THE MECHANICAL EFFECTS OF MUSCLE CONTRACTIONS ON MUSCLE BLOOD FLOW

by

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PREFACE

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ABSTRACT

To determine whether muscle contractions can increase muscle blood flow independently from metabolic factors, we isolated the diaphragmatic vasculature of 16 anesthetized and mechanically ventilated dogs. Phrenic inflow (Qphr) was controlled with a constant pressure source and the pressure (Pa) was decreased in steps to obtain the pressure-flow relation (P-Q). The vasculature was maximally vasodilated and contractions occurred spontaneously (n=6) or were induced by twitches (n=12) or tetanic trains (n=7). The P-Q relations with contractions were compared to those with vasodilatation alone. With spontaneous contractions, the pressure intercept decreased from 47.35 ± 17.44 to 33.77 ± 16.82 mmHg ($p < 0.05$) and the slope remained unchanged so that at Pa=100 mmHg, Qphr increased from 36.22 ± 34.85 to 43.91 ± 38.22 ml/min/100g ($p < 0.05$). Flow increased slightly with twitches but not with trains. We also elicited twitches, 12/min and 60/min trains in vascularly isolated gastrocnemius muscles (n=6) and found no change in flow. In conclusion, the muscle pump has only a small effect on muscle blood flow.

RESUME

Afin de determiner si les contractions musculaires augmentent le flux arteriel musculaire independamment de facteu s metaboliques, on a isole les vaisseaux sanguins du diaphragme de 16 chiens anesthésies et ventiles mécaniquement. Le flux artériel phrénique (Qphr) a ete contrôlé par une source de pression constante et on a diminué la pression (Pa) par étapes pour determiner les relations pression-flux (P-Q). On a vasodilate les vaisseaux au maximum et induit des contractions diaphragmatiques spontanément (n=6), par des secousses repetees (n=12) ou des trains d'impulsions tétaniques (n=7). Les relations P-Q obtenues lors des contractions ont été comparees a celles obtenues lors de la vasodilatation seulement. Pendant les contractions spontanees, Qphr a Pa=100 mmHg a augmenté de 36.22 ± 34.85 à 43.91 ± 38.22 ml/min/100g ($p < 0.05$). Qphr a augmenté légèrement avec les secousses mais pas avec les trains d'impulsions. On a aussi induit des secousses et des trains d'impulsions dans des jumeaux isolés (n=6) mais le flux n'a pas changé. En conclusion, la pompe musculaire a un effet modeste sur le flux artériel musculaire.

LITERATURE REVIEW

The objective of the present study was to determine whether muscle contractions can increase blood flow to skeletal muscle by squeezing blood out of the vessels and thus acting as a muscle pump.

1. BLOOD FLOW TO SKELETAL MUSCLE

Blood flow is the volume of blood which passes each minute through an organ or a tissue and is expressed in l/min or ml/min/100g tissue. In resting mammalian skeletal limb muscles, blood flow is approximately 2-5 ml/min/100g, and 5-52 ml/min/100g in in situ muscle preparations (Bockman et al., 1980 ; Folkow and Halicka, 1968; Grimby et al., 1967; Hilton et al., 1970; Shepherd, 1983). During exercise, blood flow rises to more than 300 ml/min/100g in humans and conscious animals, and reaches up to 263 ml/min/100g in in situ muscle preparations (Andersen and Saltin, 1985; Armstrong and Laughlin, 1985; Armstrong et al., 1987; Grimby et al., 1967; Hilton et al., 1970; Lind and McNichol, 1967; Rowell et al., 1986). In animals, diaphragmatic blood flow ranges from 9 to 42 ml/min/100g during quiet spontaneous breathing and increases to 15-207 ml/min/100g during inspiratory resistive loading, 169-333 ml/min/100g during treadmill running, and 70-265 ml/min/100g during electrophrenic stimulation (Armstrong and Laughlin, 1985; Bellemare et al., 1983; Buchler et al., 1985; Magder et al.,

1985; Manohar, 1986; Mush et al., 1987; Robertson et al., 1977a, 1977b; Rochester and Bettini 1976; Rochester and Pradel-Guena, 1973).

The high variability of measurements of peak blood flow to skeletal muscles in these studies can be accounted for by differences in species, surgical and muscle preparations, types of muscle fibers studied, workloads used, percentage of the active muscle mass, training states of the muscles, and techniques employed for measuring blood flow. Furthermore, there has been a marked variation between studies in the values of the determinants of blood flow. According to Poiseuille's law, the flow of fluid through a rigid tube is determined by the driving pressure and the conductance of the tube. Small collapsible arterial vessels function as rigid tubes when the difference between the pressures inside and outside the vessel (transmural pressure) is positive throughout the vascular bed. Under these conditions, the equation for arterial blood flow (Qa) is:

$$Qa=(P_I-P_O) \times 1/R_a$$

where P_I and P_O are the pressures at the inflow and outflow ends of the vascular bed, respectively, and $1/R_a$ is muscle vascular conductance (the reciprocal of arterial resistance) measured at a given P_I (Green, 1987). The contribution to muscle perfusion by each of these factors is as follows.

a. <u>Inflow Pressure</u>

The pressure at the inflow end of the muscle vascular bed is the arterial or perfusion pressure. Under resting

conditions, blood flow is independent of changes between 40 and 120 mmHg in perfusion pressure due to autoregulation (Hussain et al., 1988; Stainsby, 1962; Stainsby and Renkin, 1961). Autoregulation is usually explained by metabolic or myogenic mechanisms (Bacchus et al., 1981; Folkow, 1964; Folkow and Oberg, 1961; Goodman et al., 1978; Johnson, 1980; Mohrman and Sparks, 1974; Smiesko, 1971; Stainsby, 1961). The metabolic hypothesis attributes changes in vascular resistance to the release of tissue metabolites triggered by physiological alterations in blood flow. The myogenic theory attributes the stability of blood flow to active contraction or relaxation of the vascular smooth muscle elicited by changes in intravascular or transmural pressure.

When the ability of vessels to vasodilate is exhausted, the blood flow capacity of skeletal muscle becomes dependent on perfusion pressure (Magder, 1986; Reid and Johnson, 1983). Thus, differences between studies in the perfusion pressure used could contribute to the disparity in the "maximal blood flow" reported (Folkow and Halicka, 1968; Hilton et al., 1970). Investigators often use "maximal blood flow" to describe the peak flow obtained during a series of experiments. The term "maximal" implies a unique value representing the highest flow possible. However, due to the relationship between perfusion pressure and blood flow, it is only possible to measure a true maximal blood flow under a given set of conditions. Therefore, the use of "blood flow

capacity" is more appropriate (Laughlin, 1987).

b. Muscle Vascular Conductance

Conductance is expressed in l/min/mmHg or ml/min/100g/mmHg. The conductance of a tube is proportional to the fourth power of the radius of the tube and is inversely proportional to the length of the tube and the viscosity of the fluid. Under normal physiological conditions, the hematocrit and the length of the blood vessels are constant. Thus, a change in conductance usually results from a change in the radius of the vasculature (Green, 1987). Since direct measurement of the radius is difficult, muscle vascular conductance is calculated as the ratio of muscle blood flow to the driving pressure.

Numerous studies indicate that blood flow to skeletal muscle increases during exercise (Andersen and Saltin, 1985; Armstrong et al., 1987; Manohar, 1986; Robertson et al., 1977b). In situ studies have shown that the highest conductances in fast-twitch glycolytic muscles are obtained during twitch-type electrical stimulations whereas peak blood flows in high oxidative fast- and slow-twitch muscles are produced during tetanic trains (Folkow and Halicka, 1968; Mackie and Terjung, 1983). The conductances achieved in high oxidative muscles are even greater during contractions generated by natural impulses (which will be referred to as "spontaneous" contractions) than electrical stimulation (Armstrong and Laughlin, 1985; Laughlin, 1987).

The mechanisms of the increased arterial perfusion in body tissues during exercise (exercise hyperemia) are not yet known. It is generally accepted that active skeletal muscles release metabolites which produce vasodilatation of resistance vessels and increase blood flow proportionally to the metabolic demands (Barcroft, 1964; Shepherd, 1983). The list of potential metabolic vasodilators is extensive and includes hypoxia, carbon dioxide, hydrogen-ion concentration, lactic acid, potassium, inorganic phosphate, osmolarity, adenosine, adenine nucleotides and prostaglandins. However, none of these substances seems to be solely responsible for exercise hyperemia (Snepherd, 1983). Recent reports also point to an important role in the beds of resistance vessels for "endothelium-derived relaxing factor" (EDRF), a vasoactive autocoid produced by the vascular endothelium (Furchgott et al., 1987; Griffith et al., 1987; Pohl et al., 1987). The second messengers through which vasoactive substances may relax arterioles of skeletal muscle include cyclic 3'-5'-adenosine monophosphate (cAMP), cyclic guanosine monophosphate (cGMP), and prostaglandins. Vasodilatation induced by individual pharmacological agents (e.g. papaverine, nitroprusside) is often smaller than that achieved during contractions elicited spontaneously or by electrical stimulation (Laughlin, 1987; Laughlin and Ripperger, 1987; Magder, 1986). Since papaverine activates cAMP only (Nickerson, 1975) and nitroprusside cGMP only (Waldman

and Murad, 1987), further vasodilatation could have been obtained with the infusion of a combination of drugs (Laughlin, 1987). Adrenergic and nonadrenergic neuronal cell bodies have been described in the walls of skeletal muscle arterioles (Myers et al., 1975). These neurons, which can be activated by shearing forces and changes in transmural pressure associated with muscle contractions, may contribute to exercise hyperemia by releasing a vasodilator mediator which has not yet been identified (Honig and Frierson, 1976; Honig, 1979). However, this concept has not been universally accepted (Shepherd, 1983). Muscle contractions could also increase muscle blood flow, but this hypothesis has received little attention.

It is well known that exercise can reduce muscle vascular conductance; for example, strong isometric contractions elevate intramuscular pressure and mechanically compress blood vessels (Hill, 1948). Blood flow can be partially or totally occluded during sustained contractions. The exact tension at which the obstruction takes place varies with different muscles. In the calf, sustained forces of about 20 to 30% of that developed by a maximal voluntary contraction (MVC) are sufficient to occlude blood flow to the ankle plantar flexors (Barcroft and Millen, 1939). Hand-grip muscles must exert isometric forces greater than 70% MVC in order to arrest the circulation through the forearm (Humphreys and Lind, 1963). In the canine diaphragm, Hussain et al. (1989a) demonstrated that diaphragmatic blood flow is

not occluded even when nearly maximal transdiaphragmatic pressure is produced at a duty cycle of 0.5, compared with the normal 0.25 to 0.30.

The discrepancy between blood flow impedance in the forearm, calf and diaphragm muscles may be explained by differences in their anatomy. However, although the forearm and calf muscles are pennate, the amount of tension necessary to occlude blood flow to the forearm and calf muscles is significantly different. The most likely explanation for this discrepancy is that the blood vessels in the calf may be "nipped" (i.e. pinched) because of shortening and tautness in the soleus and gastrocnemius muscles (Barcroft and Millen, 1939; Barcroft, 1963). Arteriograms and venograms performed in maximally contracting calf muscles of dogs have shown numerous localized compressions in both arteries and veins as these vessels entered the muscle or passed between muscle fasciculi (Gray et al., 1967). The thin sheet-like diaphragm has a more favorable arrangement than other skeletal muscles. Its fibers insert over a wide area and are parallel to one another. This fanning design reduces the compression of the vasculature during contraction.

c. Outflow Pressure

In many studies, the venous pressure is taken as the pressure at the outflow end of the vascular bed. Rhythmic muscular contractions repeatedly increase the pressure in peripheral as compared to central veins and hence, repeat-

edly empty the peripheral veins toward the heart. This mechanism has been referred to as the muscle or venous pump and was first described in the 1940s (Barcroft and Dornhorst, 1949a). Since then a number of researchers have examined the muscle's capacity to promote central venous return. Pollack and Wood (1949) demonstrated that intermittent contractions of the plantar flexors during walking could reduce the venous hydrostatic pressure at the ankle and thereby enhance venous return. Barcroft and Dornhorst (1949b) reported that the ability of the calf muscles to reduce the calf volume was not affected by inflation of a pressure cuff about the thigh during rhythmic exercise. Stegall (1966) showed that the calf emptied equally well whether rhythmic plantar flexion was performed with supine subjects tilted in the head-down or head-up position. He also reported that the muscle pump allowed the outflow of venous blood from the exercising legs despite the resistance caused by a substantial increase in intra-abdominal pressure. Stegall calculated that the muscle pump could contribute at least 30% of the total systemic circulatory work required during running.

Very few studies have investigated the effects of the muscle pump on local perfusion of muscle tissue. Wiggers, in 1954, analyzed phasic changes in coronary sinus flow before and during procedures which altered myocardial contractility and/or coronary resistance in anesthetized dogs. He found that increases in systolic ventricular compression produced

higher venous outflow than increments in diastolic vascular conductance. Wiggers concluded that myocardial contractions aided flow through the heart by exerting a "massaging" (i.e. pumping) action on vessels. He could not identify the mechanisms underlying this action as metabolites were not eliminated. This descriptive study was also limited by the fact that, under certain procedures, the differences between the increments in systolic and diastolic flow were small and statistical significance cannot be established as the number of dogs studied was not specified.

Hirche et al. (1970) compared the effects of rhythmic and sustained contractions on the vascular resistance of electrically stimulated gastrocnemius muscles of dogs. They found that resistance increased significantly during sustained contractions and was more marked during isometric than isotonic contractions. During rhythmic electrical stimulation there was no significant increase in resistance for either type of contractions. Hirche et al. concluded that rhythmic contractions did not affect resistance to flow because of a "massage" effect on the vessels. In their study, the driving pressure was calculated as Pa-Pv-Pcrit where Pa is the mean perfusion pressure, Pv the venous pressure, and Pcrit the critical closing pressure. However, either Pv or Pcrit should have been used as the outflow pressure. Pcrit, the arterial pressure obtained by gradually lowering flow to zero, was determined in only 4 of the 18 maximally vasodilated and resting gastrocnemii muscles and

ranged from 9-11 mmHg. This value is lower than the 42.3 mmHg reported for the resting hindlimbs of dogs (Magder, 1990).

Folkow et al. (1970) investigated the effect of rhythmic exercise on the blood flow to isolated feline calf muscles. They showed that venous outflow increased significantly during the contraction phase and venous pressure decreased during the relaxation phase. They also found that the extent of venous filling depended on the reduction of venous pressure between consecutive contractions. This was determined by the frequency and duration of muscle contractions. For example, blood flow was limited during short relaxation periods because of inadequate venous filling time and during prolonged relaxation periods because of a rise in venous pressure prior to the next contraction. Similarly, long duty cycles and high contraction frequencies reduce diaphragmatic perfusion (Buchler et al., 1985). Other factors that enhance the venous pressure-lowering effect include the force of the contractions, the precontraction vascular volume, and the capacity and filling rate of the venous bed. Folkow et al.'s results are limited because maximal vasodilatation of the resistance vessels was produced using only electrical stimulation. Thus, it is possible that maximal vasodilatation was not attained and vasodilator metabolites contributed to the rise in flow. Furthermore, the authors measured venous pressure in a superficial vein whereas venous compression should be greater

in deeper muscle veins, as shown by Gray et al. (1967). Folkow et al. also recorded higher pressures in the femoral than small muscle veins which may seem contradictory to the muscle pump theory. This negative gradient was probably caused by the venous valves.

2. THEORY OF THE MUSCLE PUMP

If the vasculature of skeletal muscle is characterized by an arterial resistance followed by a compliant region (venules) and a venous resistance (Fig. 1a), then Poiseuille's equation for arterial blood flow can be expressed as $Qa = (Pa-Pc)/Ra$, where Pc is the pressure in the compliant region. As a result, rhythmic muscle contractions could increase muscle blood flow in the following way. The contracting muscle will compress and empty the compliant region of the vasculature (Fig. 1b). This will produce some retrograde flow but most of the blood should go out the venous side because impedance is much less than on the arterial side. During the relaxation phase, Pc decreases whereas arterial pressure does not change (Fig. 1c). Thus, the gradient for inflow is transiently augmented and mean arterial flow should increase.

3. CAVEAT

According to Poiseuille's equation for arterial inflow, flow should cease when arterial (Pa) and venous pressures (Pv) are equal. However, numerous studies have invariably

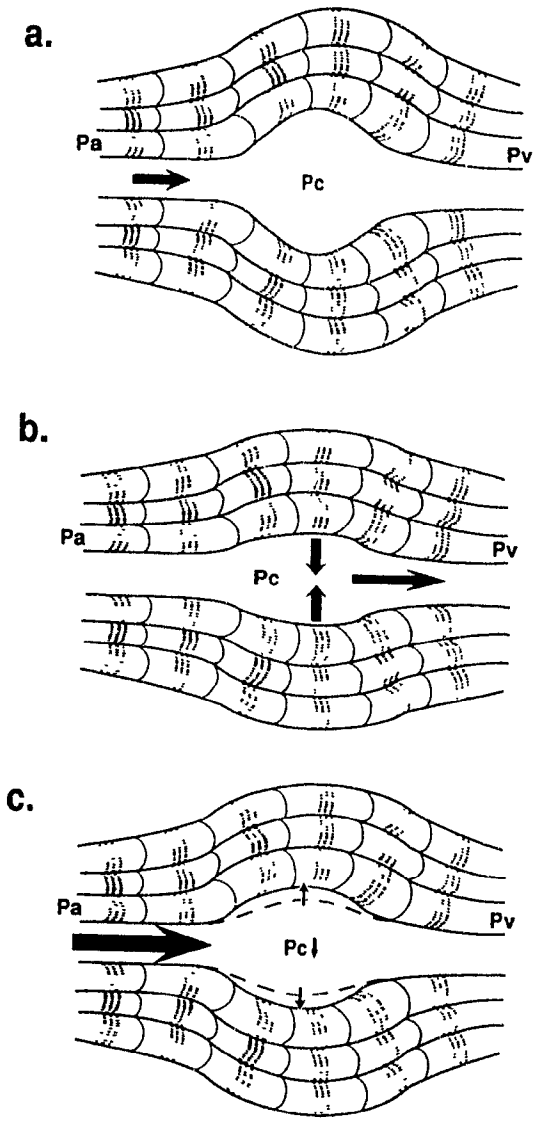

Figure 1. Schematic drawing illustrating the muscle vasculature with a compliant region between two resistances (a). The gradient for inflow is the difference between the arterial and compliant region pressures or Pa-Pc. During muscle contractions the compliant region is compressed and venous outflow is enhanced (b). During the relaxation phase, the pressure in the emptied compliant region decreases and as a result, the gradient Pa-Pc and thus inflow increases markedly (c).

shown that at zero flow Pa remains greater than Pv (Ehrlich et al., 1980; Magder, 1990; Pappenheimer and Maes, 1942; Sylvester et al., 1981; Whittaker and Winton, 1933; Yamada and Åstrom, 1959). Such findings could be due to a vascular capacitance which continues to discharge after arterial flow is arrested and thus maintains Pa above Pv (Eng et al., 1982; Spaan, 1985). These findings could also be caused by small arterial vessels acting as Starling resistors (Magder, 1990).

Flow through a collapsible thin-walled tube, or Starling resistor, is dependent on the difference in pressure between the inside and outside of the tube. As discussed previously, when the pressure inside is greater than that outside the tube, flow is proportional to the gradient $P_I - P_O$. However, when P_I is greater than the outside pressure (P_S), and P_S is greater than P_O, flow is determined by $P_I - P_S$. P_S could be estimated by the arterial pressure at zero flow calculated as the X-intercept of the pressure-flow relationship ($P_{Z=0}$). If $P_{Z=0}$ is greater than the pressure in the compliant region, $P_{Z=0}$ would become the effective downstream pressure and $Pa-(P_{Z=0})$ the gradient which moves blood flow through the arteriolar bed (Fig. 2). Under these conditions Pc would have no effect on flow.

4. RATIONALE FOR THE STUDY

Although the importance of the close matching of blood

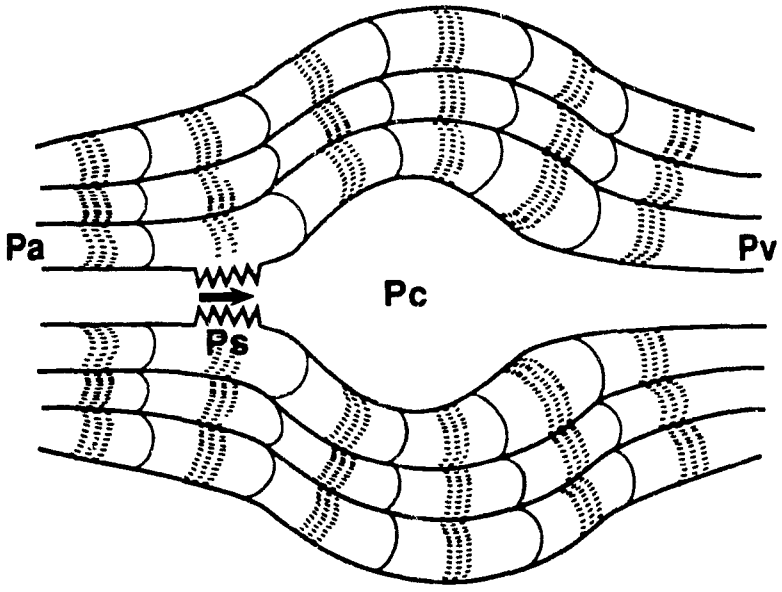

Figure 2. Model of the muscle vasculature with a precapillary Starling resistor. The pressure at the Starling resistor (Ps) is always greater than in the compliant region (Pc). Since the intravascular pressure is less than the extravascular pressure, the vessel tends to collapse and the gradient for inflow is the difference between arterial pressure and Ps or Pa-Ps. Under these conditions, Pc has no effect on inflow.

flow to the metabolic needs of the working muscle is well recognized, the mechanisms of exercise hyperemia are not well understood. In particular, little attention has been paid to the potential ability of muscle contractions to increase blood flow independently of metabolic factors.

Results from earlier studies have supported the role of a muscle pump in skeletal limb muscles but have failed to establish and, more importantly, to quantify its effect on arterial inflow (Folkow et al., 1970; Hirche et al., 1970; Laughlin, 1987). Therefore, we decided to study the mechanical effect of isometric contractions on blood flow to the gastrocnemius muscle.

None of the previous studies on the muscle pump was carried out on the respiratory muscles. Respiratory failure is an important clinical problem and occurs during cardiogenic shock, septic shock or whenever blood flow fails to meet the metabolic needs of the respiratory muscles (Aubier et al., 1981; Reid and Johnson, 1983; Viires et al., 1983). Therefore, an understanding of exercise hyperemia in the respiratory muscle is critical to the prevention and treatment of respiratory failure. The diaphragm is the primary muscle of inspiration and since it is characterized by structural, biochemical, physiological and functional properties that are different from those of other skeletal muscles, it must be studied specifically (Rochester and Briscoe, 1979, Rochester and Drash, 1985; Sant'Ambrogio and Saibene, 1970). Furthermore, breathing is an automatic pro-

cess elicited by impulses responding to sensors (e.g. chemoreceptors and mechanoreceptors) and originating from the respiratory centers in the brain stem and the central pattern generator in the brain stem and spinal cord (Feldman, 1986). As a result, the respiratory muscles are the only skeletal muscles that contract spontaneously under general anesthesia. In addition, the diaphragm has been considered to be two muscles, that is a crural and costal part (De Troyer et al., 1981). These parts have been shown to contract in sequence during spontaneous breathing (Newman et al., 1984). The sequential recruitment of muscles and muscle fibers, as occurs in spontaneous contractions, may be associated with a better coordination of muscle activation than the simultaneous recruitment which occurs during electrical stimulation (Burke, 1981; Saltin and Gollnick, 1983). Thus, the diaphragm is well suited for a study of the changes in blood flow during contractions occurring with a natural pattern of recruitment and without the involvement of autonomic nerve fibers that might be activated by electrical stimulation (Honig and Frierson, 1976). Finally, since the isolated hemidiaphragm preparation developed by Hussain et al. (1989b) allows diaphragmatic shortening, the effect of the muscle pump can be studied during pleiometric (shortening) contractions.

5. HYPOTHESES OF THE STUDY

We tested the hypothesis that electrically induced con-

tractions of skeletal muscles will augment muscle blood flow through a mechanical effect which is independent of metabolic factors. We also hypothesized that an increase in the frequency of contractions up to some optimal rate will produce a rise in flow. Finally, our third hypothesis was that muscle perfusion will be greater during spontaneous than electrically elicited contractions because of a better coordination of muscle activation.

METHODS

1. ANIMAL PREPARATION

We anesthetized 20 mongrel dogs of either sex, weighing 25-41 kg, with chloralose (60-100 mg/kg) which was supplemented as necessary. Anesthesia was initiated with either ketamine (500 mg/ml, n=7) or thiopental (50 mg/ml, n=14). The animals were supine, intubated and mechanically ventilated at a frequency and tidal volume which kept $PaCO_2$ between 35 and 45 mmHg to suppress ventilatory activity. PaO_2 was kept above 100 mmHg with supplemental O_2 and 5 cm of positive end-expiratory pressure (PEEP) was applied to maintain a normal functional residual capacity. The jugular vein was cannulated for the administration of fluids and the common carotid artery was cannulated for the measurement of systemic arterial pressure and withdrawal of blood samples for hematocrit and blood gas analysis. In some dogs, infusion of blood from a donor dog or 6% dextran solution in saline was necessary to maintain adequate arterial pressure. Core body temperature was kept at approximately 38°C with a heating pad placed under the animal.

2. SURGICAL PREPARATION

a) Hemidiaphragm

The in-situ left isolated hemidiaphragm preparation developed by Hussain et al. (1989b) was used in 16 dogs. Briefly, the left lower six ribs and interspaces were ex-

posed through a thoracotomy and the intercostal muscles were incised. The epigastric and internal mammary arteries and the intercostal vessels were ligated. The ribs were resected and the two halves of the costal diaphragm were divided to separate their vascular supply. The abdomen was opened by an incision parallel to the left costal margin. The free left costal margin was then secured to three 5 cm long metal bars. These bars were connected through ball and socket joints to three force transducers (Grass FT10) so that tension could be recorded from the anterior, middle, and posterior diaphragmatic segments.The left phrenic artery was isolated at its entrance into the left crural diaphragm and cannulated. The left phrenic nerve was left intact so that spontaneous contractions of the left diaphragm could still occur. The diaphragmatic length was adjusted at L_{FRC}, the length at functional residual capacity. This length was determined by putting four reference marks on the diaphragm while it was still intact. At the end of the experiments, the length, weight, and cross-sectional areas of each segment were determined using techniques described previously (Close, 1972; Hussain et al., 1989b).

b) <u>Gastrocnemius Muscle</u>

The right gastrocnemius was vascularly isolated in six dogs through a medial incision extending from the mid-thigh to the ankle (Stainsby et al., 1956). The right gastrocnemius was exposed and its distal tendon ligated and cut. The popliteal artery and vein were isolated and all

branches except those supplying the gastrocnemius were doubly ligated and cut between the ties. The completeness of the vascular isolation was verified by stopping inflow and outflow simultaneously (double occlusion technique) and monitoring arterial and venous pressures which should equilibrate and maintain a plateau during the 30-40 s occlusion. The right shank was amputated and the hindleg was placed vertically with a 90° angle at the hip joint. Shortening of the gastrocnemius was prevented by introducing a pin through the head of the tibia and securing it against a metal frame surrounding the dog's hindquarters. The tendon of the gastrocnemius was fastened to a force transducer placed above the hindleg and mounted on a caliper which was connected in turn to the metal frame. Thus, isometric tension could be recorded and muscle length adjusted. The distal portion of the tibial nerve was cut and a silver electrode attached.

c) <u>General Procedures</u>

At the completion of each surgical preparation, blood clotting was prevented by injecting 10,000 i.u. of heparin. The isolated muscle was wrapped with plastic film to prevent heat loss and dehydration. Muscle temperature was recorded by a thermocouple (Mon-a-Therm, St Louis, MO, USA) and kept at approximately 35°C for the diaphragm and 38°C for the gastrocnemius by heat lamps. At the end of each experiment, the animal was killed with an overdose of potassium chloride (KCl). The left diaphragm or right gastrocnemius was then

removed and weighed. The portion of the gastrocnemius which was weighed was determined by infusing Lissamine Green B (Sigma, L6382, 25 mg/100 kg) into the right popliteal artery and resecting the stained area. This was done in two dogs only as the area of perfusion was the same as that expected. Force transducers were calibrated with 100-1000 g weights. Tension was expressed in g/cm^2 or kg.

3. MEASUREMENTS

The phrenic artery was cannulated with a perfusion circuit as previously reported by Hussain et al. (1989b). Blood was diverted from the left femoral artery to the phrenic artery through a line including a catheter placed in the proximal portion of the left femoral artery, a Y connection, an electromagnetic flow probe (Carolina Medical Electronics, 1.91 mm ID), a 15 cm-long polyethylene tubing, and a catheter introduced in the distal portion of the phrenic artery. A side port was placed at the entrance of the catheter into the phrenic artery for the measurement of phrenic perfusion pressure. The other arm of the Y connection was secured, via two three-way stopcocks and an extension, to an outflow orifice at the bottom of a pressurized reservoir. The stopcocks were connected to a constant infusion pump (Harvard Apparatus, model 940) for the administration of vasodilating drugs. The reservoir was filled partially with blood delivered from the right femoral artery by a Masterflex pump (Cole Palmer Instruments, model 7523-00). The other portion

of the reservoir contained air at a pressure necessary to maintain perfusion of the phrenic artery. The amount of blood in the reservoir was kept at the lowest level possible to prevent the development of hypoxemic conditions. At the beginning of each experiment, the natural flow of the left hemidiaphragm was determined by perfusing it with blood from the left femoral artery. The catheter introduced into the left femoral artery was clamped thereafter, and the diaphragm was perfused from the blood reservoir at constant pressures.

The popliteal artery was cannulated with a line which included a Y connection and electromagnetic flow probe (10 mm ID) connected to a catheter in the left femoral artery. The perfusion circuit was similar to that of the left hemidiaphragm. In addition, the popliteal vein was cannulated with a rigid plastic tube (6.35 mm) which contained a side arm for the measurement of venous pressure and was connected to a large bore tube draining in a container through an electromagnetic flow probe (12 mm ID). Venous blood was then re-infused into the dog through the jugular vein.

Each perfusion circuit was filled with the dog's own blood before it was connected. The flow probes were connected to square-wave electromagnetic flowmeters (Carolina Medical Electronics). Zero flow levels were checked by a brief mechanical occlusion of each circuit. The flowmeter measuring venous blood was calibrated with a stopwatch and a

graduated cylinder at the beginning of the experiments. The flowmeters measuring arterial inflow were calibrated at the end of the experiment by perfusing the phrenic or popliteal artery with the Masterflex pump at a known flow rate. Blood flow was expressed in ml/min/100g and was determined by averaging the flow signal over the entire contraction and relaxation cycle. Maximal and minimal flows were also obtained. Pressure transducers, force transducers and flowmeters were connected to Hewlett-Packard preamplifiers with the output registered on a Gould 8-channel recorder. The signals were digitized manually or using a software package (Anadat) on an IBM compatible computer.

4. ARTERIAL VASODILATATION

Adenosine (ADO), acetylcholine (ACh) and sodium nitroprusside (SNP) were simultaneously infused in the perfusion circuit via the infusion pump in order to vasodilate maximally the phrenic or popliteal artery. The concentration required by each drug to produce maximal vasodilatation was identified in preliminary experiments. The doses of ACh, ADO and SNP averaged 1.43 ± 0.41(SD) mM/min, 1.43 ± 0.41 mM/min and 114.75 ± 32 µg/min in the diaphragm and 2.31 ± 1.05 mM/min, 2.31 ± 1.05 mM/min, and 196.47 ± 68.26 µg/min in the gastrocnemius. A bolus of indomethacin (3 mg/kg) was also injected in the jugular vein to block the release of prostaglandins and, thus, rule out their contribution to exercise hyperemia. Maximal

vasodilatation of the phrenic and popliteal artery was verified by the absence of reactive hyperemia after a 20 s flow occlusion.

5. MUSCLE CONTRACTION

Pleiometric contractions of the left hemidiaphragm were elicited by electrophrenic stimulation or occurred spontaneously. Electrical stimulation was delivered by a well insulated electrode placed around the left phrenic nerve and connected via a Grass stimulus isolation unit (SIU5) to a Grass Stimulator (S48). The phrenic nerve was paced with two types of electrical stimulation presented in random order. Each stimulation was applied for 60 s and was produced by impulses of 0.2 ms duration at an intensity of 4-15 V. Repeated single twitches were elicited by impulses delivered continuously at a frequency of 2-4 Hz at which no summation occurred. Trains of impulses, in which single stimuli were delivered at a frequency of 25 Hz, were applied using a constant duty cycle of 0.25 and train rates of 10-22/min. Spontaneous breathing was elicited by reducing the frequency and tidal volume of the respirator and therefore allowing PCO_2 to rise from 31.0 ± 3.8 to 39.9 ± 4.4 mmHg.

Isometric contractions of the right gastrocnemius were produced by electrically stimulating the tibial nerve with repeated twitches, 20 and 60/min trains. Supramaximal voltage was first determined by gradually increasing the voltage with a single twitch. The optimal length (L_o) was then iden-

tified using a single twitch at supramaximal voltage. The parameters of electrical stimulation were the same as those used in the diaphragm except for the voltage which was the one needed to generate half the maximum tension (8.92 ± 2.0 V) in order to reduce the effects of muscle fatigue.

6. EXPERIMENTAL PROTOCOL

After a 30 min stabilization period at the naturally occurring flows, the left diaphragm or the right gastrocnemius was perfused at constant pressure and indomethacin was injected. The pressure-flow (P-Q) relations were obtained by reducing the phrenic or popliteal perfusion pressure in four to seven steps over a range of 150 to 50 mmHg. Measurements of pressure and flow were taken when a steady state was achieved and the fluctuations in flow due to myogenic responses had occurred. Perfusion pressures were not systematically increased from 50 to 150 mmHg because steady state could not be achieved rapidly.

a) Hemidiaphragm

In one group of animals, the P-Q relations were examined in the diaphragm (n=16). First, 4-7 control P-Q points were obtained from the phrenic artery of the resting diaphragm while saline solution was infused through the perfusion circuit at the same flow rate as during the drug infusion. Maximal vasodilatation of the left phrenic artery was then induced and maintained by constant infusion of the

vasodilators. At each perfusion pressure, phrenic flow was first measured in the resting vasodilated state and then during electrical stimulation (n=12 for repeated twitches, n=7 for trains). In spontaneously breathing dogs (n=6), since it was difficult to induce and stop breathing at each pressure, P-Q relations were determined separately for each condition. The P-Q relation for vasodilatation alone was obtained before or after spontaneous diaphragmatic contractions. The P-Q relations with spontaneous contractions were also always obtained before those with electrical stimulation because the animals were allowed to breathe spontaneously as soon as the effect of the surgical anesthesia started to wear off.

b) <u>Gastrocnemius Muscle</u>

The protocol was similar in a second group of animals (n=6) except that the right tibial nerve was electrically stimulated. In each animal, a P-Q relation was first obtained from the popliteal artery of the resting gastrocnemius. When maximal vasodilatation of the popliteal artery was achieved, the P-Q measurements were made first during vasodilatation and then during twitches, 12/min and 60/min trains (n=6). Thus, as for the diaphragm, each run of contractions had its respective vasodilatation measurements to which it was compared. However, since the three electrical stimulations were applied to all animals, only one control P-Q relation was obtained prior to the vasodilated and contracting conditions.

7. DATA ANALYSIS

A linear regression was performed to analyze the pressure-flow measurements for each dog during each condition, that is while the isolated muscle was (1) in the resting state, (2) maximally vasodilated before or after each type of contraction, and, (3) contracting spontaneously and/or with electrically induced twitches and trains. The X-intercept of the least-squares line represents the arterial pressure at zero flow ($P_{Z=0}$) and the slope, the arterial conductance in ml/min/100g/mmHg. Inflow at 100 mmHg was also estimated from the regression equations. Data from the resting state were only obtained to verify the effectiveness of the drugs and were not included in the following analyses. In the gastrocnemius, the slope, X-intercept and inflow at 100 mmHg were compared using analyses of variance for repeated measures. The Newman-Keuls test was then applied for post-hoc analysis of significant differences ($p < 0.05$). In the diaphragm, the same variables as above were compared with paired t-tests corrected for multiple comparisons. Data are presented as means ± standard deviations.

RESULTS

1. HEMIDIAPHRAGM

The mean arterial blood pressure, PaO_2, and $PaCO_2$ averaged 114.1 ± 12.0, 170.4 ± 128.1, and 32.4 ± 5.3 mmHg, respectively. The pH was 7.35 and hematocrit 44.2 ± 6.9%. Rectal and muscle temperatures were 37.5 ± 1.4 and 35.5 ± 1.4 °C, respectively. All these variables remained stable during electrophrenic stimulation. During spontaneous contractions, $PaCO_2$ rose from 31.0 ± 3.8 to 39.9 ± 4.4 mmHg ($t = 4.31$, $p < 0.01$) but pH was essentially the same.

a) Resting State

The phrenic inflow at 100 mmHg, as estimated from the regression equation, ranged from 4.7 to 37.5 ml/min/100g with a mean of 12.07 ± 5.18 ml/min/100g. Despite autoregulatory mechanisms the P-Q relations in the resting state (control) were still well fitted by a linear equation (see Table 1).

b) Maximal Vasodilatation

When maximal vasodilatation was achieved, estimated phrenic inflow at 100 mmHg increased to 27.8 ± 20.1 ml/min/100g. Although we attempted to eliminate all reactivity, some myogenic responses still remained. For example, after 20 s occlusion of the phrenic artery, inflow increased by 2.1 ± 2.4 ml/min/100g while phrenic pressure declined by 15.0 ± 10.0 mmHg. Since the reservoir and venous pressures were unchanged, the fluctuations must rep-

TABLE 1. REGRESSION PARAMETERS OF PHRENIC FLOW ON PRESSURE

	Slope ml/min/ 100g/mmHg	Y-intercept ml/min/100g	X-intercept mmHg	r
TWITCHES				
Control	0.32±0.19	-19.54±13.30	57.08±10.5	0.96
Vasodilation	0.41±0.22	-15.91±10.80	39.95±16.48	0.96
Contraction	0.40±0.22	-13.62± 7.88	36.03±16.06	0.96
12/MIN TRAINS				
Control	0.27±0.12	-15.34± 8.22	55.31± 8.59	0.98
Vasodilation	0.38±0.26	-15.33±19.67	37.11±28.32	0.94
Contraction	0.34±0.26	-15.13±20.31	38.95±33.93	0.93
SPONTANEOUS BREATHING				
Control	0.34±0.20	-22.08±17.27	60.07±10.48	0.97
Vasodilation	0.61±0.52	-24.85±17.66	47.35±17.44	0.98
Contraction	0.60±0.44	-16.31±11.37	33.77±16.82*	0.98

Values are means ± SD. The regression parameters were calculated by averaging the respective regression equations obtained in each animal from 4-7 data points. There was a significant decrease in the X-intercept during spontaneous contractions with respect to the associated vasodilatation.

* $p < 0.05$

resent a decrease in arterial resistance.

c) Muscle Contractions

With repeated twitches (see Fig. 3a), tension averaged 244.6 ± 137.0 g/cm^2. There was a small elevation in perfusion pressure associated with the rise in tension. Phrenic inflow did not fluctuate with diaphragmatic contraction and relaxation but increased slightly. Since this slight increase was evident in all animals, estimated mean inflow at 100 mmHg was significantly greater ($t = 3.03$, $p < 0.05$) than during maximal vasodilatation (see Fig. 4). However, the slope and X-intercept of the P-Q relation did not change (see Fig. 5 and Table 1).

When trains of impulses were applied, tension averaged 615.0 ± 283.3 g/cm^2 and tended to be greater at higher perfusion pressures. Figure 3b shows that inflow diminished during tension development and returned to baseline during the relaxation phase. In the group as a whole, inflow, as compared to maximal vasodilatation, decreased by 58% during the contraction phase and increased by 7% during relaxation but mean estimated inflow at 100 mmHg did not change (Fig. 4) The slope and X-intercept of the P-Q relation also remained the same, as shown by Figure 5 and Table 1.

During spontaneous contractions, tension averaged 201.5 ± 132.1 g/cm$_2$ and duty cycle 0.29 ± 0.15. Figure 3c shows that transients during spontaneous contractions were similar to but smaller than those occurring during trains. However, when compared with the baseline values, inflow increased by

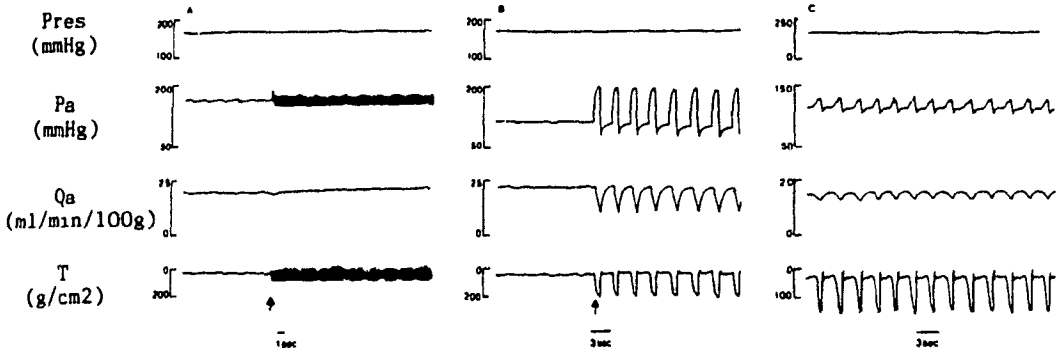

Figure 3. Example of reservoir (Pres) and perfusion (Pa) pressures, phrenic inflow (Qa), and tension in the posterior diaphragmatic segment (T). In (a) and (b), record begins with the baseline during vasodilatation and the arrow represents the start of contraction. (a) 4 Hz twitches, (b) 10-22/min trains (25 Hz), and (c) spontaneous contractions.

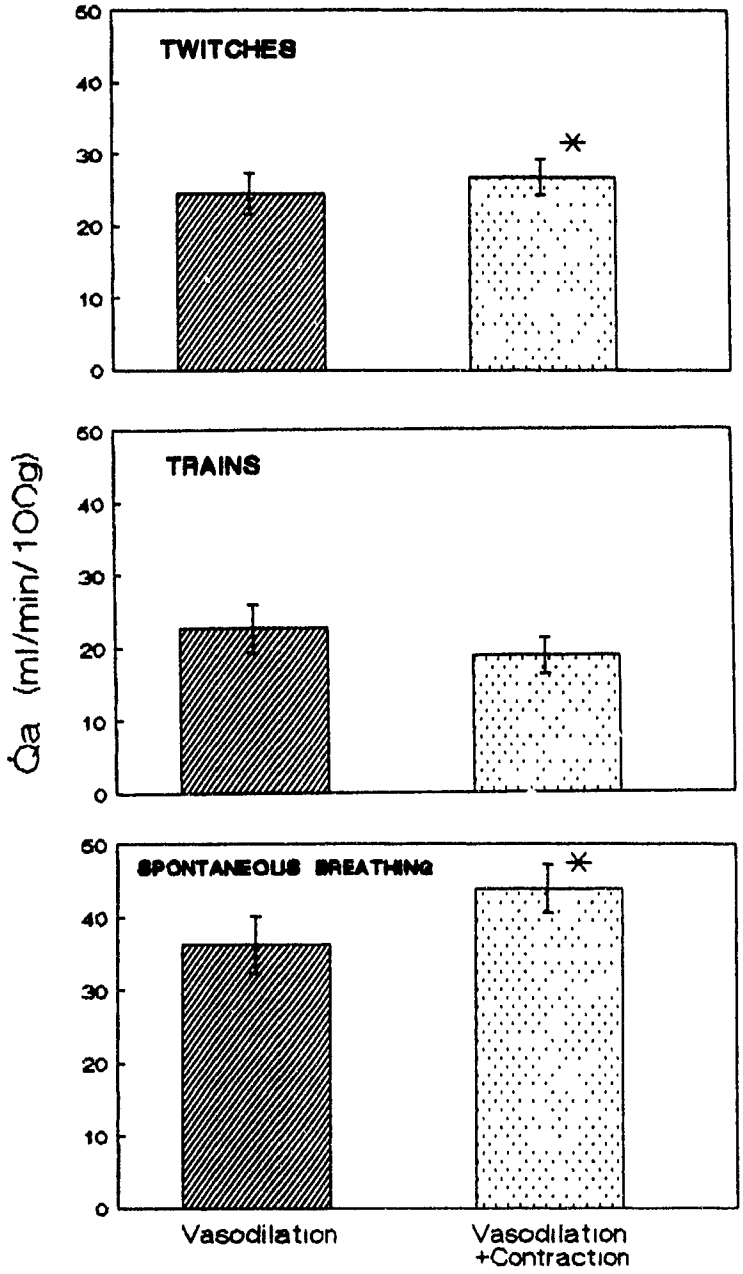

Figure 4. Mean phrenic inflow with twitches, trains, and spontaneous contractions at 100 mmHg. Flows were calculated from the regression equations in Table 1 and were significantly greater during twitches and spontaneous contractions, as compared with their respective vasodilatation. * $p < 0.05$.

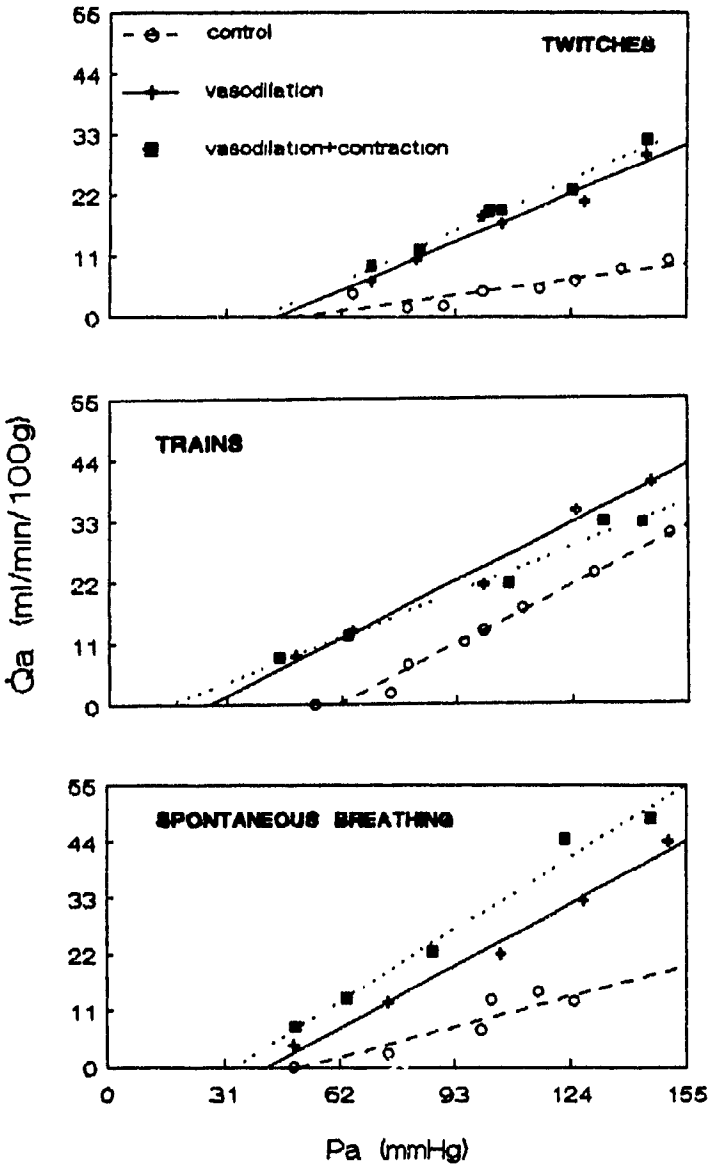

Figure 5. Examples of phrenic pressure-flow relations from three experiments. The X-intercept decreased significantly ($p < 0.05$) during spontaneous contractions but not during twitches or trains.

6% and the X-intercept decreased by 16.9% during the contraction phase. During the relaxation phase, these changes were even more marked and the average increase was 27% for inflow ($t = 5.12$, p 0.05) and the decrease 56% for the X-intercept ($t = 5.40$, $p < 0.05$). Consequently, mean estimated inflow at 100 mmHg (Fig. 4) increased significantly ($t = 3.70$, $p < 0.05$) and the X-intercept of the mean P-Q relation was also markedly lowered ($t = 4.09$, $p < 0.05$). However the slope remained unchanged (Fig. 5 and Table 1).

2. GASTROCNEMIUS MUSCLE

The mean arterial blood pressure, PaO_2 and $PaCO_2$ were 106.1 ± 11.9, 169.1 ± 30.4, and 36.8 ± 4.8 mmHg, respectively. pH and hematocrit averaged 7.35 and 44.2 ± 6.9% and rectal and muscle temperatures were 39.3 ± 0.8 and 37.0 ± 0.4°C, respectively.

a) Resting State

Popliteal arterial flow ranged from 1.0 to 7.1 ml/min/100g with a mean of 3.9 ± 2.3 ml/min/100g. As for the diaphragm, the control P-Q relations remained linear (Table 2).

b) Maximal Vasodilatation

The inflow in the maximally vasodilated gastrocnemius averaged 17.9 ± 6.4 ml/min/100g. As in the diaphragm, myogenic reactivity was not totally abolished. Double-occlusion of the popliteal artery and vein was followed by a

TABLE 2. REGRESSION PARAMETERS OF POPLITEAL FLOW ON PRESSURE

	Slope ml/min/ 100g/mmHg	Y-intercept ml/min/100g	X-intercept mmHg	r
Control	0.07±0.05	- 3.03± 3.22	46.53±12.49	0.93
TWITCHES				
Vasodilation	0.27±0.13	- 8.46± 8.21	32.43±13.96	0.94
Contraction	0.28±0.14	- 8.91± 8.33	33.55±11.93	0.95
12/MIN TRAINS				
Vasodilation	0.33±0.24**	-13.26±17.28	34.27±23.98	0.89
Contraction	0.31±0.22	-11.32±13.94	35.13±16.72	0.88
60/MIN TRAINS				
Vasodilation	0.32±0.23*	-11.08±17.02	20.31±45.10	0.86
Contraction	0.27±0.20	- 7.40±12.32	19.43±25.04	0.90

Values are means ± SD. The regression parameters were calculated by averaging the respective regression equations obtained in each dog from 4-7 data points. Slopes increased significantly during vasodilatation preceding 12 and 60/min trains than during that preceding twitches. However, there was no change in the slopes during any of the contractions. * $p < 0.05$, ** $p < 0.01$.

rise in arterial flow of 2.4 ± 2.2 ml/min/100g associated with a 16.5 ± 9.9 mmHg drop in perfusion pressure

c) **Muscle Contractions**

Maximal tension generated by a single twitch at L_o was 2.5 ± 1.5 kg. When the tibial nerve was stimulated with repeated twitches (4 Hz), tension averaged 1.2 ± 0.3 kg and mean inflow remained constant (Fig. 6). The P-Q relations during twitches and the preceding vasodilatation were essentially the same (Fig. 7).

During 12 and 60/min trains, tension averaged 6.1 ± 2.4 and 4.4 ± 1.6 kg, respectively. During 60/min trains, tension decreased rapidly within each run and with decreasing perfusion pressures. Figure 8 shows an example of the venous flow which rose rapidly with the onset of contraction, reached a minimum at the beginning of relaxation and increased slowly thereafter. The contraction-relaxation transients of arterial flow were -41 and +24% for 12/min trains and -29 and +15% for 60/min trains. Estimated mean inflow at 100 mmHg remained the same during both train rates (Fig. 6). Similarly, the X-intercept and slope of the P-Q relations during 12 and 60/min trains were not different from maximal vasodilatation (Fig. 7).

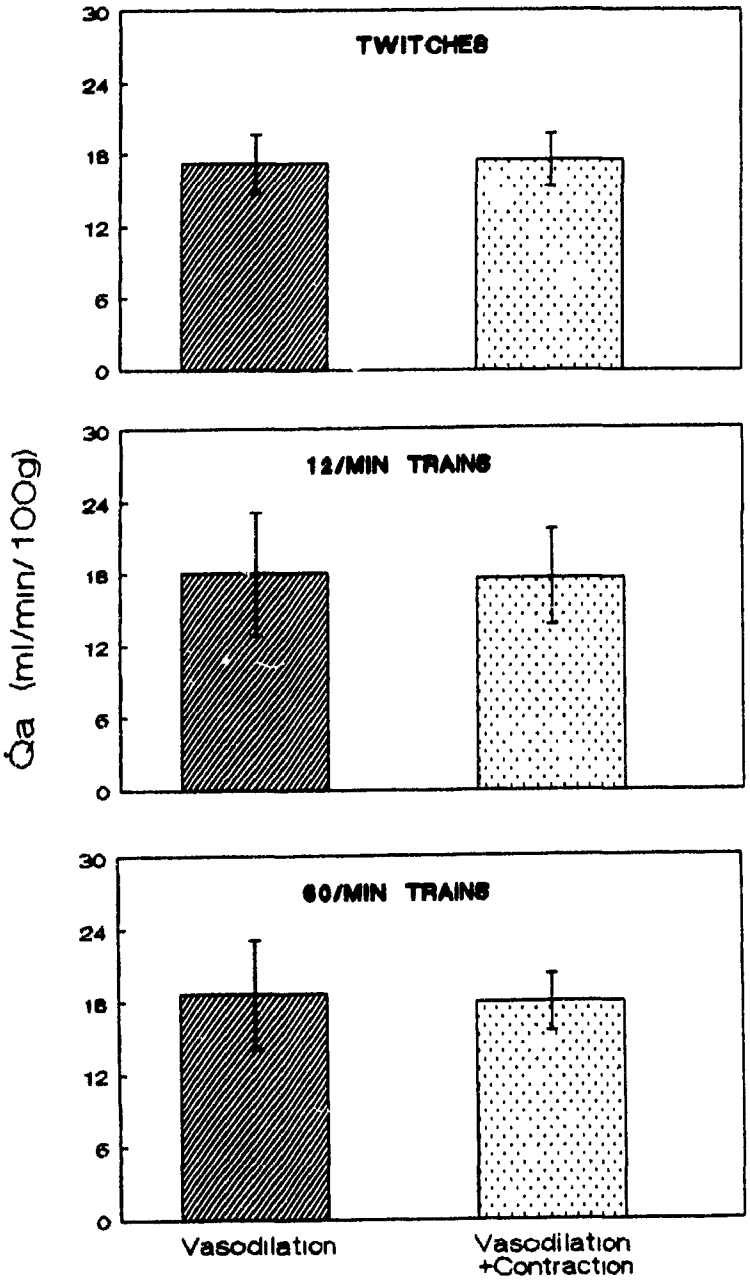

Figure 6. Mean popliteal inflow at 100 mmHg with twitches, 12 and 60/min trains. Flows were calculated from the regression equations in Table 2. There were no significant changes in flow between contractions and their respective baselines.

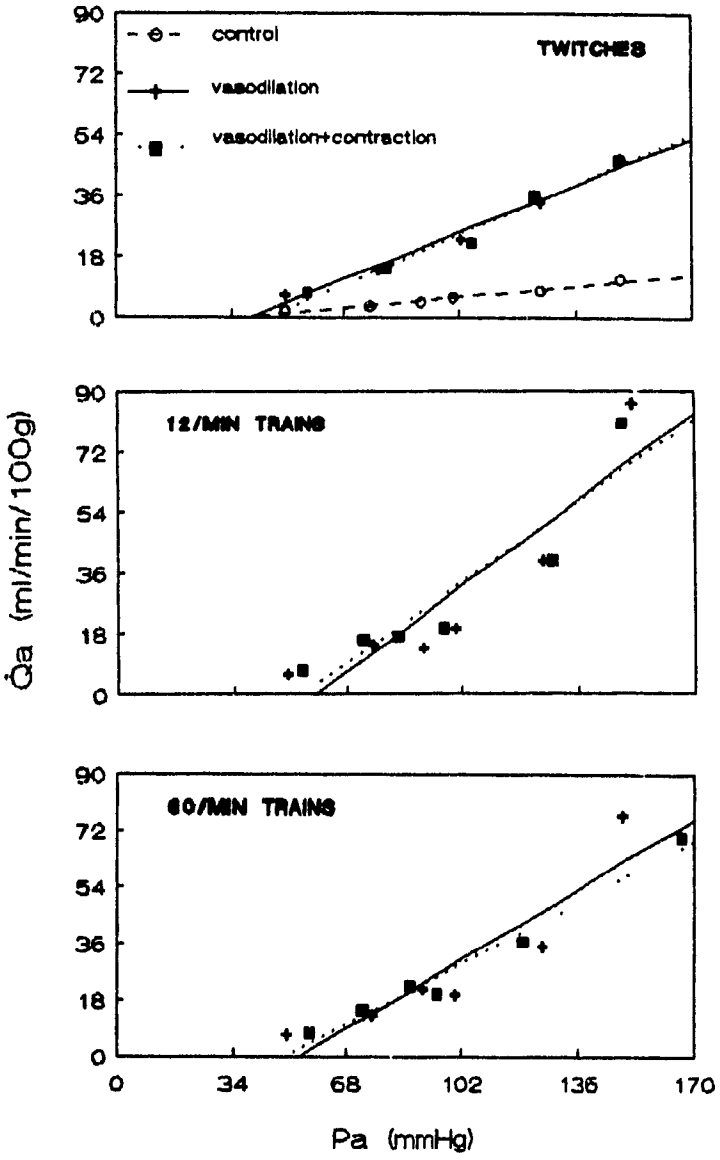

Figure 7. An example of popliteal pressure-flow relations. There were no changes in the X-intercept and slope with twitches, 12 and 60/min trains.

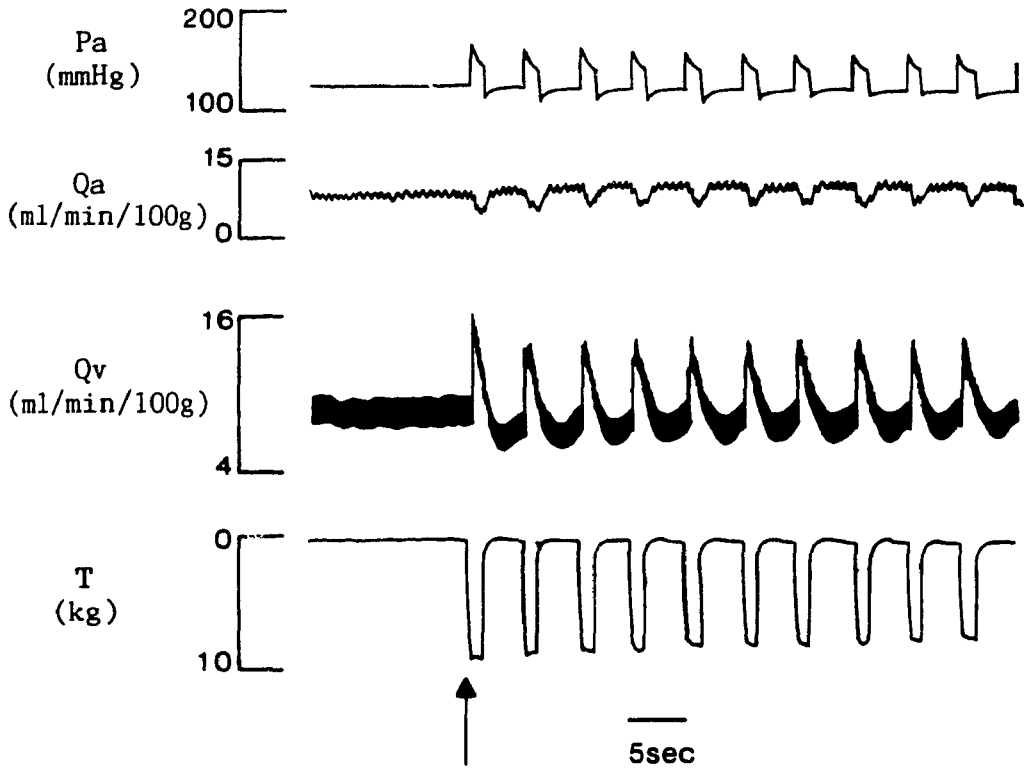

Figure 8. An example of perfusion pressure (Pa), arterial (Qa) and venous (Qv) flows, and tension (T) in the gastrocnemius muscle. During muscle relaxation, arterial inflow did not increase very much despite the large emptying of the venous segments during the contraction phase.

DISCUSSION

The main observation in this study is that the muscle pump has no effect on blood flow during tetanic trains and increases inflow slightly during spontaneous contractions.

1. Maximal Vasodilatation

An important assumption in this study was that the vasculature was maximally vasodilated prior to muscle contractions.

a) Choices of Drugs

We induced vasodilatation by maximally activating the cAMP pathway with adenosine, the cGMP pathway with nitroprusside and acetylcholine, and EDRF with acetylcholine (Waldman and Murad, 1987). We also injected indomethacin to block the prostaglandin pathway. Hypoxia, which is a potent vasodilator (Reid and Johnson, 1983), was not used because of its detrimental effect on tension development and possible depression of the ACh-induced relaxation (Furchgott and Zawadzki, 1980).

b) Evidence of Maximal Vasodilatation

Estimated flow at 100 mmHg increased by 130 and 359% in the vasodilated diaphragm and gastrocnemius, respectively. The increase was much greater in the gastrocnemius than in the diaphragm because of the lower popliteal inflow during the resting state. The marked rise in both the phrenic and popiteal inflow indicates that the drugs had a significant

vasodilating effect on the vasculature. Furthermore, trains of impulses which markedly increased tension did not augment mean inflow. The fact that no further vasodilatation was produced by endogenously released metabolites suggests that maximal relaxation of the resistance vessels was actually achieved in both muscles. This was also supported by the fact that the slope of the P-Q relations obtained during the relaxation phase of trains and/or spontaneous contractions was the same as that during maximal vasodilatation.

Although the reactive hyperemia following arterial or double occlusion could have been produced by unidentified vasodilator metabolites, it is probable that a myogenic response was elicited by the reduction in perfusion pressure (Johnson et al., 1976; Kontos et al., 1965; Tuma et al., 1977). This myogenic effect, which persisted despite maximal vasodilata- tion, could have been activated by indomethacin. Recently, Hill et al. (1990) showed that second-order arterioles distended passively in response to increased intravascular pressure but constricted markedly when exposed to cyclooxygenase inhibitors. Hill et al. (1990) concluded that indomethacin potentiates myogenic responses in skeletal muscle arterioles.

2. MUSCLE CONTRACTION

a) Muscle Tension

As previously discussed, muscle tension is one of the factors influencing the effectiveness of the muscle pump.

Hussain et al. (1989b) demonstrated that electrophrenic stimulation at 25 Hz produced diaphragmatic tension equivalent to 70% of the maximal tension at 100 Hz. Thus, we can assume that the relative tension developed in our hemidiaphragm preparation was high. In the gastrocnemius, the reduction in flow during the contraction phase was lower than in the diaphragm and suggests that the relative tension generated by the gastrocnemius was also much lower. However, the contraction phase of tetanic trains was associated with significant compression of both the phrenic and popliteal vasculatures, as indicated by the marked inhibition of inflow. Thus, the tension generated by both muscles was adequate for a muscle pump effect to be produced.

b) Type of Contraction

Repeated twitches at 4 Hz were not expected to affect inflow because of the small duration of the contraction phase, as illustrated by the lack of contraction-relaxation transients in the flow records (see Fig. 3a). The augmentation in mean phrenic inflow may have been a result of diaphragmatic shortening which diminishes vascular resistance (Supinski et al., 1986). The difference in the anatomy of the diaphragm and gastrocnemius could be responsible for their different responses to twitches. For example, the fanning design of the diaphragm could have produced less compression of the vasculature than the gastrocnemius muscle. However, differences in the types of muscle fibers could not contribute to this discrepancy since in the dog, both the

diaphragm and gastrocnemius muscles consist of 50% high oxidative slow-twitch fibers and 50% high oxidative fast-twitch fibers (Armstrong et al., 1982).

Tetanic trains did not affect phrenic or popliteal flow because the increase in flow during the relaxation phase was insufficient to compensate for the large inhibition during the contraction phase. This was probably due to the large tension generated.

When spontaneous contractions were induced, blood flow was actually increased during the contraction phase. This effect could have been potentiated by the low tension generated, the sequential contractions of the crural and costal diaphragm (Newman et al., 1984) and muscle shortening which probably averaged 8% of L_{FRC} (Newman et al., 1984). The increase in $PaCO_2$ during spontaneous contractions could have also contributed to the rise in flow. This is unlikely, however, since pH remained unchanged and CO_2 has no specific effect in the skeletal muscles of animals (Shepherd, 1983). Other metabolites can also be ruled out since none were released during the trains of impulses.

c) <u>Frequency of Contractions</u>

When 60/min trains were applied, the rise in flow during the relaxation phase was more limited than during 12/min trains. This could be attributed to the smaller duration of the relaxation phase at higher contraction rates (Buchler et al., 1985). However, mean inflow remained essentially the same during either 10-22/min or 60/min trains.

3. PREVIOUS PREPARATIONS

Our findings are in agreement with those of Hirche et al. (1970) who showed that mean resistance to flow remained unchanged when isometric or isotonic rhythmic contractions were elicited in the isolated maximally vasodilated gastrocnemius. Folkow et al. (1970), using the same preparation, found that mean blood flow increased during rhythmic contractions of the gastrocnemius. They demonstrated that the pressure in the compliant region decreased markedly during the relaxation phase and concluded that the increase in driving pressure was responsible for the rise in flow. However, they produced maximal vasodilatation with a series of muscle contractions and thus failed to rule out the contribution of metabolites to the increased blood flow. No previous studies have investigated the effect of the muscle pump on diaphragmatic blood flow.

4. FAILURE OF THE MUSCLE PUMP THEORY

Our hypothesis was that muscle contractions would increase muscle blood flow by causing changes in the pressure of the compliant region (Fig. 1a). We expected that during the contraction phase, the venules would be squeezed and venous flow augmented (Fig. 1b). Thus, during the relaxation phase, the pressure in the emptied compliant region (Pc) would be markedly decreased and the gradient Pa-Pc and thus arterial flow, significantly increased (Fig. 1c). Our results show that although outflow was large during tetanic

trains, inflow remained essentially the same (Fig. 8). These findings could be explained on the basis of a precapillary Starling resistor (Fig. 2), which may be present in the arterioles of skeletal muscle (Magder, 1990).

We estimated the pressure at the Starling resistor (Ps) by extrapolating the P-Q relation to zero flow and determining the pressure intercept ($P_{Z=0}$). Since at low perfusion pressures phrenic and popliteal inflows were very low, the error in estimating Ps should be small. The high values obtained suggest that $P_{Z=0}$ remained greater than Pc during contractions either elicited spontaneously or by trains of impulses. Thus, $P_{Z=0}$ was the effective downstream pressure and the equation for arterial inflow should have been $Qa=(Pa-(P_{Z=0}))/Ra$ (Permutt and Riley, 1963). Since Ra and Pa were essentially unchanged in our experiments, Qa was dependent on $P_{Z=0}$. When both muscle preparations were stimulated with tetanic trains, $P_{Z=0}$ and thus Qa remained the same. During spontaneous contractions of the diaphragm, there was a significant drop in $P_{Z=0}$ which produced a 20% rise in phrenic Qa at 100 mmHg, as compared with the maximally vasodilated state. The decline in $P_{Z=0}$ was most probably caused by the low tension developed, the sequential activation of the crural and costal parts and diaphragmatic shortening. Hypercapnia may have also been a contributing factor (Early et al., 1974).

In conclusion, skeletal muscle contractions produce only a modest increase in blood flow. This failure of the

muscle pump mechanism could be due to the presence of a Starling resistor mechanism which is above the venous compliant region and does not allow changes in venous pressure to affect flow into the muscle.

CONCLUSION

The muscle pump has been thought to play an important role in exercise hyperemia but its effect has not yet been quantified (Folkow et al., 1970; Hirche et al., 1970; Laughlin, 1987; Wiggers, 1954). This study has contributed to the existing body of knowledge by demonstrating that the muscle pump has no effect on blood flow during tetanic stimulation of maximally vasodilated muscle preparations and only a small effect during spontaneous contractions.

Changes in the duration, frequency or tension of isometric contractions markedly changed the pressure in the compliant region of the popliteal vasculature but had no effect on arterial flow to the gastrocnemius muscle. In addition, although some myogenic reactivity remained and endogenous metabolites were probably released during electrical stimulation, these potential confounding factors had no influence on inflow.

In the diaphragm, repeated single twitches and spontaneous contractions produced a 9 and 20% rise in flow, respectively, as compared with the maximally vasodilated state. When these changes were compared with the resting state, they averaged 17 and 64%, respectively. However, these numbers should be interpreted with caution as they can only be estimated. It would be ideal to measure the mechanical effect of muscle contractions on resting blood flow but this is impossible as metabolites would be released im-

mediately. The rise in phrenic inflow during twitches was probably enhanced by diaphragmatic shortening. Further studies are necessary to determine whether greater diaphragmatic shortening would have produced a greater increment in arterial flow. During spontaneous contractions, in addition to muscle shortening, low tension development and sequential recruitment of the costal and crural parts probably facilitated blood flow. It is possible that chloralose prevented a further augmentation in flow as it has been shown to potentiate simultaneous activation of phrenic motoneurons during the first one third of the inspiration phase (Hilaire and Monteau, 1979; Iscoe et al., 1976; St. John and Bartlett, 1979). On the other hand, the elevation of $PaCO_2$ could have aided arterial inflow. All these factors seem to have contributed to the exercise hyperemia by significantly reducing the arterial pressure at zero flow or $P_{Z=0}$. However, despite this lowering effect, $P_{Z=0}$ remained elevated and was most probably the effective downstream pressure.

In contrast to our hypothesis, this study indicates that the muscle pump mechanism can potentiate muscle inflow by changing $P_{Z=0}$ rather than the pressure in the compliant region (Pc). Hence, although the compression of the compliant region during muscle contractions counteracts venous distension caused by gravity and facilitates venous return to the heart (Pollack and Wood, 1949), it does not influence arterial inflow to skeletal muscle. Furthermore, since $P_{Z=0}$ is greater than Pc, the gradient $Pa-(P_{Z=0})$ is smaller than

Pa-Pc. As a result, the extent to which arterial inflow can increase during the relaxation phase of rhythmical muscle contractions is less than we expected.

REFERENCES

Andersen, P. and B. Saltin. Maximal perfusion of skeletal muscle in man. J. Physiol. Lond. 366: 233-249, 1985.

Armstrong, R.B., M.D. Delp, E.F. Goljan, and M.H. Laughlin. Distribution of blood flow in muscles of miniature swine during exercise. J. Appl. Physiol. 62: 1285-1298, 1987.

Armstrong, R.B. and M.H. Laughlin. Rat muscle blood flows during high-speed locomotion. J. Appl. Phys. 59: 1322-1328, 1985.

Armstrong, R.B., C.W. Saubert IV, H.J. Seeherman, and C.R. Taylor. Distribution of fiber types in locomotory muscles of dogs. Am. J. Anat. 163: 87-98, 1982.

Aubier, M., T. Trippenbach, and C. Roussos. Respiratory muscle fatigue during cardiogenic shock. J. Appl. Physiol. 51: 499-508, 1981.

Bacchus, A., G. Gamble, D. Anderson, and J. Scott. Role of the myogenic response in exercise hyperemia. Microvasc. Res. 21: 92-102, 1981.

Barcroft, H. Circulation in skeletal muscle. In: Handbook of Physiology. Circulation. Washington, DC: Am. Physiol. Soc., 1963, sect. 2, vol. II, chapt. 40, p. 1353-1385.

Barcroft, H. Circulatory changes accompanying the contraction of voluntary muscle. Austral. J. Exp. Biol. 42: 1-16, 1964.

Barcroft, H. and A.C. Dornhorst. Demonstration of the "muscle pump" in the human leg. J. Physiol. Lond. 108: 38P, 1949a

Barcroft, H. and A.C. Dornhorst. The blood flow through the human calf during rhythmic exercise. J. Physiol. Lond. 109: 402-411, 1949b.

Barcroft, H. and J.L.E. Millen. The blood flow through muscle during sustained contraction. J. Physiol. Lond. 97:17-31, 1939.

Bellemare, F., D. Wight, C.M. Lavigne, and A. Grassino. Effect of tension and timing of contraction on the blood flow of the diaphragm. J. Appl. Physiol. 54: 1597-1606, 1983.

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

Buchler, B., S. Magder, and C. Roussos. Effects of contraction frequency and duty cycle on diaphragmatic blood flow. J. Appl. Physiol. 58 : 265-273, 1985.

Burke, R.E. Motor units: anatomy, physiology, and functional organization. In: Handbook of Physiology. The Nervous System. Motor Control. Bethesda, MD: Am. Physiol. Soc., 1981, sect. 1, vol. II, part I, chapt. 10, p. 345-422.

Close, R.I. Dynamic properties of mammalian skeletal muscles. Physiol. Rev. 52: 129-197, 1972.

De Troyer, A., M. Sampson, S. Sigrist, and P.T. Macklem. The diaphragm: Two muscles. Science. 213: 237-238, 1981.

Early, C.B., R.C. Dewey, H.P. Pieper, and W.E. Hunt. Dynamic pressure-flow relationships of brain blood flow in the monkey. J. Neurosurg. 41: 590-596, 1974.

Ehrlich, W., R.W. Baer, R.F. Bellamy, and R. Randazzo. Instantaneous femoral artery pressure-flow relations in supine anesthetized dogs and the effect of unilateral elevation of femoral venous pressure. Circ. Res. 47: 88-98, 1980.

Eng, C., J.H. Jentzer, and E.S. Kirk. The effects of the coronary capacitance on the interpretation of diastolic pressure-flow relationships. Circ. Res. 50: 334-341, 1982.

Feldman, J.L. Neurophysiology of breathing in mammals. In: Handbook of Physiology. The Nervous System. Intrinsic Regulatory Systems of the Brain. Bethesda, MD: Am. Physiol. Soc., 1986, sect. 1, vol. IV, chapt. 9, p. 463-523.

Folkow, B. Description of the myogenic hypothesis. Circ. Res. 15: (Suppl. 1) 279-287, 1964.

Folkow, B., P. Gaskell and B.A. Waaler. Blood flow through limb muscles during heavy rhythmic exercise. Acta Physiol. Scand. 80: 61-72, 1970.

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

Folkow, B. and B. Oberg. Autoregulation and basal tone in consecutive vascular sections of the skeletal muscles in reserpine-treated cats. Acta Physiol. Scand. 53: 105-113, 1961.

Furchgott, R.F., M.H. Carvalho, M.T. Khan, and K. Matsunaga. Evidence for endothelium-dependent vasodilatation of resistance vessels by acetylcholine. Blood Vessels. 24: 145-149, 1987.

Furchgott, R.F. and J.V. Zawadzki. The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. Nature. 288: 373-376, 1980.

Goodman, A.H., R. Einstein, and H.J. Granger. Effect of changing metabolic rate on local blood flow control in the canine hindlimb. Circ. Res. 43: 769-776, 1978.

Gray, S.D., E. Carlsson, and N.C. Staub. Site of increased vascular resistance during isometric muscle contraction. Am. J. Physiol. 213: 683-689, 1967.

Green, J.F. Circulatory mechanics. In: Fundamental Cardiovascular and Pulmonary Physiology. Philadelphia, PA: Lea & Febiger, 1987, chapt. 4, p. 59-80.

Griffith, T.M., D.H. Edwards, R. LI. Davies, T.J. Harrison, and K.T. Evans. EDRF coordinates the behaviour of vascular resistance vessels. Nature. 329: 442-445, 1987.

Grimby, G., E. Haggendal and B. Saltin. Local xenon 133 clearance from the quadriceps muscle during exercise in man. J. Appl. Physiol. 22: 305-310, 1967.

Hilaire, G. and R. Monteau. Facteurs déterminant l'ordre de recrutement des motoneurones phréniques. J. Physiol. Paris. 75: 765-781, 1979.

Hill, A.V. The pressure developed in muscle during contraction. J. Physiol. Lond. 107: 518-526, 1948.

Hill, M.A., M.J. Davis, and G.A. Meininger. Cyclooxygenase inhibition potentiates myogenic activity in skeletal muscle arterioles. Am. J. Physiol. 258 (Heart Circ. Physiol. 27): H127-H133, 1990.

Hilton, S.M., M.G. Jeffries, and G. Vrbová. Functional specializations of the vascular bed of soleus. J. Physiol. Lond. 206: 543-562, 1970.

Hirche, HJ., W.K. Raff and D. Grun. The resistance to blood flow in the gastrocnemius of the dog during sustained and rhythmical isometric and isotonic contractions. Pflugers Arch. 314: 97-112, 1970.

Honig, C.R. Contributions of nerves and metabolites to exercise vasodilation: a unifying hypothesis. Am. J. Physiol. 236 (Heart Circ. Physiol. 5): H705-H719, 1979.

Honig, C.R. and J.L. Frierson. Neurons intrinsic to arterioles initiate postcontraction vasodilation. Am. J. Physiol. 230: 493-507, 1976.

Humphreys, P.W. and A.R. Lind. The blood flow through active and inactive muscles of the forearm during sustained hand-grip contractions. J. Physiol. Lond. 166: 120-135, 1963.

Hussain, S.N.A., C. Roussos, and S. Magder. Autoregulation of diaphragmatic blood flow in dogs. J. Appl. Physiol. 64: 329-336, 1988.

Hussain, S.N.A., C. Roussos, and S. Magder. Effects of tension, duty cycle, and arterial pressure on diaphragmatic blood flow in dogs. J. Appl. Physiol. 66: 968-976, 1989a.

Hussain, S.N.A., C. Roussos, and S. Magder. In situ isolated perfused and innervated left hemidiaphragm preparation. J. Appl. Physiol. 67: 2141-2146, 1989b.

Iscoe, S., J. Dankoff, R. Migicovsky, and C. Polosa. Recruitment and discharge frequency of phrenic motoneurones during inspiration. Resp. Physiol. 26: 113-128, 1976.

Johnson, P.C., K.S. Burton, H. Henrich, and U. Henrich. Effect of occlusion duration on reactive hyperemia in sartorius muscle capillaries. Am. J. Physiol. 230: 715-719, 1976.

Johnson, P.C. The myogenic response. In: Handbook of Physiology. The Cardiovascular System. Vascular Smooth Muscle. Bethesda, MD : Am. Physiol. Soc., 1980, sect. 2, vol. II, chapt. 15, p.409-442.

Kontos, H.A., H.P. Mauck, Jr., and J.L. Patterson, Jr. Mechanism of reactive hyperemia in limbs of anesthetized dogs. Am. J. Physiol. 209: 1106-1114, 1965.

Laughlin, M.H. Skeletal muscle blood flow capacity: role of muscle pump in exercise hyperemia. Am. J. Physiol. 253 (Heart Circ. Physiol. 22): H993-H1004, 1987.

Laughlin, M.H. and J. Ripperger. Vascular transport capacity of hindlimb muscles of exercise-trained rats. J. Appl. Physiol. 62: 438-443, 1987.

Lind, A.R. and G.W. McNichol. Local and central circulatory responses to sustained contractions and the effect of free or restricted arterial inflow on post-exercise hyperaemia. J. Physiol. Lond. 192: 575-593, 1967.

Mackie, B.G. and R.L. Terjung. Blood flow to different skeletal muscle fiber types during contraction. Am. J. Physiol. 245 (Heart Circ. Physiol. 14): H265-H275, 1983.

Magder, S. Pressure-flow relations of diaphragm and vital organs with nitroprusside-induced vasodilatation. J. Appl. Physiol. 61: 409-416, 1986.

Magder, S. Starling resistor versus compliance. Which explains the zero-flow pressure of a dynamic arterial pressure-flow relation? Circ. Res. 67: 209-220, 1990.

Magder, S., D. Lockhat, B.J. Luo, and C. Roussos. Respiratory muscle and organ blood flow with inspiratory elastic loading and shock. J. Appl. Physiol. 58: 1148-1156, 1985.

Manohar, M. Vasodilator reserve in respiratory muscles during maximal exertion in ponies. J. Appl. Physiol. 60: 1571-1577, 1986.

Mohrman, D.E. and H.V. Sparks. Myogenic hyperemia following brief tetanus of canine skeletal muscle. Am. J. Physiol. 227: 531-535, 1974.

Mush, T.I., G.C. Haidet, G.A. Ordway, J.C. Longhurst, and J.H. Mitchell. Training effects on regional blood flow response to maximal exercise in foxhounds. J. Appl. Physiol. 62: 1724-1732, 1987.

Myers, H.A., E.A. Schenk, and C.P. Honig. Ganglion cells in arterioles of skeletal muscle: role in sympathetic vasodilatation. Am. J. Physiol. 229: 126-138, 1975.

Newman, S., J. Road, F. Bellemare, J.P. Clozel, C.M. Lavigne, and A. Grassino. Respiratory muscle length measured by sonomicrometry. J. Appl. Physiol. 56: 753-764, 1984.

Nickerson, M. Vasodilator drugs. In: The Pharmacological Basis of Therapeutics, edited by L.S. Goodman and A. Gilman, New York: McMillan Publishing Co. Inc., 1975, chapt. 34, p. 727-743.

Pappenheimer, J.R. and J.P. Maes. A quantitative measure of the vasomotor tone in the hindlimb muscles of the dog. Am. J. Physiol. 137: 187-199, 1942.

Permutt, S. and R.L. Riley. Hemodynamics of collapsible vessels with tone: the vascular waterfall. J. Appl. Physiol. 18: 924-932, 1963.

Pohl, U., L. Dézsi, B. Simon, and R. Busse. Selective inhibition of endothelium-dependent dilation in resistance-sized vessels in vivo. Am. J. Physiol. 253 (Heart Circ. Physiol. 22): H234-H239, 1987.

Pollack, A.A. and E.H. Wood. Venous pressure in the saphenous vein at the ankle in man during exercise and changes in posture. J. Appl. Physiol. 1: 649-662, 1949.

Reid, M.B. and R.L. Johnson, Jr. Efficiency, maximal blood flow, and aerobic work capacity of canine diaphragm. J. Appl. Physiol. 54: 763-772, 1983.

Robertson, C.H., Jr, G.H. Foster and R.L. Johnson, Jr. The relationship of respiratory failure to the oxygen consumption of, lactate production by, and distribution of blood flow among respiratory muscles during increasing inspiratory resistance J. Clin. Invest. 59: 31-42, 1977a.

Robertson, C.H., Jr, M.A. Pagel and R.L. Johnson, Jr. The distribution of blood flow, oxygen consumption, and work output among the respiratory muscles during unobstructed hyperventilation. J. Clin. Invest. 59: 43-50, 1977b.

Rochester, D.F. and G. Bettini. Diaphragmatic blood flow and energy expenditure in the dog. Effects of inspiratory airflow resistance and hypercapnia. J. Clin. Invest. 57: 661-672, 1976.

Rochester, D.F. and A.M. Briscoe. Metabolism of the working diaphragm. Am. Rev. Resp. Dis. 119: 101-106, 1979.

Rochester, D.F. and E.C. Drash. Respiratory muscle blood flow and metabolism. In: Lung Biology in Health and Disease: Thorax, edited by C. Roussos and P. Macklem, New York : M. Dekker, 1985, Part A, chapt. 14, p. 393-436.

Rochester, D.F. and M. Pradel-Guena. Measurement of diaphragmatic blood flow in dogs from xenon 133 clearance. J. Appl. Physiol. 34:68-74, 1973.

Rowell, L.B., B. Saltin, B. Kiens, and N.J. Christensen. Is peak quadriceps blood flow in humans even higher during exercise with hypoxemia? Am. J. Physiol. 251 (Heart Circ. Physiol. 20): H1038-H1044, 1986.

Saltin, B. and P.D. Gollnick. Skeletal muscle adaptability: significance for metabolism and performance. In: Handbook of Physiology. Skeletal Muscle. Bethesda, MD: Am. Physiol. Soc., 1983, sect. 10, chapt. 19, p. 555-631.

Sant'Ambrogio and F. Saibene. Contractile properties of the diaphragm in some mammals. Resp. Physiol. 10: 349-357, 1970.

Shepherd, J.T. Circulation to skeletal muscle. In: Handbook of Physiology. The Cardiovascular System. Peripheral Circulation and Organ Blood Flow. Bethesda, MD: Am. Physiol. Soc., 1983, sect. 2, vol. III, chapt. 11, p. 319-370.

Smiesko, V. Unidirectional rate sensitivity component in local control of vascular tone. Pflugers Arch. 327: 324-336, 1971.

Spaan, J.A.E. Coronary diastolic pressure-flow relation and zero flow pressure explained on the basis of intramyocardial compliance. Circ. Res. 56: 293-309, 1985.

Stainsby, W.N. Autoregulation of blood flow in skeletal muscle during increased metabolic activity. Am. J. Physiol. 202: 273-276, 1962.

Stainsby, W.N., J.T. Fales, and J.L. Lilienthal, Jr. Effect of stretch on oxygen consumption of dog skeletal muscle in situ. Bull. Johns Hopkins Hosp. 99: 249-261, 1956.

Stainsby, W.N. and E. M. Renkin. Autoregulation of blood flow in resting skeletal muscle. Am. J. Physiol. 201: 117-122, 1961.

Stegall, H.F. Muscle pumping in the dependent leg. Circ. Res. 9:180-190, 1966.

St. John, W.M. and D. Bartlett, Jr. Comparison of phrenic motoneuron responses to hypercapnia and isocapnic hypoxia. J. Appl. Physiol. 46: 1096-1102, 1979.

Supinski, G.S., H. Bark, A. Guanciale, and S.G. Kelsen. Effect of alterations in muscle fiber length on diaphragm blood flow. J. Appl. Physiol. 60: 1789-1796, 1986.

Sylvester, J.T., R.D. Gilbert, R.J. Traystman, and S. Permutt. Effects of hypoxia on the closing pressure of the canine sys temic arterial circulation. Circ. Res. 49: 980-987, 1981.

Tuma, R.F., L. Lindbom. and K.E. Arfors. Dependence of reactive hyperemia in skeletal muscle on oxygen tension. Am. J. Physiol. 233 (Heart Circ. Physiol. 2): H289-H294, 1977.

Viires, N., G. Sillye, M. Aubier, A. Rassidakis and C. Roussos. Regional blood flow distribution in dog during induced hypotension and low cardiac output. J. Clin. Invest. 72: 935-947, 1983.

Waldman, S.A. and F. Murad. Cyclic GMP synthesis and function. Pharmacol. Rev. 39: 163-196, 1987.

Whittaker, S.R.F. and F.R. Winton. The apparent viscosity of blood flowing in the isolated hindlimb of the dog, and its variation with corpuscular concentration. J. Physiol. Lond. 78: 339-369, 1933.

Wiggers, C.J. The interplay of coronary vascular resistance and myocardial compression in regulating coronary flow. Circ. Res. 11: 271-279, 1954.

Yamada, S.I. and A. Åstrom. Critical closing pressure and vasomotor tone in the hind leg and the kidney of the cat. Am. J. Physiol. 196: 213-220, 1959.