared that oxygen delivery was inadequate to maintain the mechanical activity of the muscle in the presence of active adenosine deaminase. Tissue lactate and metabolite levels indicated severe hypoxia in the presence of active deaminase at the high

stimulation level which was not seen with the boiled control (Table VIII). Adenosine deaminase infused identically had no effect on the soleus at lower levels of muscle performance in which there was no tendency for lactate content of the muscle to increase with contraction (Figure 9). This finding is evidence against the possibility that adenosine deaminase infusion directly affected muscle performance, thereby decreasing the amount of blood flow required by the muscle. These data also support the proposal that conditions which produce a significant generation of lactate by the muscle are necessary for adenosine's involvement as a regulator of blood flow in oxidative skeletal muscle (Bockman and McKenzie, 1983).

Proctor (1984) reported that the addition of adenosine deaminase to the superfusate solution directly adjacent to the arterioles of hamster cremaster muscle attenuated the arteriolar diameter change during stimulation. The muscles were stimulated to contract at 2-10 Hz, and the reduction caused by deaminase was found to depend on the frequency of muscle contraction. The hamster cremaster muscle is composed primarily of fast-twitch, low-oxidative fibers (Sarelius, et al., 1983) and would be expected to resemble the cat gracilis muscle in its adenosine production capability. Although adenosine deaminase had no effect on the gracilis muscle in the present study, the stimulation parameters used were much lower than those used by Proctor. A significant amount of fatigue was seen in the gracilis muscle even at 0.4 Hz stimulation (Figure 12). The greatest effect seen with deaminase in the cremaster muscle in Proctor's study was during 10 Hz stimulation, which would have been likely to produce considerable fatigue to the point of total loss of muscle performance in the muscles of the present study.

The vascularly-isolated soleus technique allowed continuous measurement of blood flow throughout the infusions, as well as an assessment of the effect of adenosine deaminase on muscle oxygen consumption. From these experiments, it was concluded that adenosine deaminase did not directly affect muscle metabolism. Resting oxygen consumption and muscle blood flow were unchanged after 10 minutes of adenosine deaminase infusion, indicating that the infusion itself did not alter either the metabolism or perfusion of the muscle. However, adenosine deaminase did attenuate exercising levels of muscle oxygen consumption and blood flow (Figure 14). This attenuated hyperemic response during exercise was apparent throughout the stimulation period (Figure 13). Because oxygen extraction by the muscle was unaltered by this infusion, attenuated blood flow is most likely to be responsible for the decreased oxygen consumption. Thus, it appears that adenosine may play a significant role in mediating active hyperemia in the soleus muscle at this level of stimulation throughout the entire response.

It is important to note that adenosine deaminase infusion did not completely abolish the active hyperemic response, even in the oxidative soleus muscle. Although adenosine appears to be one important mediator of active hyperemia in muscles composed of Type I fibers, the occurrence of partial dilation points to the likelihood that other factors also participate in the response.

Mohrman et al. (1973) concluded that several factors, including osmolarity, potassium ion, and a substance associated with oxidative metabolism interact to mediate hyperemia in muscle after brief tetanic contractions. Gorczynski and Duling (1978) estimated that 45-60% of the hyperemia was correlated with nonoxidative processes during short

periods of submaximal exercise. The vasculature rapidly becomes refractory to potassium (Duling, 1975) and hyperosmolarity (Sparks, 1980), so adenosine might progressively become the most important factor mediating the sustained hyperemia as the transient effects of other substances wane.

Since several potential vasodilators in skeletal muscle do exist, negation of the action of adenosine might be compensated for by other mediators. Such a situation could result in an apparently normal hyperemic response. Blocking the effect of one mediator, therefore may provide only a minimum estimate of its fractional contribution to the normal regulatory process. In fact, Bockman suggested (1983) that the apparent "overperfusion" of the gracilis muscle relative to its oxygen consumption might result from an inappropriate accumulation of vasodilator metabolites. Its limited ability to extract oxygen sufficient to meet its energy needs could lead to the production of several varieties of vasodilators, including adenosine. Although this possibility clearly exists, the differential effect of deaminase that was seen between the soleus and gracilis muscles indicates that even if adenosine is involved in mediating a portion of the response in the gracilis, the percentage of its involvement relative to other metabolites would seem to be greater in the soleus.

Another consideration in drawing conclusions from the present study is that adenosine deaminase may not have been able to deaminate all of the adenosine released from the muscle. Because the adenosine receptor affinity and deaminase Km are both in the micromolar range (Proctor, 1984), even a great excess of exogenous adenosine deaminase in the perivascular space could not inactivate adenosine molecules in

the immediate vicinity of the receptor. Thus, the possibility that some adenosine could have conceivably been released from the muscle and acted at vascular smooth muscle sites before inactivation seems reasonable. If so, this situation may have been more pronounced in the gracilis as compared to the soleus muscle.

The basis for a differential role of adenosine as a mediator of active hyperemia in the soleus and gracilis muscles seems to be related to differences in the concentration and regulation of the enzymes responsible for its formation. It appears likely that the enzymes responsible for the conversion of AMP to either adenosine or IMP are carefully regulated in skeletal muscle. Measured activities of the enzymes in muscle would suggest that even in highly oxidative dog skeletal muscle, in which AMP deaminase activity is 300-fold greater than 5'-nucleotidase activity (Bockman and McKenzie, 1983), virtually all AMP would be degraded to IMP. One would predict, therefore, that essentially no adenosine would be produced. Yet, it has been shown that the adenosine content of dog skeletal muscle can increase as much as 20-fold under conditions of muscle contraction with restricted flow (Belloni et al., 1979). In addition, Rubio et al. (1973) have shown that relatively more AMP degradation occurs through the adenosine pathway than the IMP pathway in dog skeletal muscle. These findings strongly suggest that 5'-nucleotidase is activated during conditions of contraction and ischemia in dog skeletal muscle and AMP deaminase activity is substantially inhibited.

Wheeler and Lowenstein (1979) reported that inorganic phosphate at concentrations occurring <u>in vivo</u> strongly inhibit AMP deaminase. This would account for the relative inactivity of the

enzyme in resting muscle. However, it also presents a problem in explaining the markedly increased AMP deaminase activity resulting in an increased IMP production in glycolytic muscle during exercise (Meyer and Terjung, 1979; Meyer and Terjung, 1980; Rubio et al., 1973). The level of inorganic phosphate would be expected to increase due to creatine phosphate breakdown (Meyer and Terjung, 1979; Meyer and Terjung, 1980; Sahlin et al., 1978), thereby inhibiting AMP deaminase. On the other hand, AMP deaminase is activated by ADP, AMP, and H^+ (Chung and Bridger, 1976; Makarewicz and Stankiewicz, 1974; Ronca-Testoni et al., 1970). In glycolytic muscles studied by Meyer and Terjung (1979), AMP content changed little during exercise. However, a decrease in intracellular pH during muscle contraction may explain the apparent activation of AMP deaminase. Sahlin et al. (1978) estimated that intracellular pH decreased from 7.0 to 6.4 during fatiguing exercise in humans. Wheeler and Lowenstein (1979) found that such a decrease in intracellular pH resulted in a fourfold increase in AMP deaminase activity, even in the presence of inorganic phosphate. Therefore, changes in intracellular pH may be an important factor in the activation of AMP deaminase in glycolytic muscles. It is possible that pH changes are attenuated in more oxidative muscles due to lower levels of lactate and hydrogen ion production that occur during stimulation at a given level of muscle performance. This would be consistent with the recent report of Dudley and Terjung (1985) that the increase in H^+ concentration, accompanying excessive lactate accumulation that normally occurs in vivo did not appear to play an important role in AMP deaminase activation in high-oxidative muscle. In low-oxidative, fast-twitch white skeletal muscle, lactate accumulation does play an important role in AMP deaminase activation.

Rubio et al. (1979) have reported that creatine phosphate strongly inhibits 5'-nucleotidase and that this inhibition is relieved by magnesium, a product of ATP metabolism. Enzyme activity is also increased as energy charge decreases. Sahlin et al. (1978) have reported that a significant reduction in creatine phosphate content and energy charge occurs in fatiguing exercise in humans. Total adenine nucleotide content also decreases, which would be expected to increase free magnesium concentration. Similar changes have also been reported in rat fast-twitch, glycolytic muscles (Meyer and Terjung, 1979; Meyer and Terjung, 1980). If it is assumed that cat gracilis muscle undergoes similar changes, then one would expect 5'-nucleotidase to be activated. The results of this study suggest that creatine phosphate levels are reduced in the gracilis muscle even at low stimulation levels (Table IX). However, Bockman and McKenzie (1983) observed a low rate of nucleoside production in cat gracilis muscle. This indicates that inhibitors other than those previously described may be operating in vivo to maintain a low 5'-nucleotidase activity in cat gracilis muscle.

Conversion to IMP with the concomitant production of ammonia as the predominant pathway of AMP degradation in gracilis would be particularly beneficial for this fiber type. Ammonia is known to stimulate glycolysis by activating phosphofructokinase, one of the rate-limiting enzymes of glycolysis (Lowenstein, 1972). This stimulation of glycolysis decreases glycogen and glucose stores and increases the production of pyruvate and ATP. Because of the actions of ammonia to enhance the formation of pyruvate, it is reasonable that muscles relying primarily on glycolytic metabolism would favor AMP degradation toward IMP and ammonia. At least two steps in the TCA

cycle appear to be affected by ammonia. Isocitrate dehydrogenase, which catalyzes the conversion of citrate to α-ketoglutarate, is inhibited by ammonia (Katanuma et al., 1966). Similarly, pyruvate dehydrogenase, a mitochondrial enzyme that catalyzes oxidative decarboxylation of pyruvate to acetyl CoA for entry into the TCA cycle may also be inhibited (Mutch and Banister, 1983). It would be equally reasonable, therefore, that oxidative muscles that rely on oxidative phosphorylation of substrates would favor AMP degradation toward adenosine. Oxidative metabolism would not be inhibited, and the formation of adenosine and subsequent vasodilation would ensure adequate substrate for oxidative conversion of energy.

In summary, the infusion of adenosine deaminase in order to specifically degrade adenosine to vasoinactive inosine significantly attenuated the normal hyperemic response to 2.0 Hz stimulation in the soleus muscle. This reduction in active hyperemia was accompanied by loss of muscle performance and a metabolic profile which is characteristic of hypoxia. The initial amount of force developed by the muscle was unchanged, indicating that the contractile mechanism was not directly affected by the presence of the enzyme. Adenosine deaminase had no effect on resting oxygen consumption or metabolite levels, suggesting that the enzyme had no direct effect on muscle metabolism. In contrast, adenosine deaminase infusion did not alter the hyperemic response, muscle performance, or metabolic profile of the soleus at 0.5 Hz stimulation. There was also no attenuation of active hyperemia in the gracilis muscle at either 0.2 or 0.4 Hz stimulation.

These findings support a role for adenosine in the mediation of active hyperemia in oxidative muscles at levels of stimulation which

result in significant increments in lactate production. A similar role for adenosine is not supported in glycolytic muscle. These data indicate that the oxidative capacity of the muscle and the intensity of the metabolic stimulus influence the extent to which adenosine mediates the regulation of skeletal muscle blood flow during active hyperemia.

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