# THE EFFECTS OF NALOXONE ON MUSCLE BLOOD FLOW DURING LOW INTENSITY EXERCISE IN RATS

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Walter B. Cannon in "The Way of an Investigator" presents the idea that the search for understanding is an adventure or, more commonly, a series of adventures--namely advertures in ideas.

> If an attempt in one direction fails, the failure is not discouraging to an eager explorer... When the goal is reached, there is occasion for joy and exultation. A conquest has been achieved. New knowledge has been gained which deeply satisfies both the explorer's adventurous spirit and his persistent curiosity.

Understanding the mechanisms that control the various muscle blood flow patterns during exercise has been the aim of many recent research projects in the laboratories of Drs. Laughlin and Armstrong. Determining the role played by the endogenous opioids brings us one step closer in this search for understanding. As a result of this study, new knowledge has been gained in this area.

I wish to express my sincere gratitude to all who assisted me in this project.

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# CHAPTER I

## INTRODUCTION

Contraction of skeletal muscle, both in situ and in vivo studies, is associated with a rapid and dramatic increase in blood flow. In 1961, Barcroft (17) stated in the Handbook of Physiology that "the mechanism of the increase in blood flow associated with exercise is not yet understood" and that "it is the most important problem in the field of skeletal muscle circulation." More recently, Stainsby (95) observed that "rather little research into local control mechanisms has been very probing, and new ideas seem to be few relative to the amount of experimentation." In spite of the effort devoted to solving this problem, the mechanism(s) is (are) still not fully understood (70).

Recent research endeavors have also been directed toward a better understanding of the multi-faceted role of the endogenous opioids in physiological processes of "extreme circumstances" such as physical exercise (32). Emerging evidence suggests that endogenous opioid peptides influence diverse functions many of which may be linked with exercise (25). There are many studies which report increases in blood levels of opioids as a result of exercise (1,5,25,27,30,31,32,41,46,48,78).

No studies, however, have yet investigated the relationships between the endogenous opioids and blood flow within and among muscles during low intensity exercise. Thus, the role that the endogenous opioids may play in the control of muscle blood flow needs to be investigated more thorougly.

## Statement of the Problem

The purpose of the present study is to determine if endogenous opioids produce the same cardiovascular effects in rats as seen in other species. Additionally, it will be determined what effect endogenous opiates may have on muscle blood flow control during exercise.

## Hypotheses

If the opioid peptides are involved in the control of muscle blood flow during exercise, blocking the opioid receptors with naloxone should significantly alter muscle blood flow distribution at the .05 significance level across conditions in the 61 samples taken. Arterial blood pressure is not expected to vary significantly across conditions at the same level of significance.

## Extent of the Study--Delimitations, Limitations, and Assumptions

1. The study was conducted in the Department of Physiology, Oral Roberts University School of Medicine, Tulsa, Oklahoma.
2. The experimental data were collected on male Sprague-Dawley rats (approximately $395 \pm 24$ g body weight).

3. Anesthetized rats were used to determine the effects of opioids on heart rate, blood pressure, and regional blood flows.
4. Baseline and exercise data were collected on two groups of rats: those injected with naloxone and those given an injection of saline.
5. The data to be collected included mean aortic pressure, inter- and intra-muscular blood flow, and blood flow to other selected tissues. Resistance was calculated for anethetized rats.
6. Assumptions of the microsphere technique include: 1) the microspheres are trapped in small vessels during the first passage through the tissues; 2) the tissue and reference blood samples contain adequate numbers of microspheres to assure acceptable errors and confidence limits; 3) the microspheres are uniformly distributed in the suspension medium prior to infusion into the blood; 4) the microspheres are well mixed with the blood upon infusion so that the concentration of microspheres reaching all branch sites is the same; 5) the infusion of the microsphere suspension must not disturb the cardiovascular system and the circulation of the blood; 6) the best microsphere size is 15 μm (71).

## Definition of Terms

1. Adrenergic - Term applied to nerve fibers of the sympathetic nervous system.

2. Agonist - A drug that can interact with receptors and initiate a drug response.
3. Analgesia - Absence of sensibility to pain.
4. Beta-endorphin - belongs to the family of endorphins. (H-TYR-GLY-GLY-PHE-MET-THR-SER-GLU-LYS-SER-GLN-THR-PRO-LEU-VAL-THR-LEU-PHE-LYS-ASN-ALA-ILE-ILE-LYS-ASN-ALA-HIS-LYS-LYS-GLY-GLN-OH).
5. Blood-brain barrier - The endothelial cells of capillaries in the brain that are joined by tight junctions and provide an effective barrier to passage of some substances.
6. Catecholamine - One of a group of similar compounds having a sympathomimetic action.
7. Catheter - A slender, flexible tube used to remove or introduce fluids by way of a blood vessel.
8. Endogenous - Originating or produced within the body.
9. Endorphin - Family that includes all endogenous peptides whose sequences include an enkephalin pentapeptide and share some common actions at presumptive opiate receptors as defined by naloxone antagonism; includes methionine enkephalin, leucine enkephalin, and beta-endorphin.
10. Heparin - A mucopolysaccharide with the ability to keep blood from clotting.
11. Hyperemia - The presence of an increased amount of blood in a muscle or an organ.
12. in Situ - In position.
13. in Vivo - Within the living body.

14. Leucine enkephalin - Belongs to the family of endorphins. (H-TYR-GLY-GLY-PHE-LEU-OH).
15. Methionine enkephalin - Belongs to the family of endorphins. (H-TYR-GLY-GLY-PHE-MET-OH).
16. Microspheres - Refers to radiolabled microspheres of 15 μm diameter.
17. Motoneurons - Refers to motor neurons.
18. Motor unit - A motoneuron and the fibers it innervates.
19. Naloxone - A drug that competitively inhibits the actions of the opioids.
20. Neuromuscular junction - The synapses between the axons of motor neurons and skeletal muscle fibers.
21. Neurotransmitter - Any substance that aids in transmitting impulses between two nerve cells or between a nerve and a muscle.
22. Opiate receptors - Regions of the brain which have the capacity to bind opiate agonists.
23. Radioimmunoassay (RIA) - A method of analysis such as determination of the concentration of opioid peptides in plasma, through the use of radioactive antibodies.
24. Receptor - A constituent in a cell that combines with specific drug, resulting in a change of the cell's function.
25. Sympathectomy - Surgical removal of a portion of a sympathetic nerve or of a sympathetic ganglion.
26. Sympathetic - Denoting the sympathetic part of the autonomic nervous system.

27. Vasoconstriction - Narrowing of the lumen of blood vessels, especially of arterioles.
28. Vasodilation - Widening of the lumen of the blood vessels, especially of the lumen of arterioles, leading to increased blood flow to a part.

# CHAPTER II

# REVIEW OF THE LITERATURE

## Muscle Blood Flow

The cardiovascular response to exercise is one of the most dramatic physiological responses that occurs in normal life. Marked increases occur in oxygen consumption, ventilation, cardiac output, and the distribution of cardiac output throughout the body is altered (20,28,29, 86,88,89,100). The basic reason for these changes is to support the increased muscle metabolism that accompanies exercise. If the exercise is to be maintained, it is necessary that the muscle contractions be fueled by aerobic metabolism, which is dependent upon muscle circulation. Thus, muscle blood flow must be sufficient to provide adequate oxygen and substrates (e.g., free fatty acids and glucose) for oxidative metabolism, to remove metabolites, and to maintain temperature homeostasis. The available information indicates that blood flow during exercise is directed primarily to the active oxidative fibers in the muscles (70).

### Blood Flow in Resting Muscles

#### Muscle Fiber Type Properties and Distribution

Mammalian skeletal muscles are composed of fibers with different physiologic, morphologic, and biochemical characteristics. There is evidence of a relationship between muscle fiber type and blood flow in resting and exercising muscle (70).

The locomotory muscles of the hindlimb of the rat are composed of three reasonably distinct types of fibers (11). In describing the relationship of blood flow and muscle fiber type, the fiber type terminology suggested by Peter and co-workers in 1972 (83), has been used in muscle blood flow studies conducted by Laughlin and Armstrong (8,9,10, 12,67,68,69,71,72). This system includes both the metabolic and physiologic properties of muscle fibers. Histo-chemical analysis of the muscles for mitochondrial enzyme and myofibrillar adenosine triphosphatase (ATPase) activities permit indentification of fibers as fast-twitch oxidative glycolytic (FOG), fast-twitch glycolytic (FG), and slow-twitch oxidative (SO) (11). Fiber type populations and abbreviations of muscles and parts of muscles are presented in TABLE I.

Several general patterns of distribution of the three fiber types have been described (6,7,16,68). The deepest portions of limb muscles are typically composed of high proportions of SO and FOG fibers and the most superficial regions of the FG type (Figures 1 and 2). Also, in antigravity extensor muscle groups, the deepest muscle within each group has a relatively high population of SO fibers, whereas the more superficial muscles are primarly fast-twitch (FOG and FG). Thus, in physiological extensor muscle groups, there is a typical pattern of deep SO and

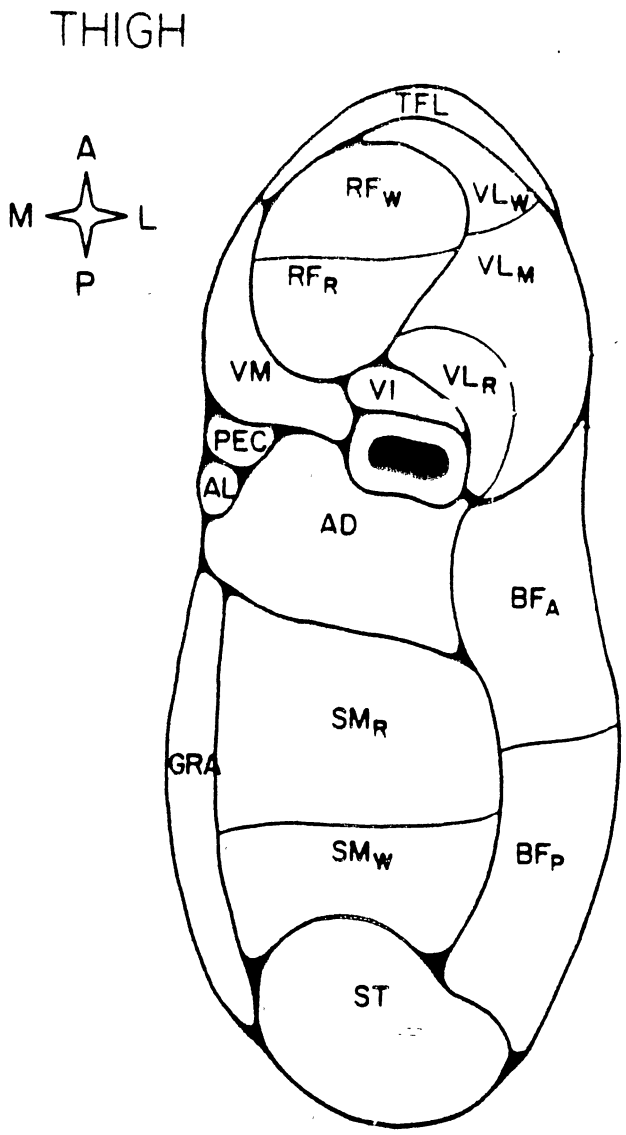
TABLE I

FIBER TYPE POPULATIONS AND ABBREVIATIONS OF RAT MUSCLES AND PARTS OF MUSCLES EXAMINED

| Muscles | Abbreviations | Fiber Types, % SO | FOG | FG |
|---|---|---|---|---|
| Knee Extensors | | | | |
| Vastus intermedius | VI | 59 ± 9 | 40 ± 9 | 1 ± 1 |
| Vastus medialis | VM | 3 ± 3 | 20 ± 5 | 77 ± 3 |
| Vastus lateralis, red | $VL_R$ | 9 ± 3 | 56 ± 4 | 35 ± 5 |
| Vastus lateralis, white | $VL_W$ | 0 ± 0 | 3 ± 3 | 97 ± 3 |
| Vastus lateralis, mixed | $VL_M$ | 1 ± 1 | 36 ± 3 | 63 ± 3 |
| Rectus femoris, red | $RF_R$ | 7 ± 1 | 53 ± 9 | 40 ± 8 |
| Rectus femoris, white | $RF_W$ | 1 ± 1 | 25 ± 6 | 74 ± 6 |
| Knee Flexors | | | | |
| Biceps femoris, cranial | $BF_A$ | 1 ± 1 | 29 ± 5 | 70 ± 5 |
| Biceps femoris, caudal | $BF_P$ | 7 ± 1 | 27 ± 5 | 66 ± 5 |
| Semitendinosus | ST | 7 ± 2 | 45 ± 6 | 48 ± 7 |
| Semimembranosus, red | $SM_R$ | 7 ± 2 | 43 ± 5 | 50 ± 5 |
| Semimembranosus, white | $SM_W$ | 1 ± 1 | 22 ± 8 | 77 ± 9 |
| Thigh Adductors | | | | |
| Adductor longus | AL | 81 ± 4 | 18 ± 4 | 1 ± 1 |
| Gracilis | GRA | 23 ± 3 | 24 ± 4 | 53 ± 5 |
| Pectineus | PEC | 10 ± 2 | 35 ± 4 | 55 ± 4 |
| Adductor magnus and brevis | AD | 4 ± 4 | 33 ± 4 | 63 ± 6 |
| Ankle Extensors | | | | |
| Soleus | SO | 87 ± 4 | 13 ± 4 | 0 ± 0 |
| Plantaris | PL | 9 ± 1 | 50 ± 3 | 41 ± 3 |
| Gastrocnemius, red | $G_R$ | 30 ± 2 | 62 ± 3 | 8 ± 4 |
| Gastrocnemius, white | $G_W$ | 0 ± 0 | 16 ± 3 | 84 ± 3 |
| Gastrocnemius, mixed | $G_M$ | 7 ± 1 | 28 ± 5 | 65 ± 5 |
| Tibialis posterior | TP | 2 ± 1 | 48 ± 4 | 50 ± 4 |
| Flexor digitorum longus | FDL | 7 ± 2 | 42 ± 4 | 51 ± 3 |
| Flexor hallicus longus | FHL | 4 ± 1 | 33 ± 6 | 63 ± 5 |
| Ankle Flexors | | | | |
| Tibialis anterior, red | $TA_R$ | 5 ± 1 | 57 ± 8 | 38 ± 9 |
| Tibialis anterior, white | $TA_W$ | 1 ± 1 | 27 ± 6 | 72 ± 6 |
| Extensor digitorum longus | EDL | 2 ± 1 | 42 ± 7 | 56 ± 8 |
| Peroneals | PER | 11 ± 2 | 41 ± 7 | 48 ± 8 |

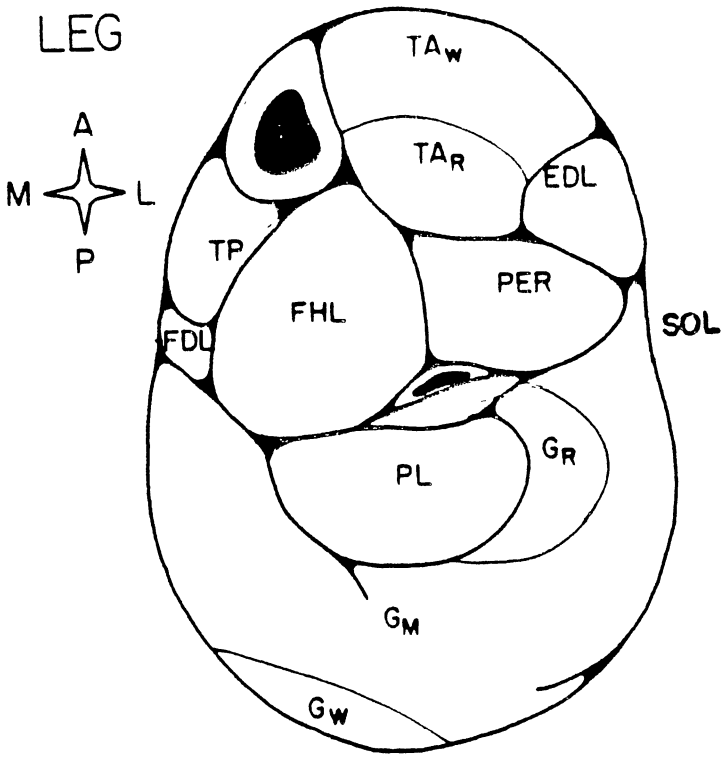
Values are means ± SEM.

Source: (11)




Source: (69).

Figure 1. Diagram of a transverse section through the thigh of the rat, depicting the methods of sampling of the muscle. A, M, L, and P are anterior, medial, lateral, and posterior, respectively, in reference to a standing rat. Abbreviations for muscle samples are the same as those presented in TABLE I.




Source: (69).

Figure 2. Diagram of a transverse section through the leg of the rat, depicting the method of sampling of the muscles. A, M, L, and P are anterior, medial, lateral, and posterior, respectively, in reference to a standing rat. Abbreviations for muscle samples are the same as those presented in TABLE I.

FOG to superficial FG, both within and among muscles (11).

Blood flows to intact muscles of various fiber types are quite similar if the muscles are completely inactive. There is evidence that surgical manipulation can cause increases in blood flow to resting muscles and that this effect may be greater in muscles primarily composed of the SO fiber type (70). The blood flow distribution in rat muscles closely corresponds to patterns of fiber recruitment (68).

A factor that appears to influence pre-exercise muscle blood flow values in conscious rats is the type, intensity, and duration of training of the rats prior the the experiments. The anticipatory response of the rat before the treadmill is turned on seems to influence blood flow in the muscles. Also, following prolonged treadmill training (13-17 weeks), rats have higher pre-exercise blood flows in deep red muscles and muscle parts (10,70). Therefore, when resting muscle blood flow is discussed, it is important to define exactly what is meant by resting, since even minimal levels of motor unit activity result in increases in muscle blood flow (70).

## Muscle Blood Flow During Exercise

When a muscle starts to contract, vascular resistance decreases, which is associated with increases in muscle blood flow (70,94). Blood flow distribution during exercise is not homogeneous within or among muscles (9,68, 69,70,72). The radiolabeled microsphere technique has demonstrated a marked nonhomogeneity in blood flow in

muscles of animals in which the fiber types are regionally concentrated within muscles (9,68,70,75).

## Muscle Fiber Recruitment Patterns During Exercise

When an animal is standing prior to exercise, most of the force is supplied by SO fibers in the muscles (70,97, 101). When the animal walks on the treadmill, FOG fibers are recruited to provide the increased forces (13,70), while SO fibers in the muscles continue to be active (70, 93,101). FG fibers are not active during slow locomotion. At slow trotting speeds, the force appears to be produced by SO and FOG fibers in most muscles (13,70,97). During galloping, a progressive recuitment of FG muscle fibers occurs so that when the animal is obtaining maximum speeds, FG fibers in the most superficial parts of the muscles are activated to produce force (13,70,97). Thus during terrestrial locomotion, there is an additive recruitment of SO to FOG to FG fibers as the animal increases running speed (24,70). It is apparent from the metabolic characteristics of the fiber types that as long as the animal can perform the exercise with SO and FOG fibers, the exercise can be continued for relatively long periods of time (24,70). However, once the exercise intensity is high enough to require recruitment of significant numbers of FG motor units, the duration of exercise is limited (34,70,97).

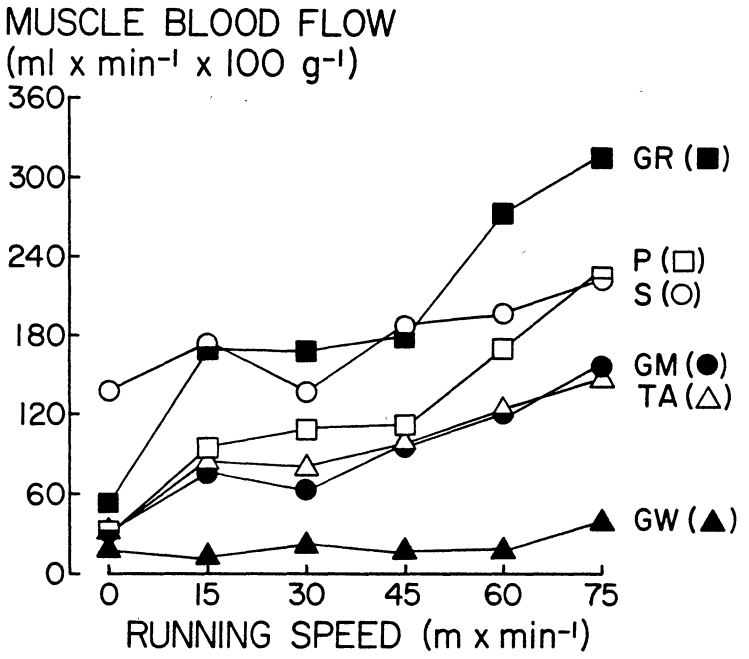
## Muscle Blood Flow During Physiological Exercise

Until recently, techniques for measuring muscle blood

flow during locomotory exercise did not permit study of blood flow distribution within and among muscles (70). Fixler et al. (43) and Flaim et al. (44) used the radio-labeled microsphere technique to measure muscle blood flow (70). Their findings demonstrated that there are differences in the magnitude of the elevations in blood flow to various skeletal muscles during locomotory exercise These authors, however, did not systematically sample muscles within groups or regions with widely divergent fiber populations, so it was not possible to determine relationships between blood flow and the fiber types or muscle fiber recruitment patterns (70).

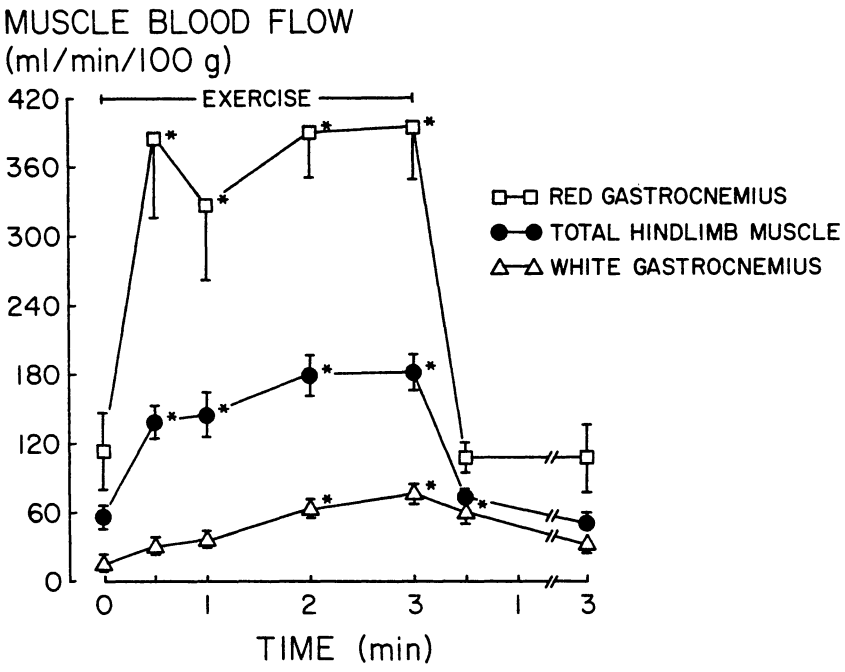
In rats standing on the treadmill anticipating exercise, muscle blood flow is proportional to the SO fiber population in the muscle (9,68,70). During treadmill locomotion, blood flows increase as a function of speed in most muscles (Figure 3), which is directly related to the FOG fiber percentage in the muscle (68,70). The data from these studies (9,68) indicated that the amount of blood flow received is proportional to the oxidative capacities of the fibers, and that muscle blood flow is preferentially distributed to the active oxidative fibers within muscles (70).

Blood flow continues to be directed to active FOG and SO fibers within extensor muscles in rats during prolonged, low-intensity treadmill exercise (69,70). In addition, the blood flow response as a function of time during slow treadmill exercise is different within and among muscles (69,70) (Figure 4). Muscle blood flow has also been




**Source:** (68,70).

Figure 3. Rat muscle blood flows as a function of running speed. GR, GM, and GW are red, middle, and white gastrocnemius muscle samples, respectively. P, S, and TA are plantaris, soleus, and tibialis anterior muscles, respectively.




**Source:** (9,70).

**Figure 4.** Muscle blood flows as a function of time in rats running at 60 meters/minute on a treadmill.

investigated in rats as a function of time during high-speed running (9). Blood flow increases rapidly in the deep red extensor muscles at the start of high-speed running. Through three minutes of high-speed running (60 meters/minute), extensor muscle blood flow is a function of the FOG fiber population. At high running speeds, blood flow to FG fiber areas also increases, but the magnitude of the increase in the white portions of the muscles is much less (9,70).

In swimming rats, the blood flows to the flexor muscles are relatively high compared to those in the extensor muscles, and the flexor muscle blood flows are a linear function of the populations of oxidative fiber types of the muscles (70,72). Unlike terrestrial locomotion, during swimming the extensor muscle blood flows are not related to muscle fiber type (70,72).

## Control of Muscle Blood Flow

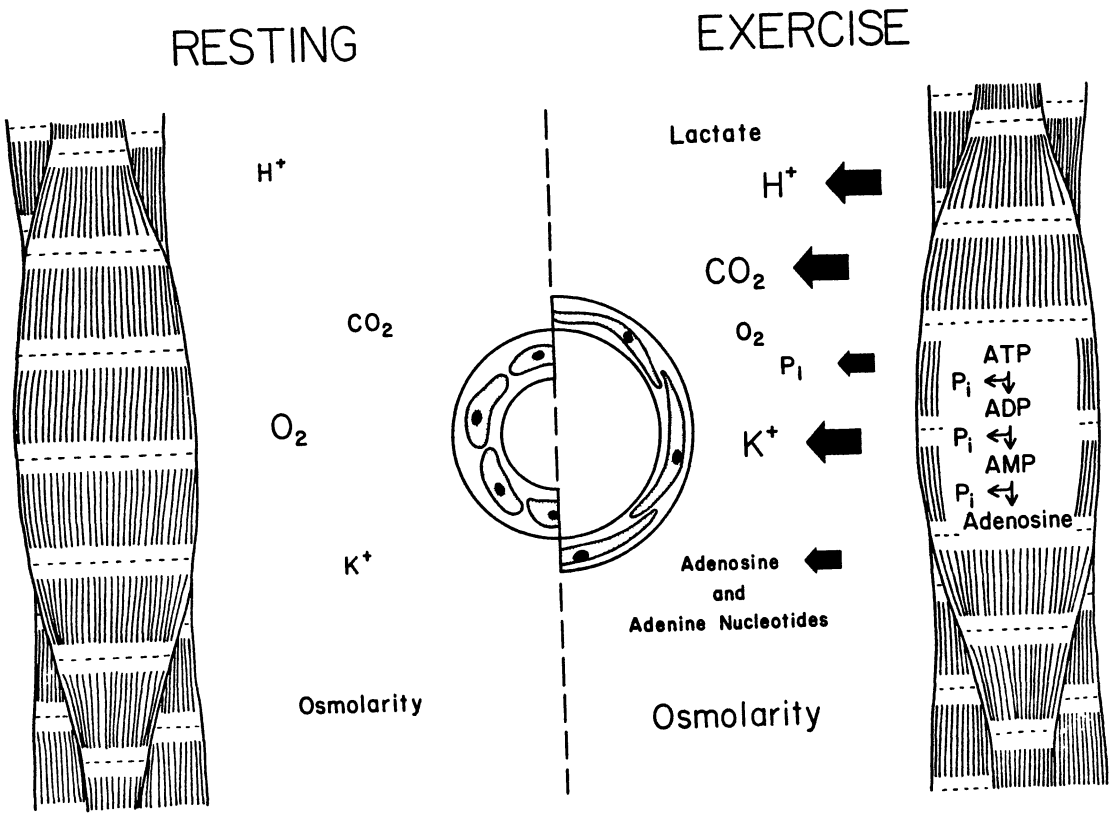
### Resting Muscle Blood Flow

Blood flow to any tissue is determined by the perfusion pressure (i.e., arterial pressure - venous pressure) and the resistance to flow. Since mean arterial pressure is maintained within normal limits under resting conditions, resting muscle blood flow is determined by the resistance to flow that exists in the vascular bed. Poiseulle's law predicts that alterations in resistance to flow are normally due to changes in radius of precapillary resistance vessels (62,70).

The radius of the resistance vessels in turn is controlled by variations in the contractile tension developed by the vascular smooth muscle cells of the arterioles, terminal arterioles, and functional precapillary sphincters (70). The many factors that influence their contractile activity can be divided into three general categories: myogenic, neurohumoral, and local metabolic. The myogenic factors are the inherent characteristics of the vascular smooth muscle which includes spontaneous activity, responses to stretch, and responses to temperature change (64,70,99).

Neurohumoral control includes sympathetic nervous system activity to intact resting skeletal muscle. This activity is high relative to other tissues, since there is a constant discharge of the sympathetic postganglionic neurons that terminate in the proximity of the vascular smooth muscle cells (70,91,92). These neurons release norepinephrine that binds to alpha receptors on the vascular smooth muscle cell membranes causing contraction. Interruption of sympathetic tone either with alpha adrenergic blocking drugs or with sympathetic denervation results in a transient 50 to 100 percent increase in resting muscle blood flow (45,62,70,87,91,92).

As illustrated in Figure 5, several tissue metabolic factors have been proposed as mediators between muscle metabolism and blood flow. Those that cause vasodilation include low partial pressure of oxygen ($PO_2$) in the blood or tissue, increased hydrogen ion (H+) concentration, increased carbon dioxide ($CO_2$) concentration, changes in




Source: (70).

Figure 5. Metabolic factors proposed to be involved in the local control of muscle blood flow under resting consitions (left) and during exericse (right). The relative concentrations of metabolites in the interstitial fluid are illustrated by the size of the letters. When the skeletal muscle cells are inactive, the $O_2$ concentration is high, the metabolite concentrations are low, and the resistance vessels (arterioles) are constricted. During exercise, metabolites are released from the skeletal muscle cells causing the vascular smooth muscle cells to relax resulting in vasodilation.

tissue osmolarity, and release of adenosine, adenine nucleotides, potassium ions, prostagladins, histamine, kinins, and phosphates (70).

## Muscle Blood Flow During Exercise

There are marked reductions in resistance in the vascular beds of muscle during contraction. This decrease in resistance may result from myogenic, neurohumoral, and local metabolic interactions as previously discussed (70).

Initial Phase. During the first few seconds of low-intensity exercise, muscle blood flow increases rapidly in high-oxidative and mixed muscles, and is transiently elevated above the exercise steady state levels for 30 to 60 seconds (69,70). At the slower running speeds, the overshoot in initial hyperemia appears to be independent of exercise intensity (68,70). After five minutes of exercise, the blood flows decrease to steady state levels that are proportionate to exercise intensity (10,69,70). These observatins suggest that at the slower running speeds there is an "on" signal that activates hyperemia in the red muscle that is independent of the amount of contractile activity in the muscle (70).

Studies on animals with sympathectomized hindlimbs argue against a significant involvement of the sympathetic nervous system in the initial hyperemia (33,70). In a study by Peterson et al. (84), in which muscle blood flow was measured in lumbar sympathectomized rats, it was found that the sympathetic innervation is not required for the rapid increase in muscle blood flow observed at the start

of low speed exercise in the red oxidative muscles (70, 68). The effects of circulating epinephrine released from the adrenal glands was investigated by Laughlin and Armstrong (unpublished observations). Neither adrenal-medullectomy alone nor adrenalmedullectomy in combination with hindlimb sympathectomy significantly alters the initial blood flow response in rat muscles during exercise (70).

Although the sympathetic nervous system appears to have no net effect on the initial hyperemia in oxidative muscle during low-speed exercise, preferential beta blockage does alter the magnitude of the response. Use of the beta-adrenergic blocking drugs propranolol ($\beta_1$ and $\beta_2$) or butoxamine ($\beta_2$) decreases the initial elevation in blood flow by 20 to 30 percent during slow-speed treadmill exercise in rats (67,70).

A direct neural linkage between the motor units and their vascular supply has been proposed as a mechanism for rapidly matching blood flow to recruited muscle fibers (4,70). In a recent study, Armstrong et al. preferentially blocked recruitment of FOG and SO fibers in the deep extensor muscles during the initial stage of low-speed treadmill exercise with low doses of curare. The amount of glycogen lost in deep red muscles was decreased during exercise. This reduced recruitment and/or metabolism of the deep red muscle fibers of the curarized animals was not accompanied by reduced blood flow (12). Since curare blocks at the neuromuscular junction (56), and the alpha-motoneurons to the SO and FOG fibers presumably continued to be activated, these findings are consistent

with the notion that there is a direct link between the motoneurons and the vascular smooth muscle in adjacent resistance vessels (70).

The most popular concept for the control of muscle blood flow has been the metabolic control theory described earlier. There are many experiments suggesting that each of the various local metabolic factors (Figure 5) may individually serve as a vasodilator. However, none acting alone can produce the degree of vasodilation that occurs with muscle contractions or in normal exercise. Rather, there is an orchestration of multiple factors (70).

The results of Sullivan and Armstrong (97) suggest that as running speed is increased, the metabolic demands of the FOG fibers in the red muscles are progressively increased due to changes in force requirements related to gait, stride length, and stride frequency. Thus, elevated metabolic vasodilator release could cause progressive dilation of the resistance vessels (70).

Whatever the mechanisms are for the initial muscle hyperemia in exercise, there can be no question that the flow is specifically directed to the recruited fibers within and among the muscles (9,10,68,69,70).

Steady-State Phase. During the early stages of steady state submaximal exercise, it is probable that blood flow in muscles is reasonably well-matched to the oxidative metabolism of the muscle fibers (69,70). It has been proposed that sympathetic activity is increased to all tissues during exercise (96) so that blood flow to most nonmuscular tissues and to inactive skeletal muscles

decreases at the same time that blood flow is elevated in the contracting muscles (68,70). Evidence in support of this comes from experiments done on rats with hindlimb sympathectomies (84). Even though sympathectomy has no effect on the initial hyperemia during low-intensity exercise, from 5 to 15 mintues of exercise, blood flows in the high-oxidative muscles of the sympathectomized rats are elevated above those in normal animals (70).

There is evidence that the mechanisms of control of muscle blood flow vary with the fiber type of the muscles (52,58,70,77). Finally, there may also be differences among muscles of different fiber types in blood flow control in treadmill exercise (9,70). FG muscle blood flow does not show the initial overshoot response to low-intensity exercise (69,70). With high-intensity exercise, FG muscle blood flow does not increase as rapidly as does blood flow to FOG and SO muscle. Also, blood flow to FG muscle does not return to pre-exercise levels for several minutes after exercise, whereas FOG and SO muscle tissue have blood flows equal to pre-exercise values within 30 seconds of exercise termination (9,70). While it seems unlikely that the control mechanisms are totally different in qualitative terms, these observations suggest that the relative importance of each control mechanism may differ among muscles of different fiber types (70).

## Endogenous Opiates

Few other scientific fields have been plagued by widespread dissemination in the popular press, and phrases

such as "the exercise high" have become part of the current exercise mythology (98). While scientists and lay persons alike may believe that exercise can produce a "high", and while the concept of a "runner's high" has become a popular topic of conversation, discussions to date have been vague and nebulous (78). As a result, we find ourselves entering into one of the newer fields of exercise endocrinology--that of the endogenous opioids.

In 1975, Hughes et al. (63) reported the isolation of the first endogenous opiate peptides, methionine enkephalin and leucine enkephalin in pig brain. These studies utilized classical pharmacological bioassays and demonstrated that these opioids possessed naloxone-reversible activity (54). This resulted in an explosion of interest in the nature and physiology of opiate peptides and their receptors (66).

There is evidence of four classes of endogenous opioid peptides (TABLE II), each with characteristic precursors, distribution, and receptor affinities (21,54).

Radioimmunoassay (RIA) has become the most important technique for the measurement of opioid peptides in body fluids or extracts with the related immunohistochemical techniques allowing precise localization in tissue sections (21). All human studies to date have used radioimmunoassay, with one exception (40).

Since the discovery of the opiate peptides, vast amounts of research have been carried out which attempt to discover more about the nature, function, and action of the peptides. The term opioid is used to designate a group of

TABLE II

PEPTIDES OF THE SUPERFAMILY ENDORPHIN

| | |
|---|---|
| I. | Peptides of the pro-opiamelanocortin series |
| | A. Opioid peptides |
| | β-endorphin |
| | α-endorphin |
| | γ-endorphin |
| | B. Nonopioids |
| | βMSH |
| | γMSH |
| | $\gamma MSH_3$ |
| II. | Enkephalins |
| | Met-enkephalin |
| | Leu-enkephalin |
| | Met-Arg-Phe-enkephalin |
| III. | C-terminally extended enkephalins |
| | Dynorphin |
| | α-neo endorphin |
| | β-neo endorphin |
| IV. | Others |
| | Kyotorphin |
| | Dermophin |
| | Casei-Morphin |

Source: (21)

drugs that are to varying degrees, opium- or morphine-like in their properties (50).

The opioids are employed primarily as analgesics, and have been known to produce a state of euphoria (78). But, they have many other pharmacological effects as well. The opiates can also create dependence (42). Several of these characteristics may be important during the stress of exercise (42,50).

The superfamily "endorphin" includes all endogenous peptides whose sequences include an enkephalin pentapeptide (either methionine enkephalin or leucine enkephalin) and share some common actions at presumptive opiate receptors as defined by naloxone antagonism (21).

Opioids interact with what appear to be several closely related receptors (50). It is generally recognized that there are several opioid receptor types, --mu, kappa, sigma, delta, and epsilon receptors--responding differentially to various opiate agonists, with the primary mu agonist being morphine. The delta receptor responds mostly to the enkephalins, and the epsilon receptor's primary agonist is beta-endorphin (80). Some of these receptors (the mu, kappa, and delta sites) can be assessed by both functional response and binding assays (21).

It has been shown that some specific agonists can affect several different receptor types, and a single receptor type can be affected by more than one agonist (80). The same review reports that various species have varying kinds and amounts of the multiple receptors and that there are even differences among strains within the

same species. It can be concluded that the overall picture concerning opiate receptors is still not clear.

The opioid peptides are nearly ubiquitous, as they are produced in the brain, hypothalamus, spinal cord, pituitary gland, adrenal gland, gastrointestinal tract, and other sites (54,66,78,80,90). Evidence indicates that endorphins are released from the pituitary gland in response to stress concomitantly with the hormone adrenocoricotropin (ACTH) (32). High levels of plasma methionine enkephalin are found in the adrenal vein, and extremely high concentrations of enkephalins are co-stored and co-released with catecholamines in the adrenal medulla (54). However, the adrenal cannot be the sole origin of circulating methionine enkephalin, as there is no obvious change in the levels of circulating methionine enkephalin in adrenalectomized subjects (54,66). However, most evidence suggests that the majority of circulating methionine enkephalin originates in the sympathetic nervous system (54). The fact that endorphins are produced by so many sources has made it difficult to interpret peripheral levels of endorphins (90).

The literature regarding enkephalin transport through the brain endothelial wall (the blood-brain barrier) is conflicting (81). Endorphins from the brain, hypothalamus, and spinal cord may not be reflected in peripheral levels because endorphins do not cross the blood-brain barrier (90). It does remain possible, however, that the blood-brain barrier may permit centrally produced substances to enter the peripheral circulation during exercise (90).

Central to the ultimate interpretation of the potential involvements of the endogenous opiates is the issue of whether plasma-borne endorphins are "functional" as judged by opiate receptor pharmacology (21). This question has potential answers at two levels of currently unanswerable competency: 1) blood-borne endorphins either do or do not enter the central nervous system. If they do, then a large number of potential central nervous system phenomena are open to manipulations of peripheral endorphin fluctuations. 2) If they do not cross the blood-brain barrier, then blood-borne peptides must act at peripheral receptors. This could occur either through direct peripheral actions or through actions on parts of the central nervous system that are not guarded by the blood-brain barrier (21).

Effects of Exercise

It has been convincingly demonstated that the physiological stress of exercise in humans is consistently associated with increases in plasma beta-endorphin (65). Studies during exercise have suggested that increases in serum concentrations of endorphins occur and that they may have important physiological actions (98). An increase in the peripheral plasma levels of beta-endorphin in humans after exercise has been noted by all investigators to date (40).

The adrenal response to exercise is representative of a general response to stress that also occurs with anxiety. The central trigger for this response is brain or hypo-

thalamic production of proopiomelanocortin that contains both adrenocorticotropin (ACTH) and beta-endorphin within its structure (90). Of the studies in which blood endorphins have been measured, none used the same protocol in terms of intensity, duration, and mode of exercise. The exact length of runs are not clearly stated in many of the studies and in most, the runners are of undetermined or subjective fitness levels. Therefore, comparisons between the different studies is difficult.

Researchers are somewhat plagued by the fact that despite extensive efforts, the basic physiological functions of the endogenous opiate systems are as yet unclear (40). Farrell (40) evaluated nine male and five female runners before and after a ten mile road race. The mean plasma leucine enkephalin-like radioreceptor activity (leu-ERA) increased from 22.2 pmol/ml prior to the race to 26.1 pmol/ml following the race. Appenzeller and co-workers (5) reported a 71 percent increase in endorphin levels after a 28.5 mile race which coursed along a mountain trail at high altitudes. In another study, Colt et al. (30) measured plasma endorphins in trained long distance runners before and after both a strenous and an easy run. He reported percent increases of 9 to 241. Another study utilized five untrained subjects, who ran 20 minutes on a treadmill at variable speeds to maintain heart rate at 80 percent maximum (47). There was a 440 percent mean increase in circulating beta-endorphin immuno-reactivity after exercise.

Fraioli et al. (46) measured endorphin levels in eight "fit" subjects during and after a maximal graded exercise test on a treadmill (8 to 15 minutes). The author found relatively large increases in endorphins for the relatively short period of exercise duration.

Currently available measurements of beta-endorphin among exercising women are in excellent agreement and indicate a two to three-fold increase over basal levels (76). However, there appears to be a difference between males and females. Gambert et al. (48) found that 20 minutes of submaximal treadmill running was associated with an elevation in plasma levels of beta-endorphin immunoreactivity. This increase was greater in men (14.9 ± 3.4 fmole/ml) than women (2.6 ± 1.2 fmole/ml) exercising at the same percentage of their predicted maximum heart rates. It was reported in another study that plasma levels incresed as much as 440 percent, again with greater changes in men than women (49,80).

Endogenous opiate levels also appear to be elevated following bouts of low intensity exercise. Dearman et al. (32) reported a 25 percent increase following a two mile run, while a six mile run produced a 132 percent increase. Colt et al. (30) also compared exercise at two different intensities. Following an easy run, beta-endorphin immunoactivity increased from 11.8 ± 1.8 to 17.6 ± 3.1 pg/ml. These levels were increased from 8.2 ± 1.03 to 28.0 ± 6.3 pg/ml following a stenuous run. In still another study conducted by Boarder et al. (22), mild stress consisting of two minutes of deep knee bends

produced a two-fold elevation in overall circulating opioid activity.

Running is not the only type of exercise in which opioid levels are reported to increase. Many studies utilize swimming in rodents (27,31,40,78,99,102). Beta-endorphin has been shown to be secreted by the pituitary of the rat in parallel with adrenocorticotropin (ACTH) in response to the stress of swimming 20 to 30 minutes in water of 20$^{\circ}$ C (102). In a study by Cooper et al. (31), the stress of swimming produced opioid-related analgesia in as short as 15 seconds in rodents. With longer swims, the magnitude of analgesia increases. Decreasing the water temperature also increases the analgesia slightly.

The hormonal changes previously reported with endurance activities also occur with burst activity exercise. Elliot et al. (35) reported significant increases in beta-endorphin/beta-lipotrophin immunoactivity following weight training.

The rise in peripheral peptide levels is reported to be facilitated by training (25,32,41,42,47,48,90). Berk et al. (18) reported that trained athletes demonstrated increased levels of endorphins of 169 percent following a maximal graded exercise test. Untrained athletes were reported in the same study to have had an increase in endorphins of 130 percent. Carr et al. (25) reported a 57 percent increase in endorphin levels in fit subjects after they exercised on bicycles at 85 percent $\dot{V}O_2$ maximum for 45 minutes. He also concluded that training augments the increases in plasma levels of beta-endorphins and its

precursor, beta-lipotropin.

It is firmly established that as exercise intensity increases, plasma levels of most hormones increase (40). Some studies show that unlike most other endocrines, plasma levels of beta-endorphin do not increase proportionally to work intensity (40). Others indicate that the more physically strenuous the activity, the greater the percent increase of endorphin levels (30,32).

Changes in plasma levels of endorphin immunoreactivity may be responsible for some of the euphoria and analgesia anecdotally associated with running (48).

Hypothalamic beta-endorphin but not adrenocorticotropin (ACTH) decreases significantly after stress. This decrease may represent a net shift of beta-endorphin from its site of synthesis in hypothalamic neuronal cell bodies to the beta-endorphin fiber system which extends throughout the brain, and may be related to the reported phenomenon of stress-induced analgesia in rats (102).

One author has postulated that the stress of exercise acts as a stimulus to greater endorphin secretion, a reduction in its degradation, or a combination of these, which leads to increased levels of these ligands in the blood (42).

Opiate Antagonism

Naloxone, the most specific opioid antagonist will promptly reverse or prevent some effects of the opioid agonists (80). A 10 μg/kg dose was found to consistently

and significantly antagonize completely all of the arteriolar and precapillary actions of all the opiate agents (3) as well as prevent the reduction in neurointermediate lobe immunoreactive beta-endorphin levels otherwise found following prolonged stress (74).

Since naloxone does generally increase blood pressure, it has been used to treat shock. In almost all instances it reversed the shock and prolonged survival time, regardless of the cause of the shock (80,59).

Cardiovascular Responses

A surge of interest in opiate-cardiovascular interactions followed the discovery of endogenous opiate systems (59). A family of opioid peptides were found to be located at sites suggesting an autonomic action (103); also, opiate receptors were shown to be densely distributed in the brainstem and hypothalamus in close proximity to cardiovascular centers as well as endogenous opiate pathways (14,51).

In the brainstem, several nuclei are integrally related in the functional maintence of cardiovascular homeostasis. The central integration of various inputs ultimately results in an orchestration of autonomic cardiovascular responses through alterations in variables such as heart rate, cardiac contractility, peripheral resistance, and adrenal medullary outflow (59). It is possible that vascular smooth muscle contain opiate receptors (2), and preliminary evidence suggests that different cardiovascular responses are mediated by different receptors (59).

In a recent study by Eulie et al. (38), a drop in blood pressure was associated with an intravenous injection of methionine enkephalin in anesthetized rabbits. This resulted in increased vascular resistance in most visceral tissue and decreased vascular resistance in skeletal muscle tissue, and hence increased skeletal muscle blood flow.

Wong et al. (104) reports that beta-endorphin, enkephalins, and morphine dilated the arterioles of the micro-circulatory system of the hamster cheek pouch studied in vivo. Still other studies have shown that beta-endorphin (79,85) and the enkephalins (15) lowered blood pressure in anesthetized normal and hypertensive animals.

It has been proposed by Holaday et al. (19,59,60) that endogenous opiates, released by the severe stress accompanying shock, would contribute to the decrease in circulatory function that characterizes the shock syndrome. The opiate antagonist naloxone improves cardiovascular function in several models of shock (19,59, 60,80).

Opiate receptors were found in the cardiac muscle in the right ventricle, and it was proposed that they may have some function in the syndrome of shock, although the specific mechanism involved was not postulated (23,80).

The endogenous opioid peptides also appear to play a role in exercise thermoregulation (65). Increasing exercise thermoregulatory stress has been associated with increased peripheral beta-endorphin concentration (65).

Enkephalins in the circulation appear to come pre-

dominantly from the adrenal medulla. By contrast, beta-endorphin in the circulation originates from the anterior and intermediate lobes of the pituitary gland (59). Concern has been expressed about measuring plasma beta-endorphin and correlating those concentrations with central nervous system or behavioral phenomena like euphoria or analgesia because it was felt that beta-endorphin does not penetrate the blood-brain barrier (30). However, in an exercise context, it has been demonstrated that stress of sufficient intensity can breach the blood-brain barrier to certain molecules (76). Thus, it is possible that exercise might facilitate the entry of compounds, otherwise excluded, into the brain.

Although they share some important structural similiarities, their differential synthesis, distribution, and release in central and peripheral tissues suggests that endogenous opiates may function as neurotransmitters, hormones, or neuromodulators with effects upon a variety of physiological and behavioral systems involved in regulatory blood flow (59). There is also evidence that suggests that endorphins are involved in the control of catecholamine release from the adrenal medulla (32). Exercise-induced changes in circulating catecholamines are markedly enhanced by the opiate antagonist naloxone (53).

The data of Eulie et al. (38) suggest that the hypotensive effects of methionine enkephalin are due to vasodilation in skeletal muscle vascular beds. Opiates can exert concentration-dependent vasodilator effects on intact terminal arterioles and pre-capillary sphincters, but not

venules (3). This strongly suggests the presence of opiate receptors in the microcirculation. Pert and Bowie (82) reported an increase in opiate receptor occupancy in rats forced to perform "mild" exercise by running in an activity wheel for 15 minutes. They concluded that this was due either to an increased release of enkephalin or endorphin onto opiate receptors or else a slowing of their dissociation rate from receptors.

Methionine enkephalin reduced the rate of sympathetic nerve discharge in parallel with the reduction of blood pressure (37). These data suggest that methionine enkephalin might reduce sympathetic tone selectively so that specific vascular beds might be selectely dilated. Another study by the same authors showed a decrease in resistance in skeletal muscle beds only (38). This suggests that a generalized sympathetic withdrawal is not the mechanism.

The available data, therefore, indicate that opioids can cause vasodilation in the vascular beds of skeletal muscle both by direct actions (3,82) and by indirect influences via the sympathetic nervous system (37). This fact combined with the fact that exercise stress causes increased blood levels of opioids make it reasonable to propose that the endogenous opioids may be involved in the control of muscle blood flow during exercise.

# CHAPTER III

# METHODS AND PROCEDURES

## Experimental Protocol for Anesthetized Animal Studies

### Drug Dose

Intravenous injections of methionine enkephalin (Sigma Chemical Company, St. Louis, MO) were given to anesthetized male Sprague-Dawley rats. The drug was dissolved in saline just prior to the experiment to give doses ranging from 20 to 2000 μg/kg of the enkephalin. The drug was injected via the catherized femoral vein (PE-10) and flushed with saline. Approximately five minutes were allowed between doses, or until cardiovascular parameters stablized at baseline, whichever was longer. Blood pressure was measured via a catheter (PE-50) placed in the carotid artery using an Ailtech pressure transducer and a Gould Brush chart recorder (model 2800).

A dose response curve was obtained for methionine enkephalin which demonstrated that a dose of 1000 μg/kg produced the greatest drop in blood pressure. It was also confirmed that a standard naloxone (Sigma Chemical Company, St. Louis, MO) dose of ten μg/kg (42) completely blocked the blood pressure response produced by enkephalin injection. Dosing with the enkephalin began ten minutes

after pre-treatment with naloxone (36,37).

Cardiovascular Response

It was necessary to to determine if the effects of injected enkephalin were consistent with those in other species. Therefore, a pilot study was done using five anesthetized male Sprague-Dawley rats (629 ± 20 g body weight).

Experimental Procedure

The rats were anesthetized by injection of pentobarbital sodium (Nembutol) (30 mg/kg) into the abdominal cavity.

Each rat was ventilated with a positive pressure respirator (Havard) using room air, and instrumented with the following: 1) a PE-50 catheter advanced into the right femoral artery for blood pressure measurement and reference blood withdrawal, 2) a PE-10 catheter in the right femoral vein for drug infusions, and 3) a PE-50 catheter in the right carotid artery for microsphere infusion. Radiolabeled ($^{46}Sc$, $^{85}Sr$, $^{113}Sn$, $^{57}Co$, $^{153}Gd$, and $^{95}Nb$) microspheres (New England Nuclear and 3M), 15 μm diameter were used for these studies. The microsphere suspensions consisted of 500,000 spheres in 0.1 ml of saline containing less than 0.1 percent Tween 80. The suspensions were mixed in a Mettler Electronics ultrasonicator (HE1AR) for ten minutes and then on a Vortex for 15 seconds prior to infusion to assure a uniform distribution of spheres (61,73). Each microsphere infusion was performed as

follows: 1) the reference withdrawal was started at a rate of 0.618 ml/min; (2) ten seconds later, 0.1 ml of microsphere suspension was injected into the carotid catheter; 3) 1.0 ml of warm (37° C) saline was infused over a period of 20 to 30 seconds; and 4) the reference withdrawal continued until a 1.0 ml sample was obtained. This approach to the use of the microsphere technique has been shown to be valid by Laughlin et al. (71). Figure 6 demonstrates interfibrillar locations of the microshperes.

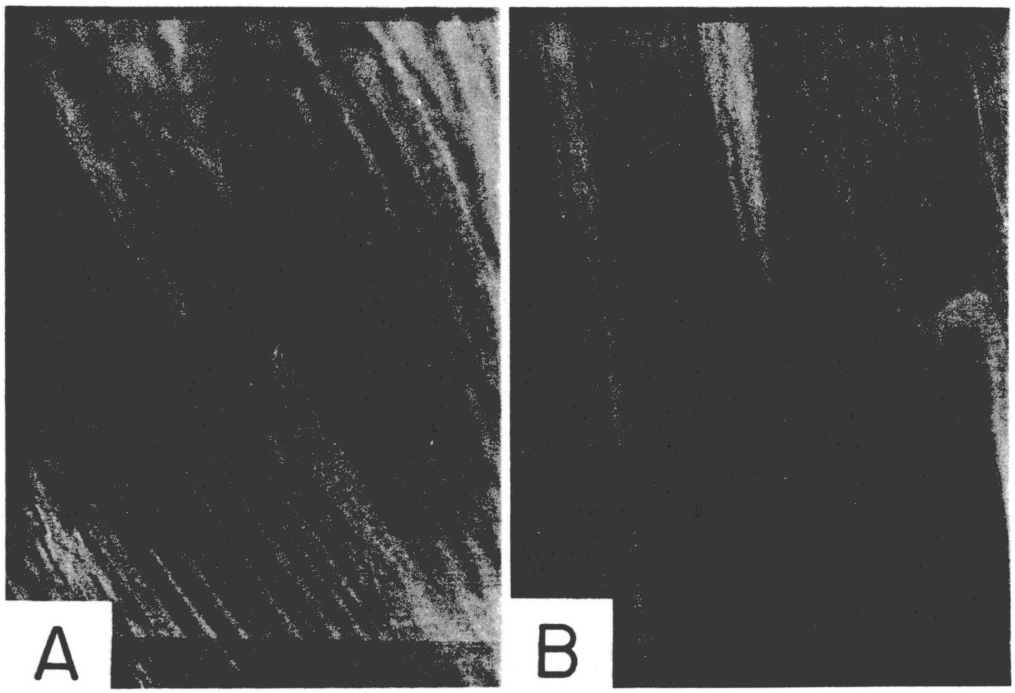
The radiolabeled microspheres were given at three different times: baseline, during enkephalin infusion (1000 µg/kg) using a Harvard infusion/withdrawal pump (Model 902) at a rate of .591 ml/min., and following a ten µg/kg injection of naloxone.

Twelve selected muscle samples and both kidneys were dissected, blotted, weighed, placed in counting vials, and along with the reference blood sample were transferred to a three-channel Beckman 8000 Gamma counter. Blood flow was computed from counts/minute and tissue wet weight with a Digital Equipment PDP 11/03 coumputer according to standard procedures (57,71). Vascular resistance was also calculated ($R = \Delta BP/BF$).

## Experimental Protocol for Conscious Animal Studies

### Animal Care and Training

Male Sprague-Dawley rats (250-350 g body weight) were housed in cages in a room maintained at 23 ± 1° C with days artificially divided into 12 hours each of light and




Source: (71).

Figure 6. Photomicrographs demonstrating interfibrillar locations of single (A) and paired (B) microspheres in 60-μm sections of soleus muscle (x600).

darkness. Food and water were provided ad libitum. All animals were exercised on a motor-driven treadmill at 15 meters/minute for ten minutes/day, for two weeks prior to the experiments. At the end of this period, the rats were not considered to be "exercise trained" in a physiological sense, but "trained to exercise".

Surgical Procedure

At the end of the training period, each rat (353-439 g body wt) was anethetized with pentobarbital sodium (initial dose of 30 mg/kg IP, supplemented doses administered as required). A 1.5 centimeter incision was made on the ventral surface of the rat's tail about one centimeter from its origin. Polyethylene tubing (PE-50) filled with sodium heparin was inserted into the ventral tail artery for a distance of seven to eight centimeters. The tubing was then fixed in the artery and sutured to the tailskin, reflected dorsally and anteriorly, and led subcutaneously through a 25 centimeter length of 15-gauge hollow-needle tubing to emerge at the dorsal cervical region, as described by Chiueh et al. (26).

A second catheter, the microsphere infusion catheter, a 19 centimeter section of silastic tubing (ID 0.6 mm, OD 1.0 mm) was filled with heparinized saline and inserted approximately 30 to 35 millimeters into the right carotid artery. A suture was placed through the sternohyoideus muscle and around the catheter and tied securely. The ventral incision was closed using nine millimeter wound clips. The catheter was exteriorized adjacent to the

caudal catheter. To secure the extruding catheters, a stitch was made using a 0 silk suture, six millimeters posterior to the catheter exit site and tied loosely around the catheters. The rat was given 0.1 ml Bicillin ($3 \times 10^{-5}$ U/ml). This technique was developed and described by Laughlin et al. (71). Figure 7 illustrates the catheter placement.

Both catheters were filled with a 50 percent glucose solution containing 500 U/ml sodium heparin, and flushed and refilled twice the following day.

Because catheter patency is a problem in the use of chronically instrumented animals, and because trouble existed with the rats pulling their catheters or plugs out, the shortest possible time for surgical recovery (one day) was allowed.

Experimental Protocol

The rats were randomly divided into two groups: a control group given a sham saline injection, and an experimental group given a 10 µg/kg dose of naloxone.

The rat was given either saline or naloxone and the exercise was started. Following five minutes of walking at a speed of 15 meters/minute, the first microsphere injection was given. The treadmill was then turned off and after five minutes of rest, at which point the rat's blood pressure had returned to pre-exercise levels, the baseline microsphere injection was given.

Following the last microsphere injection and reference withdrawal, the rats were given a one ml dose of Nembutol

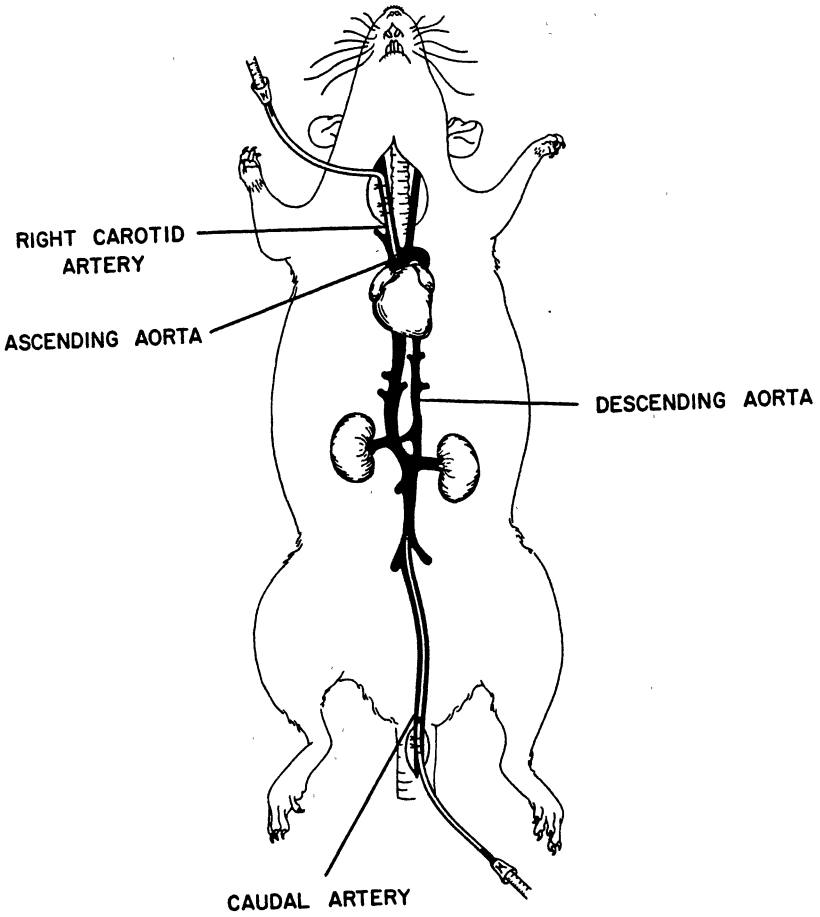



Figure 7. Locations of right carotid artery catheter for microsphere infusion into ascending aorta and caudal artery catheter for withdrawal of reference samples.

and decapitated. Sixty-one tissue samples were taken with some of the muscles subdivided into red, white, and mixed portions. They were blotted, weighed, placed in counting vials, and, along with the blood samples transferred to a Packard Auto-Gamma counter (5780). Blood flow was again computed from counts/minute and tissue wet weight using an IBM PC computer according to standard procedure (57,71).

Physiological Measurements

Throughout each experiment, mean aortic blood pressure was recorded from the carotid catheter with an Ailtech pressure transducer and a Gould Brush chart recorder (Model 2400S). Only mean aortic pressure is reported because long, small-bore silastic catheters were used for the measurements, precluding accurate determination of systolic and diastolic pressures.

Data Analysis

The Mann-Whitney U test was used to compare blood flows within muscles or tissues across conditions at the 0.5 significance level. The same test was also used to compare blood pressure across conditions.

# CHAPTER IV

# RESULTS AND DISCUSSION

## Anesthetized Animal Results

The results of the preliminary study showed that methionine enkephalin did cause vasodilation in skeletal muscle vascular beds. This was shown by decreased resistance in all muscles sampled except red and white portions of the gastrocnemius (TABLE III). This vasodilation could be blocked by a standard dose of naloxone. Heart rate did not differ significatly, whereas methionine enkephalin caused a decrease in blood pressure (TABLE IV). These results are similar to those reported for other species (36,37).

## Conscious Animal Results

### Blood Pressure

Arterial blood pressure data for control and naloxone pre-treated groups are presented in TABLE V. Mean arterial pressure was not significantly changed in the two groups.

### Baseline Muscle Blood Flow

Muscle blood flow data during baseline conditions are presented in TABLES VI and VII, and Figure 8. Baseline

TABLE III

CALCULATED RESISTANCE IN SELECTED MUSCLES
OF ANESTHETIZED ANIMALS

| Muscle | BL (5) | ENK (6) | NAL (6) |
|---|---|---|---|
| VI | 27 ±9 | 21 ±6 | 54 ±20 |
| VM | 46 ±20 | 38 ±19 | 224 ± 166 |
| $VL_R$ | 13 ±6 | 13 ±7 | 36 ±8 |
| $VL_W$ | 71 ±40 | 31 ±13 | 232 ± 170 |
| $VL_M$ | 45 ±17 | 26 ±12 | 58 ±15 |
| $BF_A$ | 46 ±9 | 39 ±22 | 83 ±20 |
| $BF_P$ | 59 ±21 | 38 ±15 | 43 ±10 |
| $G_R$ | 37 ±28 | 54 ±24 | 63 ±36 |
| $G_W$ | 24 ±6 | 45 ±30 | 150 ±65 |

Values are means ± SEM in ml/min/100g at baseline conditions (BL) and methionine enkephalin (ENK) and naloxone (NAL) injections. The number of animals is given in parenthesis. Abbreviations for muscle samples are the same as those presented in TABLE I.

TABLE IV

HEART RATE AND BLOOD PRESSURE RESPONSE
OF ANESTHETIZED ANIMALS

| | BL (6) | ENK (6) | NAL (6) |
|---|---|---|---|
| HR | 249 ±1 | 250 ±1 | 250 ±1 |
| $\bar{P}_a$ (torr) | 141 ±12 | 105 ±8 | 137 ±9 |

Values are means ± SEM at baseline conditions (BL), and methionine enkephalin (ENK) and naloxone (NAL) injections. Mean arterial pressure ($\bar{P}_a$) and heart rate (HR) were recorded from the femoral artery prior to microsphere infusions. The number of animals is given in parenthesis.

TABLE V

MEAN ARTERIAL PRESSURE AT FIVE MINUTES OF TREADMILL RUNNING AT 15 METERS/MINUTE AND AT BASELINE CONDITIONS IN CONTROL AND EXPERIMENTAL GROUPS

| | 5 m | | BL | |
|---|---|---|---|---|
| | Control (6) | Naloxone (4) | Control (5) | Naloxone (4) |
| $\bar{P}_a$ (torr) | 118 ±10 | 125 ±14 | 115 ±15 | 120 ±17 |

Values are means ±SEM. Mean arterial pressure ($\bar{P}_a$) was recorded from the carotid artery prior to microsphere infusions. The number of animals is given in parenthesis.

TABLE VI

BLOOD FLOW IN THIGH MUSCLE SAMPLES AT FIVE MINUTES OF TREADMILL RUNNING AT 15 METERS/MINUTE AND AT BASELINE CONDITIONS IN CONTROL AND EXPERIMENTAL GROUPS

| Muscle | 5 m | | BL | |
|---|---|---|---|---|
| | Control (6) | Naloxone (4) | Control (5) | Naloxone (4) |
| Knee Extensors | | | | |
| VI | 311 ±60 | 357 ±40 | 55 ±28 | 41 ±8 |
| VM | 118 ±32 | 150 ±30 | 10 ±5 | 7 ±1 |
| $VL_R$ | 267 ±52 | 325 ±56 | 28 ±16 | 16 ±3 |
| $VL_W$ | 17 ±5 | 10 ±1 | 7 ±2 | 5 ±1 |
| $VL_M$ | 110 ±26 | 141 ±26 | 10 ±4 | 8 ±1 |
| $RF_R$ | 185 ±35 | 190 ±32 | 22 ±11 | 7 ±1 |
| $RF_W$ | 64 ±13 | 66 ±12 | 10 ±3 | 7 ±1 |
| Knee Flexors | | | | |
| $BF_A$ | 11 ±3 | 8 ±3 | 6 ±1 | 4 ±1 |
| $BF_P$ | 37 ±11 | 41 ±18 | 8 ±2 | 7 ±1 |
| ST | 42 ±8 | 38 ±3 | 8 ±2 | 7 ±1 |
| $SM_R$ | 57 ±17 | 73 ±38 | 14 ±4 | 6 ±1 |
| $SM_W$ | 35 ±8 | 37 ±16 | 8 ±1 | 6 ±1 |
| Thigh Adductors | | | | |
| AL | 279 ±74 | 216 ±66 | 138 ±46 | 158 ±28 |
| GRA | 26 ±6 | 16 ±1 | 14 ±5 | 9 ±2 |
| PEC | 52 ±15 | 44 ±25 | 32 ±14 | 12 ±4 |
| AD | 58 ±22 | 59 ±28 | 12 ±4 | 8 ±2 |

Values are means ±SEM in ml/min/100 g. The number of animals is given in parenthesis. Abreviations for muscle samples are the same as those shown in TABLE I.

TABLE VII

BLOOD FLOW IN LEG MUSCLE SAMPLES AT FIVE MINUTES OF TREADMILL RUNNING AT 15 METERS/MINUTE AND AT BASELINE CONDITIONS IN CONTROL AND EXPERIMENTAL GROUPS

| Muscles | 5 m | | BL | |
|---|---|---|---|---|
| | Control (6) | Naloxone (4) | Control (5) | Naloxone (4) |
| Ankle Extensors | | | | |
| SO | 259 ±53 | 290 ±69 | 78 ±25 | 81 ±18 |
| PL | 109 ±35 | 120 ±44 | 8 ± 2 | 8 ± 2 |
| $G_R$ | 238 ±54 | 291 ±54 | 34 ± 8 | 42 ±20 |
| $G_W$ | 11 ± 3 | 10 ± 6 | 8 ± 3 | 4 ± 1 |
| $G_M$ | 85 ±24 | 103 ±35 | 12 ± 2 | 12 ± 2 |
| TP | 113 ±15 | 77 ± 3 | 14 ± 4 | 11 ± 4 |
| FDL | 58 ±20 | 55 ±29 | 16 ± 5 | 9 ± 5 |
| FHL | 35 ± 6 | 35 ± 8 | 11 ± 3 | 10 ± 2 |
| Ankle Flexors | | | | |
| $TA_R$ | 167 ±29 | 180 ±64 | 14 ±10 | 6 ± 2 |
| $TA_W$ | 71 ±12 | 83 ±18 | 12 ± 4 | 9 ± 2 |
| EDL | 43 ± 6 | 30 ± 5 | 10 ± 3 | 8 ± 1 |
| PER | 100 ±26 | 105 ±38 | 8 ± 2 | 9 ± 2 |

Values are means ± SEM in ml/min/100g. The number of animals is given in parenthesis. Abbreviations for muscle samples are the same as those presented in TABLE I.

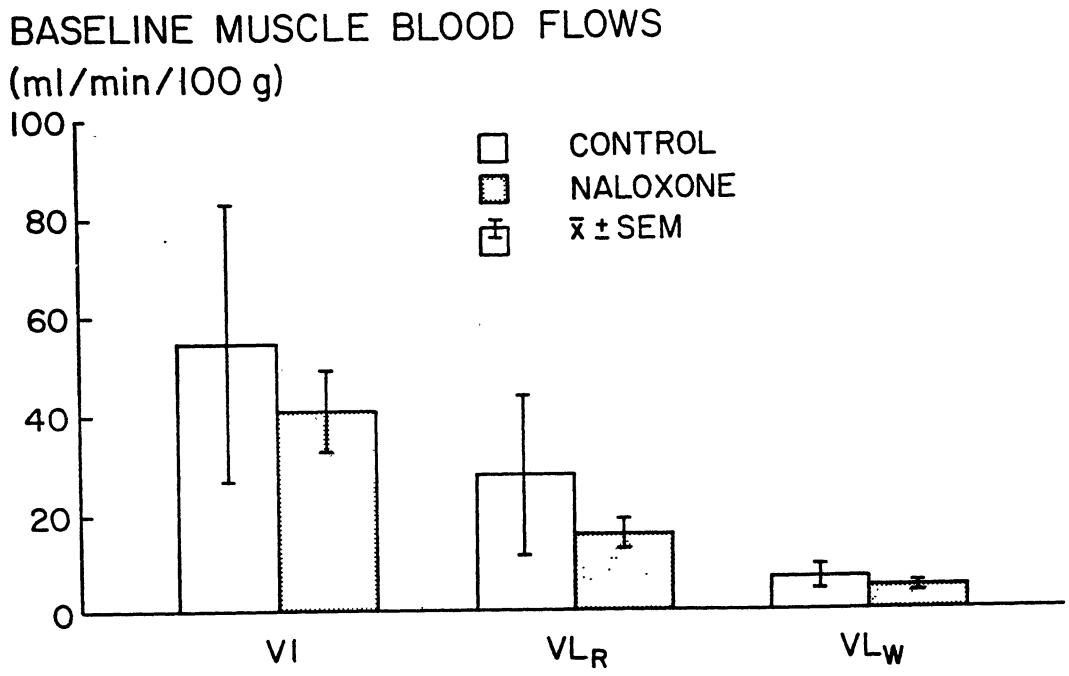



Figure 8. Baseline muscle blood flow of muscles representative of the three muscle fiber types in control and naloxone pre-treated groups. Values are means ± SEM in ml/min/100g. Abbreviations for muscle samples are the same as those presented in TABLE I.

blood flows ranged from 6 ($BF_A$) to 138 (AL) ml/min/100g in the control rats, and from 4 ($BF_A$ and $G_W$) to 158 (AL) in the experimental rats. These values for baseline conditions are comparable to those reported by Laughlin et al. (71). There were no differences between blood flow in the control and naloxone pre-treated rats.

Exercise Muscle Blood Flow

Muscle blood flow data at five mintues of treadmill exercise at 15 meters/minute are also presented in TABLES VI and VII, and in Figure 9. Figure 10 illustrates total muscle blood flow. Exercise blood flows ranged from 11 ($BF_A$ and $G_W$) to 311 (VI) ml/min/100g in the control rats, and from 8 ($BF_A$) to 357 (VI) in the experimental groups.

Statistical analysis showed blood flow to be significantly different in only one muscle between the two conditions. The particular muscle was the red portion of rectus femoris. In light of the fact that 32 muscle samples were taken, a significant difference in only one suggests that naloxone does not affect muscle blood flow during low intensity exercise.

Organ Blood Flow

There were no significant differences between the blood flows of the control and naloxone pre-treated rats. These data appear in TABLE VIII. Ileum blood flow is illustrated in Figure 11.

## Discussion

The purpose of this study was to determine the role the

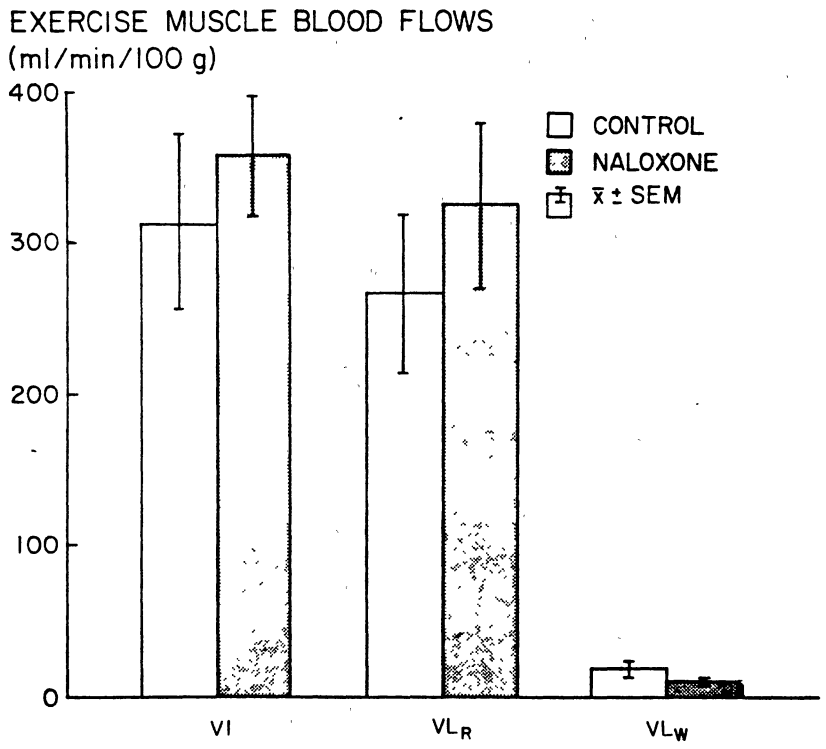



Figure 9. Exercise muscle blood flow of muscles representative of the three muscle types in control and naloxone pre-treated groups. Values are means ± SEM in ml/min/100g. Abbreviations for muscle samples are the same as those presented in TABLE I.

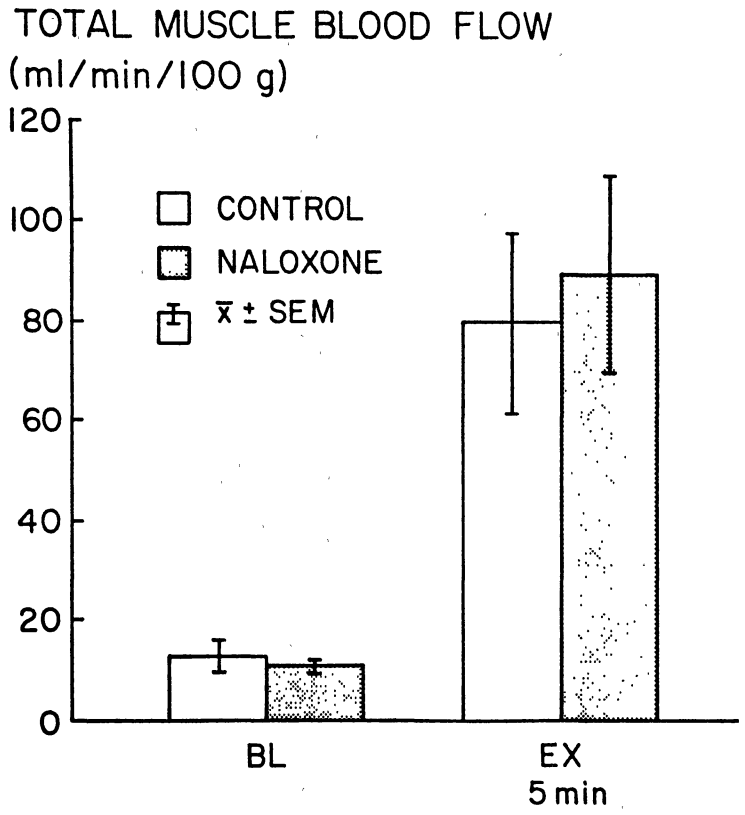



Figure 10. Total muscle blood flow during baseline (BL) and exercise (EX) in control and naloxone pre-treated groups. Values are means ± SEM in ml/min/100g.

# TABLE VIII

## ORGAN BLOOD FLOWS AT FIVE MINUTES OF TREADMILL RUNNING AT 15 METERS/MINUTE AND AT BASELINE CONDITIONS IN CONTROL AND EXPERIMENTAL GROUPS

| Organ | 5 m | | BL | |
|---|---|---|---|---|
| | Control (6) | Naloxone (4) | Control (5) | Naloxone (4) |
| Brain | 137 ±43 | 140 ± 74 | 106 ±34 | 87 ±36 |
| Diaphram | 100 ±28 | 109 ± 53 | 82 ±28 | 57 ±24 |
| Liver | 66 ±20 | 79 ± 37 | 54 ±15 | 82 ±30 |
| Spleen | 103 ±22 | 138 ± 36 | 282 ±42 | 317 ±129 |
| Kidney | 487 ±95 | 637 ±105 | 540 ±27 | 431 ±44 |
| Epididymal Fat | 7 ±4 | 8 ±5 | 11 ±3 | 9 ±4 |
| Inguinal Fat | 19 ±4 | 24 ±8 | 26 ±7 | 22 ±10 |
| Skin | 7 ±1 | 7 ±2 | 7 ±1 | 8 ±2 |
| Lung | 91 ±72 | 118 ± 121 | 58 ±24 | 26 ±9 |
| Stomach | 65 ±5 | 71 ±20 | 69 ± 6 | 72 ±17 |
| Duodenum | 286 ± 41 | 400 ± 115 | 312 ±31 | 323 ±68 |
| Jejunum | 250 ±45 | 325 ± 83 | 276 ±47 | 290 ±76 |
| Ileum | 175 ±23 | 230 ± 51 | 226 ±34 | 242 ±64 |
| Cecum | 197 ±38 | 203 ± 60 | 224 ±17 | 208 ±44 |
| Colon | 102 ±23 | 87 ± 17 | 90 ±8 | 101 ±22 |
| Testes | 23 ±3 | 27 ± 5 | 30 ±4 | 32 ±3 |

Values are means ± SEM in ml/min/100 g. The number of animals is given in parenthesis.

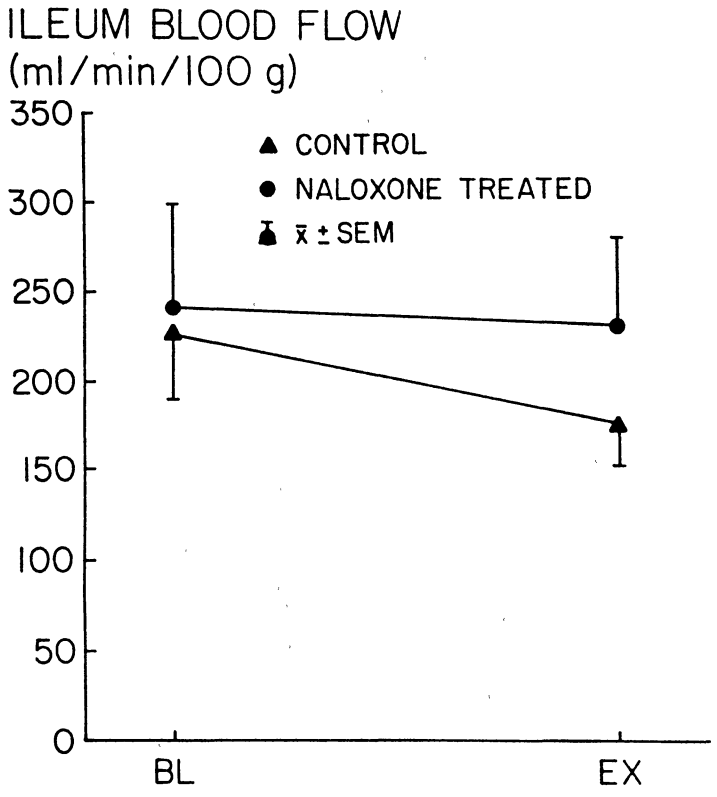



**Figure 11.** Ileum blood during baseline (BL) and exercise (EX) in control and naloxone pre-treated groups. Values are means ± SEM in ml/min/100g.

endogenous opioids play in the control of muscle blood flow. Although much research has been done to elucidate the factors that regulate blood blood flow in muscles, a clear unified picture has not emerged.

The changes seen in blood flow to skeletal muscles as a result of exercise are due to reductions in resistance in the vascular beds. This decrease in resistance may result from myogenic, neurohumoral, and local metabolic interactions as discussed earlier.

Emerging evidence suggests that endogenous opiate peptides may be linked to various functions associated with exercise. There are several studies which document increases in the opioid levels in the blood as a result of exercise (1,5,25,27,30,31,32,41,46,48,78). Eulie data (38) indicate that the effects of opioids on blood pressure may be the result of changes in the skeletal muscle vascular beds. Therefore, it seems possible that opioids contributed to the control of muscle blood flow during exercise.

The rationale for this study was based on the fact that if opioid peptides are involved in the control of muscle blood flow, blocking the opioid receptors with naloxone should alter muscle blood flow distribution during exercise.

No significant differences were observed in muscle blood flow between the control group and the naloxone pretreated group. It can be concluded that naloxone blockade does not affect the distribution of muscle blood flow during low intensity exercise in rats, and hence, the

endogenous opioids are not involved in producing the normal exercise hyperemia of skeletal muscle in rats.

While it is clear that opioids are not involved in the normal exercise hyperemia of skeletal muscle of rats at this intensity of exercise, two other considerations must be kept in mind in interpreting these data. It is possible that the exercise intensity and time chosen were not stressful enough to cause a release of the endogenous peptides. Thus, the opioids may be involved at higher exercise intensities and at longer durations of low intensity exercise. However, in a study by Cooper et al. (31), the stress of swimming produced opioid-related analgesia in as short as 15 seconds in rodents. The metabolic cost ($\dot{V}O_2$) of swimming in rats has been shown to be equivalent to the rats walking on a treadmill at 15 meters/minute by Armstrong (70). Therefore, it is not unreasonable to expect that opiates are released into the peripheral circulation at the intensity of exercise used in this study. On the other hand, it must be emphasized that while similar in metabolic cost, the stress of swimming is different than that of walking. Thus, the two modes of exercise may not be equally stressful.

# CHAPTER V

# SUMMARY, CONCLUSION, AND RECOMMENDATIONS

## Summary and Conclusion

The objective of this study was to determine the role played by the endogenous opioid peptides in the control of muscle blood flow during low intensity exercise in rats. The radiolabeled microsphere technique developed by Laughlin et al. (71) was used. Male Sprague-Dawley rats were chronically instrumented with two catheters: one catheter in the caudal artery for reference blood sample withdrawal, and one catheter in the ascending aorta via the right carotid artery for microsphere infusion. The rats were randomly divided into control and experimental groups with the experimental group pre-treated with naloxone to block opioid receptors.

The results obtained indicated no significant differences in blood flow between the two groups. Hence, it can be concluded that the endogenous opioids are not involved in the control of muscle blood flow during low intensity exercise in rats.

## Recommendations for Further Research

In order to more fully answer the question of whether endogenous opioids play a role in the regulation of muscle

blood flow, the following studies would be in order: 1) a comparison of muscle blood flow in control and naloxone pre-treated rats at a higher intensity of exercise, for example, 60 meters/minute; 2) a comparison of muscle blood flow in control and naloxone pre-treated rats for a longer duration of exercise at 15 meters/minute; 3) a combination of higher intensity and longer duration of exercise; 4) a comparison of muscle blood flow in control and naloxone pre-treated rats during swimming; and 5) measurement of blood endorphin levels at the intensity and duration of this study, and the intensities and durations of the proposed studies.

Future studies of the control of muscle blood flow should also include consideration of the possible effects on muscle fiber type within the specific muscles (9,68,70).

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# VITA

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Professional Organizations: Professional Association of Diving Instructors; YMCA Underwater Activities Association; National Speleological Society--Cave Diving Section; Underseas Medical Society; Divers Alert Network.