ANTAGONISM OF THE EFFECT OF BRADYKININ BY NOREPINEPHRINE ON MICROVASCULAR FLUID FLUX

Thesis for the Degree of M. S.
MICHIGAN STATE UNIVERSITY
JAMES JOHN MACIEJKO
1976

ABSTRACT

ANTAGONISM OF THE EFFECT OF BRADYKININ BY NOREPINEPHRINE ON MICROVASCULAR FLUID FLUX

By

James John Maciejko

Bradykinin (0.8 or 10 mcg/min) infused into the brachial artery of the canine forelimb for 60 minutes, causes marked increases in forelimb weight, lymph flow and lymph total protein concentration. The mechanism is by an increase in the transmural capillary hydrostatic pressure gradient and by a decrease in the transmural colloid osmotic pressure gradient due to an increased microvascular permeability to plasma protein. In contrast, systemically (intravenous) administered bradykinin, even in blood concentrations equal to or exceeding those achieved by the local infusion, failed to increase forelimb lymph flow and lymph total protein concentration. Thus, there exists a route-dependent differential action of bradykinin on transvascular fluid flux.

Possible explanations for this differential action between the two modes of infusion are: destruction of bradykinin by the lungs occurring with the intravenous infusion; inactivation by factors in the plasma before reaching the microvessels with the intravenous infusion; an effective antagonism by substances (i.e. catecholamines) released during a sympathoadrenal discharge subsequent to the hypotension produced by the intravenous infusion.

Infusing bradykinin (140 to 280 mcg/min) into the vena cava or the left ventricle of the heart slightly increased flow and total protein concentration of the lymph, as compared to the local infusion route. There was little difference in lymph flow and lymph protein concentration between intravenous or left ventricular infusion, although left ventricular infusions increased these parameters slightly more than the intravenous infusions. This would seem to indicate that at these dosages of bradykinin, pulmonary inactivation plays a minor role in its destruction.

The greater transit time required for bradykinin to reach the microvasculature during a systemic infusion, as compared to a local infusion, would allow more time for factors in the blood to inactivate bradykinin. However, this cannot account for the route-dependent differential actions of bradykinin. This can be explained by the fact that large increases in lymph flow and lymph protein concentration are observed with local infusions of bradykinin into forelimbs perfused at constant inflow. In these experiments, bradykinin must travel through a one to two meter length of polyethylene tubing before reaching the forelimb. Since this distance is greater than the distance bradykinin must travel during systemic infusions and if factors within the blood were destroying bradykinin, then the marked increases in flow and protein concentration of the lymph would not be expected during the local bradykinin infusions at constant inflow.

To investigate the possibility of an antagonism by substances released during a hypotensive sympathoadrenal discharge on the microvasculature, hypotension was produced for 60 minutes by hemorrhage.

This w

It sag

size a

abou

suddq

rescu

Keep

Inside

brady

accep

time.

forel

weigh

scrib

and

Ther

This

trad

cust

Use

the

tra

This was followed by a 60 minute local infusion of bradykinin (0.8 and 10 mcg/min, I.A.) into forelimbs perfused at constant inflow. Flow rate and protein concentration of the lymph increased very slightly to about the same levels as observed with the systemic infusions.

To further examine the hypothesis that substances released during a sympathoadrenal discharge antagonize bradykinin at the microvascular site, weight, hemodynamic and lymph studies were conducted. Norepinephrine (4 mcg base/min) was infused simultaneously with bradykinin (0.8 mcg/min) into the forelimb brachial artery. In contrast to bradykinin (0.8 mcg/min) infused alone, the concurrent infusion with norepinephrine failed to alter lymph flow and lymph protein concentration. In the weight and hemodynamic experiments at natural inflow, forelimb weight did not change, whereas at constant inflow, forelimb weight increased due to an augmented venous resistance (active venoconstriction by norepinephrine), thereby increasing microvascular pressure and filtration.

Hence, it is concluded that norepinephrine prevents the marked increase in extravascular fluid volume that is produced by bradykinin. This antagonism could be due to a direct blockade of the action of bradykinin on the microvascular membrane, a shunting of blood flow from nutritional to non-nutritional channels, or a combination of both. Also, this antagonistic action of norepinephrine may, in part, explain the differential effects of locally and systemically administered bradykinin on lymph flow, protein efflux and microvascular fluid flux.

ANTAGONISM OF THE EFFECT OF BRADYKININ BY NOREPINEPHRINE ON MICROVASCULAR FLUID FLUX

By

James John Maciejko

A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE

Department of Physiology

1976

To my parents

Without their love, encouragement and support, my education and this thesis would not have been possible.

ACKNOWLEDGMENTS

For their many contributions in this endeavor, I express my sincere appreciation to Dr. Jerry B. Scott, Dr. C. C. Chou, Mr. Edward Gersabeck, Mr. Douglas L. Marciniak and Mr. Daniel P. Sak.

I also wish to thank Miss Darlene DenHollander for her assistance in typing.

I am deeply indebted to Dr. George J. Grega, my advisor, for his counsel and valuable assistance throughout this undertaking.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF SYMBOLS AND ABBREVIATIONS

NF = Naturally perfused forelimb.

CF = Forelimb perfused at constant inflow.

LH = Drug infusion into the left ventricle of the heart.

IA = Drug infusion intra-arterially into the forelimb.

IV = Drug infusion into the vena cava.

cm = Centimeter.

mm = Millimeter.

μm = Micrometer.

kg = Kilogram.

gms = Grams.

mgm = Milligram.

mcg = Microgram.

ml = Milliter.

min = Minute.

Å = Angstrom.

mmHg = Millimeters of mercury pressure.

B0.8 = 0.8 mcg/min of bradykinin.

B10 = 10 mcg/min of bradykinin.

B140 = 140 mcg/min of bradykinin.

B280 = 280 mcg/min of bradykinin.

N4 = 4 mcg/min of norepinephrine base.

* = $p \leq 0.01$ relative to 0 minute control.

† = $p \leq 0.05$ relative to 0 minute control.

ω = $p \leq 0.01$ relative to 60 minute control.

Ω = $p \leq 0.05$ relative to 60 minute control.

INTRODUCTION

The pharmacological effects of the venom of Bothrops jararaca (South American serpent) had been under investigation in 1949 by Rocha e Silva and led to the finding that incubation of the venom with the globulin fraction of blood plasma from a dog resulted in a potent vasodilator and smooth-muscle-stimulating substance (47). The material was named bradykinin, owing to its slow muscle contracting action on pig ileum, and the globulin fraction from which the bradykinin was released was named bradykininogen. In 1960, bradykinin was isolated by Elliot (13) from ox serum treated with trypsin and later synthesized by Boissonnas (5).

Bradykinin is known to stimulate certain types of smooth muscle, to cause vasodilation, to increase capillary permeability, and to produce pain when brought into contact with pain fibers (34). This study is principally concerned with those actions of bradykinin pertinent to pathological conditions which are manifested by abnormal fluid fluxes causing tissue edema.

It is well established that bradykinin, administered locally (0.8 or 10 mcg/min, I.A.) increases the efflux rate of water from capillaries and the immediate post-capillary venules leading to massive edema formation with the higher dose. The mechanism of this effect is

both by a rise in the transmural capillary hydrostatic pressure gradient subsequent to arteriolar vasodilation and by a fall in the transmural colloid osmotic pressure gradient owing to an increased microvascular permeability to plasma protein. However, when bradykinin is administered systemically (iv), it fails to increase the rate of transcapillary fluid movement (8). There are obvious differences which may account for the observed route dependent differential actions of bradykinin on fluid movement. First of all, in the studies pertaining to the intravenous infusions of bradykinin on fluid fluxes, the concentrations were too small to equal the concentrations used in the local infusion studies. Also, during intravenous infusion, bradykinin passes through the pulmonary circuit before reaching the systemic microvasculature. It is well established that the lungs metabolize or biotransform bradykinin (1, 17). Furthermore, bradykinin has a short half-life in plasma, and the transit time from the point of infusion to the microcirculation with systemic infusions could result in destruction. Finally, locally administered bradykinin has little if any effect on systemic arterial pressure, whereas intravenously infused bradykinin causes marked hypotension. Since the fall in systemic blood pressure is associated with a sympathoadrenal discharge, it is possible that substances liberated subsequent to hypotension effectively antagonize the edemogenic action of bradykinin.

Consequently, it is the goal of this investigation to construe the mechanisms of the route dependent differential action of bradykinin[1] on transvascular fluid movement.

[1] Due to the excessive cost of commercially available bradykinin ($7.00 to $15.00/mgm), and the large quantities used in the systemic infusion studies, these experiments had to be kept to a minimum. Only those systemic experiments of utmost importance were carried out.

SURVEY OF THE LITERATURE

Furnishing the tissues of the body with blood in amounts sufficient to meet the requirements for oxygen, nutrients and the removal of metabolic byproducts is the foremost aim of the cardiovascular system. The capillaries, which form an interconnecting network of tubes between the arterioles and venules, together with the immediate postcapillary venules, accomplish this function by allowing exchange to occur between the blood and interstitium. Exchange of substances between blood and tissues can occur by filtration, diffusion or pinocytosis.

Movement of fluid across the capillary wall and into the interstitium is based on the balance between hydrostatic and osmotic forces. The degree of filtration or reabsorption is dependent on the sum of these physical forces which can be related in the following equation derived by Starling (52):

$$F = k(Pc - Pi - \pi p - \pi i),$$

where

F = the rate of fluid movement;

k = filtration coefficient (This coefficient is a measure of the permeability of the microvascular wall to isotonic fluid. It is determined by the product of capillary permeability and surface area available for diffusion.) (33);

Pc = capillary hydrostatic pressure;

Pi = interstitial hydrostatic pressure;

πp = plasma colloid osmotic pressure;

πi = interstitial colloid osmotic pressure.

According to Starling's hypothesis, when the algebraic sum of this equation is positive, filtration occurs; when it is negative, reabsorption occurs.

Capillary hydrostatic pressure (Pc) is directly dependent upon capillary blood volume and compliance. Clough et al. (7) have indicated that capillaries are quite rigid. They report a change of only 0.1 μm in radius in capillaries of cat mesentery during systole. This rigidity results from the environment circumjacent to the capillaries. The basement membrane and gel matrix surrounding these microvessels give the capillaries little, if any, compliance (18). Since compliance is relatively constant in capillaries, changes in capillary blood volume are the primary factor in determining Pc. Capillary blood volume is influenced by systemic arterial pressure, venous pressure and the pre and post capillary resistances. The interrelationship of these factors is expressed in the following equation of Pappenheimer and Soto-Rivera (33):

$$Pc = (Pa - Rv)\frac{Rv}{Ra + Rv} + Pv,$$

where

Pa = systemic arterial pressure;

Pv = venous pressure;

Ra = arterial resistance (precapillary);

Rv = venous resistance (post capillary).

An increase in Pa or Pv will increase Pc. A given increase in Pv,

however, has a five to ten fold greater effect on Pc (33). Increasing Rv will raise Pc, whereas increasing Ra will lower Pc. Vessel resistances are indirectly related to vessel caliber. This caliber is determined mainly by active changes in vascular smooth muscle activity and passively by changes in effective transmural pressure. Effective transmural pressure is the pressure in the interstitial fluid environment of the capillary subtracted from the intraluminal pressure in the capillary. Changes in blood viscosity also affect resistance to blood flow. Blood viscosity is determined by the hematocrit and the dissolved materials in the plasma.

The hydrostatic pressure of the interstitial spaces is determined by tissue compliance and interstitial fluid volume. Classically, it is accepted that this pressure is positive and will therefore, oppose fluid filtration out of the capillaries. However, Guyton (23) using implanted perforated spheres in various tissues, has concluded that this pressure is sub-atmospheric (-7 mmHg). This issue is under much criticism and further investigation is needed to resolve this dilemma.

Plasma colloid osmotic pressure (oncotic pressure) is the pressure due to the concentration of dissolved proteins in the blood. Under normal conditions, the total osmotic pressure of plasma is about 6,000 mmHg, with the oncotic pressure contributing about 25 mmHg (4). This oncotic pressure is responsible for vascular fluid retention. It is achieved because the plasma proteins are largely confined to the intravascular space and therefore, create an active tonicity within the vasculature. The ions which constitute the bulk of the remaining total

osmotic pressure pass freely through the capillary membrane, creating no tonicity between the two body fluid spaces (43).

Albumin, globulin and fibrinogen constitute the major plasma proteins. Albumin, which is found in the greatest abundance, has an average molecular weight of 69,000 and a concentration of about 4.6 gms %. Globulin has an average molecular weight of about 140,000 with a concentration of 2.5 gms %. Fibrinogen is the largest of the plasma proteins, having a molecular weight of about 400,000 but is found in a concentration of only 0.3 gms %. Of the total oncotic pressure, 19 mmHg are attributable purely to the proteins, and the remaining 6 mmHg are due to the cations which bind to the proteins by electronegative forces. This phenomenon is known as the "Donnan Effect". Since albumin is in the greatest concentration of the three proteins, it constitutes the largest fraction (70%) of the total oncotic pressure.

Interstitial fluid colloid osmotic pressure is contingent on the protein concentration of the interstitial fluid. Normal concentration of the interstitial proteins is not uniform; it varies from 0.4 gms % to 3.3 gms %, depending on the tissue (33). In skin and skeletal muscle, the average interstitial protein concentration is about 2.0 gms %, which yields an oncotic pressure of about 5 mmHg. More recent findings suggest that the total protein concentration of interstitial fluid is about 3 gms % and the colloid osmotic pressure about 10 mmHg (58). In the liver, where capillary protein permeability is high, large amounts of the plasma proteins cross the microvascular membrane, producing an interstitial fluid oncotic pressure of about 16 mmHg or more. This corresponds to a minimal protein concentration of about

3.3 gms %; in fact, this value is often greater. Electron microscopy has revealed that the ultrastructure of the capillaries is not the same in all parts of the circulation (40). Three different types of capillary walls have been identified; they are termed continuous, discontinuous and fenestrated. The continuous capillary wall is the most common type observed in smooth and skeletal muscle, adipose tissue, connective tissue and pulmonary tissue. The capillary wall is a continuous membrane of endothelial cells with numerous intercellular channels, 40 to 50 Å wide, connecting the lumen of the capillary with the interstitial space around it. Fenestrated capillaries have intracellular fenestrations (openings) in the endothelial wall. The openings are about 0.1 μm in diameter, and may have thin membranes closing them. These types of vessels are found in the intestinal mucosa and the renal glomeruli. In discontinuous capillaries, the endothelial wall is interrupted at intervals by large gaps. These gaps are of such diameter that formed elements of the blood and fluid can freely pass. These capillaries are characteristic of bone marrow, the spleen and the hepatic sinusoids.

Discussion of absolute values for interstitial fluid colloid osmotic pressure is under considerable debate. Measurements using implantable devices such as perforated capsules that theoretically equilibrate with interstitial fluid, may be inaccurate because of the possibility of contamination by plasma, or that the fluid sampled may not necessarily contain all the osmotically active substances. The most common method for measuring interstitial oncotic pressure is lymph analysis. This method makes the assumption that lymph is a true reflection of the interstitial fluid contents. Critics of this view

argue tha

terminal

tein conc

(5) hav

size are

when inj

that the

capillar

that exc

trunks.

true re

vessel

both di

fluid

squeeze

that c

muscle

of the

During

about

ted be

sion

molecu

greate

argue that changes could occur in lymph composition as it flows from terminal lymphatics upward to larger vessels due to gradients of protein concentration within the interstitial spaces. Renkin and Garlick (45) have shown that dextran molecules of known molecular weight and size are in equal concentration between lymph and interstitial fluid when injected into a tissue. This observation allowed them to conclude that there is no significant protein concentration gradient beyond the capillaries. Garlick and Renkin (20) have performed studies showing that exchange occurs only at lymph nodes and not in the lymphatic trunks. If lymph is sampled before it reaches a node, it should be a true reflection of what is at the terminal lymphatic vessel.

Valves exist throughout all lymphatic channels. When a lymph vessel is compressed by pressure, lymph in the channel is squeezed in both directions. Since the valves are only unidirectional to flow, the fluid that will be transported from the terminal lymphatics is that squeezed in the central direction which flows past the valve. Factors that can compress the lymphatics and evoke the movements of lymph are: muscle contraction, arterial pulsations, passive movements of the parts of the body, and compression of the body tissues from the outside (22). During exercise, therefore, lymph flow can increase substantially to about 14 times normal.

The most important mechanism by which substances are transported between the plasma and interstitial fluid is by diffusion. Diffusion is a process which is dependent only on the thermal movement of molecules; that is, the greater the concentration difference, the greater the diffusion. This mode of exchange can be described by the

Fick Law of Diffusion, which states that the quantity of substance moved per unit time is equal to the free diffusion coefficient of the molecule, the concentration gradient, and the area of the capillary membrane. These factors are related in the following equation:

$$\frac{ds}{dt} = D \cdot A \cdot \frac{dc}{dt},$$

where

$\frac{ds}{dt}$ = amount of substance moved per unit time;

D = free diffusion coefficient for a molecule. (This value is inversely proportional to the square root of the molecular weight.);

A = area of capillary membrane;

$\frac{dc}{dt}$ = concentration gradient.

The site of diffusion of a molecule depends on whether the substance is water soluble or lipid soluble. Water soluble substances pass through pores in the endothelial cell. For small molecules such as water, ions, and urea, diffusion is free and rapid. However, for lipid-insoluble molecules of increasing size, diffusion becomes progressively more restricted, such that molecules above a molecular weight of 60,000 are almost completely impermeable. Lipid soluble molecules such as CO_2, O_2 and anesthetic gases pass freely through the intact cell. As a result, lipid-soluble molecules pass with great ease and rapidity between the capillary and interstitium. The ease with which a lipid soluble substance passes through the capillary endothelium is dependent on its oil to water partition coefficient.

Pinocytosis is a very slow active transport process, which is believed not to contribute much to total transcapillary exchange.

Electron microscopy has revealed that this mechanism involves vacuoles which traverse across the endothelial cell of the capillary wall. The vacuoles are formed by being pinched off the surface membrane of the cell. They are believed to contain macromolecules which cannot be readily exchanged by either filtration or diffusion. Presently, conclusive evidence is lacking to support the significance of pinocytosis in transcapillary protein movement.

Any physical stimulus or substance which can influence the contractile property of vascular smooth muscle or alter the microvascular permeability to plasma protein, can modify fluid movement in the capillary bed. Fluid movement is determined by the hydrostatic pressure difference between the blood and interstitial fluid. The contraction or relaxation of vascular smooth muscle will decrease or increase capillary blood volume respectively, thereby decreasing or increasing capillary hydrostatic pressure. Increasing the permeability of the microvascular membrane to protein will result in the escape of plasma protein from the vasculature to the interstitial fluid and raise the oncotic pressure of the interstitium. This augmentation of interstitial fluid oncotic pressure will enhance fluid movement out of the capillaries. In the body, vasoactive agents affect fluid movement by exerting a relaxing or contracting effect on the vascular smooth muscle and also by enhancing capillary permeability to protein. Two of the most important of these vasoactive agents are histamine and bradykinin.

Over the past five decades, vasoactive substances have been implicated in many pathological conditions, such as inflammation, shock and tissue injury. Of particular interest is the role of vasoactive

substances in inflammation. The inflammatory response is characterized by: vasodilation, increased vascular permeability, pain and migration of leukocytes (34).

Histamine is significantly involved in the mediation of the inflammatory reaction. It is found in the basophilic leukocytes of the blood and the mast cells of tissues. Histamine produces vasodilation, increased vascular permeability, migration of leukocytes and is generally conceded to be the principal mediator of the immediate inflammatory response to injury. Although histamine initiates inflammation, it is believed not to sustain the vascular changes because it has been isolated only from tissue exudates in the early stages of acute inflammation (46). However, Kahlson and Rosengren (30) have shown that histamine is also involved in the latter stages of inflammation. They observed that in rats and guinea pigs, the histamine formation capacity of the basophilic leukocytes increases with the onset of inflammation, and once the new histamine is produced, it can be released and continue mediating inflammation. Thus, the histamine present in the mast cells of inflammed tissues is utilized to initiate inflammation, and the histamine found in the leukocytes, due to an increase in the rate of synthesis, mediates the latter stages of inflammation. Therefore, between the initial and latter stages, no histamine is present, and the plasma kinins are speculated to mediate the inflammatory reaction during this period (60).

The observation that urine injected intravenously causes hypotension led to the discovery of the plasma kinins. In the late 1920's Frey and associates (58) characterized this hypotensive substance, and

also noted that it was found in a number of tissues. The material was first named kallikrein because it was found in abundance in the pancreas. Werle et al. (1937) (57) observed that kallikrein had an indirect effect by acting as an enzyme that cleaved off a pharmacologically active substance from a precursor present in the plasma. This substance was named kallidin. The discovery of bradykinin came in 1949, when Rocha e Silva (47) observed that trypsin or snake venoms released a peptide from a plasma substrate. The name bradykinin referred to the ability of the peptide to produce slow contraction of guinea pig ileum in vitro. Kallidin and bradykinin together with the plasma kinins are collectively referred to as the kinins.

Bradykinin has the following amino acid sequence:

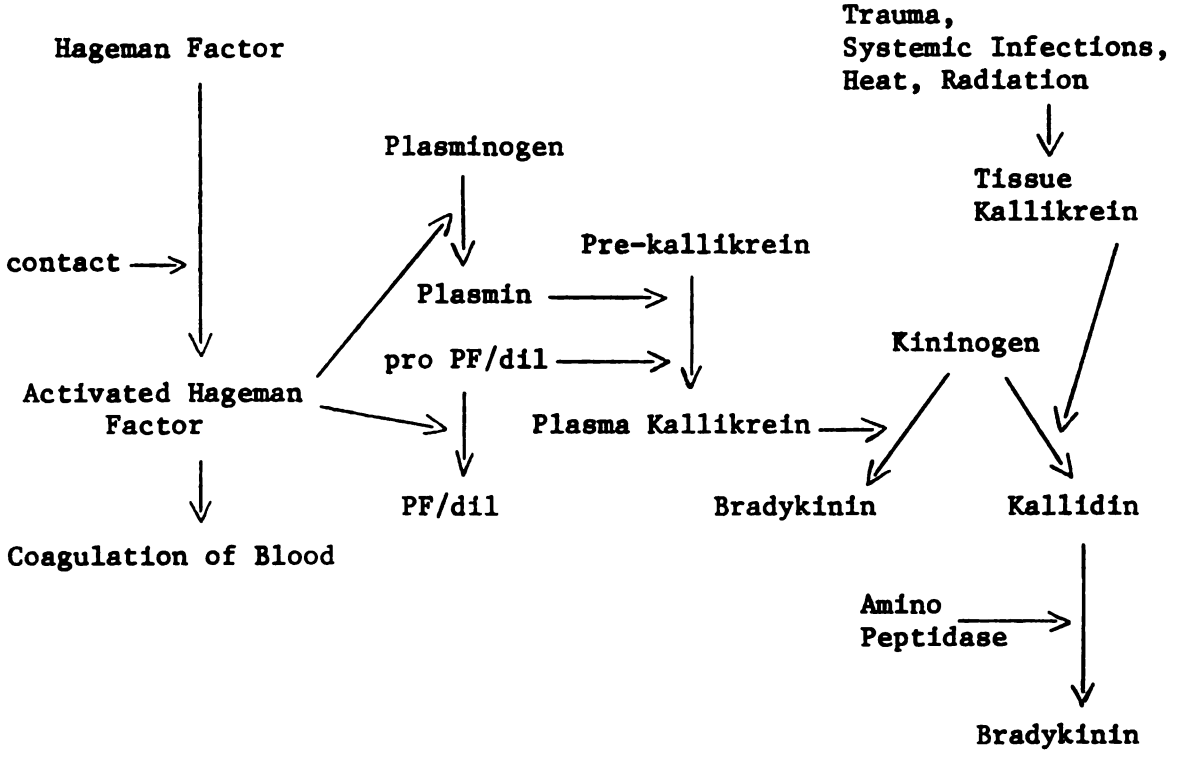
H_2N - Arg - Pro - Pro - Gly - Phe - Ser - Pro - Phe - Arg - OH.

The other kinins exhibit this nonapeptide sequence and differ only in having additional amino acid residues on the N- or C- terminal.

Clinical interest in bradykinin has been evoked because of its ability to mimic the main features of the inflammatory response and because it occurs in significant amounts in a wide range of diseases. Bradykinin is cleaved from a precursor termed kininogen, which is found in the plasma α_2- globulin fraction. The cleavage occurs by a group of enzymes collectively termed kininogenases and includes kallikreins, trypsin, pepsin as well as proteases in snake venoms and bacterial by-products. Of the kininogenases, the most important are the kallikreins, which are widely distributed and divided into tissue kallikreins and plasma kallikrein. Tissue kallikreins are often secreted in an active form from the salivary gland, pancreas, skin (sweat), small and large

bowl and the kidney, generally in response to systemic disorders (trauma, heat, infection). Compared with tissue kallikreins, plasma kallikrein differs physiochemically, in that it is formed from an inert precursor (prekallikrein). Activation of plasma kallikrein involves a series of enzymes, which are sequentially converted from pre-enzyme, with the latter successfully activating the next enzyme in the series. Activation is initiated by the Hageman factor (factor XII), which is also involved in blood clotting. The entire sequence of plasma kallikrein activation is presented in Figure I.

Figure I



Activated Hageman factor can transform prekallikrein to kallikrein either by activating plasmin or PF/dil. Plasmin is a proteolytic enzyme involved in the clot-lysing system; it dissolves fibrin and

subsequently destroys the clot. PF/dil is a plasma factor which can lead to cell lysis and the release of kininogenases.

Bradykinin inactivation is very rapid; the half-life in circulating blood is less than 15 seconds (11). The kininases are a group of enzymes which inactivate bradykinin. Plasma contains two kininases: carboxypeptidase N, which removes the C- terminal arginine group, and a dipeptide hydrolase that cleaves the proline-phenylalanine bond. However, inactivation of bradykinin occurs more effectively in the lungs than in the plasma. Friedli et al. (17) and Alabaster et al. (1) have reported up to 95% destruction of bradykinin following a single passage through the pulmonary circuit. Furthermore, the breakdown of bradykinin in the lung appears to be related to age. Friedli et al. (17) have shown that a high degree of inactivation occurs in mature ewes (93%), whereas newborn lambs show significantly less inactivation (68%). Fetal lambs at birth show 46% inactivation, and pre-term fetuses (110-128 days gestation) demonstrate no bradykinin inactivation in the pulmonary vascular bed.

Bradykinin is conceded to be a prominent mediator of inflammation, because of its ability to cause vasodilation, increased vascular permeability, pain and local accumulation of leukocytes, when injected intradermally (48). Furthermore, the probable activation of Hageman factor and other prekininogenases by contact with injured tissues, would explain the formation of kinins in inflammation. However, there is difficulty in demonstrating bradykinin in inflammatory exudates because of its rapid inactivation by kininases (34). Bradykinin has been claimed to also play a possible role in many other disease states.

In ca

conce

compa

blood

blood

holid

to de

blood

fact

uman

samp

from

minu

leve

by a

kini

toxi

(i.e

the

plas

mola

rela

sys

hyp

in

eff

In carcinoid tumors, which have metastasized to the liver, bradykinin concentrations range from 9-25 mcg/100 ml of hepatic venous blood, as compared to a normal range of 0.1-7.9 mcg/100 ml of hepatic venous blood (59). Bradykinin is believed to cause vasodilation and increased blood to the area of the tumor, supporting the tumor's enhanced metabolic requirements. In endotoxin induced shock, bradykinin is believed to decrease peripheral vascular resistance, which causes decreased blood pressure (59). Endotoxin is believed to activate the Hageman factor, which causes enhanced bradykinin levels. In studies using the unanesthetized Rhesus monkey, bradykinin levels in arterial blood samples increased during endotoxin shock (41). Bradykinin increased from a control value of 0.0 mcg/ml to 0.011 mcg/ml, after a 30 to 40 minute infusion of 10 mcg/kg Escherichia coli endotoxin. Bradykinin levels were shown to increase in rabbits subjected to endotoxin shock by also activating the Hageman factor (15). In these studies, assayed kininogen levels decreased 40% from control, after injection of endotoxin, due to the release of bradykinin. In traumatic shock states (i.e. traffic accidents) the kininogen level is lowered in man from the normal value of 15 to 20 mcg/ml plasma to 14.5 to 9.5 mcg/ml plasma, indicating the release of bradykinin (55). Therefore, on a molar basis, up to 32,500 mcg of bradykinin could theoretically be released in the average man (blood volume = 5,000 ml).

Bradykinin has two important effects on the cardiovascular system which have previously been eluded to. The first is a pronounced hypotension with systemic administration, and the second is an increase in the capillary membrane's permeability to protein, such that water efflux occurs, with local administration.

fall

Refer

4

4

4

1

1

Sir

fro

res

The intravenous (systemic) infusion of bradykinin results in a fall in systemic arterial pressure. See Figure II.

Figure II

Reference	Species	Dosage of Bradykinin	Blood Pressure	Mode of Infusion
40	Dog	2 mcg/kg	↓ of 41% from control	10 min infusion into femoral vein
40	Cat	10 mcg/kg	↓ of 55% from control	"
40	Chimp	1.5 mcg/kg	↓ of 28% from control	"
16	Dog	1.2 mcg/kg	control 120 mmHg inf. 55 mmHg	Bolus injection into jugular, femoral or brachial vein
16	Dog	0.5 mcg/kg	control 120 mmHg inf. 75 mmHg	"
8	Dog	2.4 mcg/min	control 113 mmHg inf. 79 mmHg	1.5 min infusion intravenously

In the experiments where bradykinin was infused over a 10 minute period, blood pressure slowly waned to control levels in 3 to 6 minutes. Where bradykinin was administered in bolus injections, blood pressure returned to normal almost immediately.

Since cardiac output is increased (12, 16, 42), the hypotension results from a decreased peripheral vascular resistance. Total peripheral resistance decreases only because of an increase in blood vessel

radiu

and v

creas

relax

have

fusio

vanis

This

enha

regu

pres

stro

incr

(26)

incr

forc

on s

dia

ind

dir

mec

hyp

cha

til

enh

radius, since hematocrit does not change or slightly increases (50), and vessel length is suspected not to change significantly. The decreased resistance occurs mainly at the arteriolar level, owing to the relaxation of vascular smooth muscle. Several investigators, however, have noted that the hypotension manifested during the intravenous infusion of bradykinin is not maintained (14, 42, 50). This is due to a waning of the effect of bradykinin on peripheral vascular resistance. This suggests bradykinin could evoke compensatory responses, such as enhanced sympathoadrenal activity, enhanced kininase activity, or autoregulation, which would antagonize the steady decline in arterial blood pressure (24).

The increase in cardiac output results from the elevation of stroke volume and perhaps stroke frequency (24). Stroke volume is increased mainly because of the decrease to ejection. Harrison et al. (26) have observed in the open-chest dog, that stroke volume may also increase by reason of small gains in left ventricular contractile force. Bradykinin in physiological concentrations has little effect on stroke frequency in the isolated heart (24). The increase in cardiac frequency seen with intravenous infusions results probably from indirect mechanisms, since no evidence exists showing that bradykinin directly enhances the frequency rate of the heart. One possible mechanism is activation of the baroreceptors as a result of the reduced hypotension. This activated mechanism will enhance sympathetic discharge (norepinephrine) and thus increase the heart rate and contractile force. Epinephrine secretion by the adrenal medulla is also enhanced through the baroreceptor mechanism and will cause an increase

in stroke frequency. Lewis (34) has shown that bradykinin acts directly on the adrenal glands to cause the release of catecholamines. While this phenomenon seems likely to increase cardiac frequency during systemic bradykinin infusion, the question as yet has not been critically examined.

Surprisingly, there are little data on the effects of systemically administered bradykinin on fluid flux and extravascular fluid volume. Most investigators simply assumed that local and systemically infused bradykinin would exert qualitatively the same effect on fluid filtration. Data in the literature suggest that this may not be so. Daugherty et al. (8) noted that intravenous infusions of bradykinin (20.6, 41.2 and 82.4 mcg/min) did not change weight and failed to increase small vein pressures in collateral-free, innervated canine forelimbs. The infusion period for each of the bradykinin concentrations employed averaged about 1.5 minutes. These studies indicate that intravenously administered bradykinin is unsuccessful in promoting edema formation in both skin and skeletal muscle, because of a failure to increase transmural capillary hydrostatic pressure and/or decrease transmural oncotic pressure. However, it is possible that results would be different if bradykinin were infused systemically over a longer period of time and at a higher dosage range.

Absolute plasma volume during the systemic infusion of bradykinin has not been measured. However, because of the similarity in the effects observed between systemic bradykinin and systemic histamine infusions (marked hypotension), it would not be surprising if bradykinin also failed to affect plasma volume. Deyrup (9) injected histamine

(3 t

Plas

effe

volu

was

capi

frou

flu

lar

inc

cen

32)

in

the

are

sug

The

cap

dir

ma

is

pr

a

(3 to 12 mcg/kg) subcutaneously into the thigh of the canine hindlimb. Plasma volume changes were assessed as an indication of histamine's effect on transcapillary fluid flux. The results show that plasma volume was unchanged or moderately increased, and in a few cases it was slightly reduced. She also observed no evidence for increased capillary permeability, since the escape of albumin-bound dye T-1824 from the vasculature did not increase.

Local infusions of bradykinin clearly cause rapid efflux of fluid from many systemic vascular beds. The increased net transvascular fluid efflux has been inferred from the development of edema, increases in organ weight, and increases in flow rate and protein concentration of lymph in forelimbs infused with bradykinin (2, 8, 10, 32). The increased net fluid filtration is attributable to both a rise in the transmural capillary hydrostatic pressure gradient and a fall in the transmural colloid osmotic pressure gradient.

Small vein pressures and blood flow in skin and skeletal muscle are markedly increased by bradykinin in the canine forelimb (8, 32), suggesting that capillary hydrostatic pressure is greatly increased. The increased microvascular pressure is attributable to an augmented capillary inflow subsequent to arteriolar vasodilation. However, direct measurements of capillary hydrostatic pressure have never been made.

The fall in the transmural colloid osmotic pressure gradient is attributable to an increased microvascular permeability to plasma protein. Flow rate and total protein concentration of lymph draining a vascular bed increases markedly from control during the infusion of

bradyk

kinin,

The in

size l

microv

tence

due t

cellu

recen

These

route

evid

poss

micr

effl

have

cula

pres

the

sen

sho

bra

que

tha

bradykinin (0.8 or 10 mcg/min) (32). With the higher dosage of bradykinin, the lymph protein concentration approaches plasma protein values. The increased protein efflux is usually attributed to an increased pore size by a direct action of bradykinin on the immediate post-capillary microvascular membrane (19). Microscopic studies demonstrate the existence of gaps appearing between adjacent endothelial cells, perhaps due to "rounding up" of the cells, thereby creating an increased intracellular cleft (35).

The theory that bradykinin causes an increased pore size has recently been challenged by Renkin and his co-workers (6, 29, 44). These investigators believe that augmented pinocytosis is the major route of protein efflux from the microvasculature. However, definitive evidence for this hypothesis is still lacking.

There is some controversy in the literature surrounding the possibility that increased capillary hydrostatic pressure increases microvascular permeability and therefore that the increased protein efflux is due to an indirect action of bradykinin. Various studies have presented evidence consistent with the concept that the microvascular surfaces become more permeable to macromolecules as microvascular pressure is increased. The increase in permeability occurs mainly at the level of the venous capillary and venule. Rowley (51) has presented findings consistent with the "stretched pore phenomenon" (52), showing that the increased capillary hydrostatic pressure observed with bradykinin, forces the opening of microvascular pores and the subsequent loss of plasma protein. He believes that this is the major way that bradykinin acts on the microvascular membrane to decrease the

transmural oncotic pressure gradient, since he observed no increase in macromolecular efflux when capillary hydrostatic pressure remained constant.

Recent studies by Kline et al. (31) have presented data suggesting that increased microvascular pressure is not associated with increases in microvascular permeability to plasma proteins as is seen with bradykinin. In fact, bradykinin increased protein efflux greatly in forelimbs perfused at constant flow. Under this condition, microvascular pressure either failed to increase relative to control or decreased slightly; yet marked protein efflux occurred (32). Furthermore, there was no evidence of venous constriction in any of the experiments reported by these investigators (32). Thus, local infusions of bradykinin cause edema by two contributing mechanisms. From experiments comparing weight gain, lymph flow and protein concentration in forelimbs infused with bradykinin (0.8 or 10 mcg/min, I.A.) at natural or constant inflow, it was estimated that a larger proportion of the edema was due to a decreased transmural oncotic pressure gradient or a direct action of bradykinin on the microvascular membrane promoting protein efflux (32). The increased capillary hydrostatic pressure, forcing fluid (water) out of the microvasculature and into the interstitium, exerts the lesser effect in edema formation.

It is also thought that capillary surface area increases with locally infused bradykinin. The increased surface area would enhance the volume of fluid filtered per unit time, thereby adding to the edema (24).

Daugherty et al. (8) noted that in comparing intra-arterial and intravenous infusions of bradykinin, that large increases in hindlimb

weight occurred during intra-arterial administration, whereas during intravenous infusion, weight did not change. Since locally administered bradykinin increases microvascular permeability and subsequently causes net fluid filtration even when capillary hydrostatic pressure remains constant at a normal level, it is bewildering why net fluid filtration does not occur during systemic administration of bradykinin. There are several possible explanations for this route-dependent differential action. First of all, by infusing bradykinin intravenously or downstream to the lung, it must pass through the pulmonary circuit where up to 95% destruction can occur, thereby allowing very little or no drug to enter the capillary beds. Secondly, if bradykinin was given intravenously in large enough quantities so that a significant amount escaped pulmonary inactivation and reached the arterial vasculature causing acute hypotension, catecholamines would be released. The mechanism for this release would be by increased sympathoadrenal discharge resulting from the activation of the baroreceptor phenomenon and by a direct action of bradykinin on the adrenals to cause epinephrine secretion. The release of the catecholamines could effectively antagonize the microvascular effects of bradykinin. Finally, there are many other substances released in response to hypotension such as renin, angiotensin II, vasopressin (ADH), aldosterone, etc., which may also antagonize the effects of bradykinin on the microvascular membrane.

The catecholamines released during hypotension (49) are also secreted in situations of hypoxia, asphyxia and emotional stress. They are vasoconstrictors, and there is considerable disagreement in the literature as to their effect on transvascular fluid flux. In general,

the c

resis

phrir

skele

perfu

obse

orga

vein

weig

phri

more

woul

trat

cles

and

reab

that

meab

how

leg

inc

and

per

of

the catecholamines constrict the capacitance vessels (veins) and the resistance vessels (arteries and arterioles). One exception is epinephrine, which in low concentrations dilates precapillary vessels in skeletal muscle (27). When norepinephrine is infused into naturally perfused forelimbs and ileum segments, small vein pressures have been observed to increase, decrease or remain unchanged. The weight of the organ (forelimb or ileum) altered directly with the change in small vein pressure. This association between small vein pressure and organ weight can be explained in terms of the varied effects of the norepinephrine on pre and post-capillary resistances. If the venules constrict more than the arterioles, capillary outflow would be impeded. This would increase capillary hydrostatic pressure and cause increased filtration of fluid and thus an increase in organ weight. If the arterioles constrict more than the venules, capillary inflow would be impeded and hydrostatic pressure would decrease, thereby favoring net fluid reabsorption.

Studies performed by Mellander and Nordenfelt (37) have shown that capillary surface area available for diffusion and capillary permeability to proteins were unaffected by norepinephrine. Järhult (28), however, has observed that in denervated skeletal muscle of the lower leg of the cat hindlimb perfused at constant inflow, norepinephrine increases capillary surface area for diffusion. In contrast, Appelgren and Lewis (3) have reported a decrease in capillary surface area and permeability in naturally perfused human skeletal muscle when solutions of 0.4 mcg/ml of norepinephrine were infused locally.

intr

as e

osmo

pres

ical

move

rout

brad

pulm

in p

intr

was

reac

isto

acti

kin

the

eit

act

STATEMENT OF THE PROBLEM

It is well established that bradykinin administered locally, intra-arterially, increases the rate of transcapillary fluid movement, as evidenced by edema formation, by decreasing the transmural colloid osmotic pressure gradient and increasing the transmural hydrostatic pressure gradient. However, when bradykinin is administered systemically (iv), it fails to increase the rate of transcapillary fluid movement. This suggests that the edemogenic action of bradykinin is route dependent.

Studies by Friedli (17) and Alabaster (1) have shown that bradykinin is inactivated up to 95% through the passage of a single pulmonary circuit. This is due to the high concentration of kininases in pulmonary tissue. These investigators also suggest this may be why intravenous bradykinin fails to promote edema. However, if bradykinin was administered in large amount, so that a significant quantity would reach the arterial side of the vasculature, or if bradykinin was administered upstream to the lungs (left ventricle) to bypass pulmonary inactivation, transcapillary fluid movement may be modified. If bradykinin fails to promote edema via this mode of systemic administration, then it is quite possible that an autoregulatory mechanism is activated either indirectly, owing to the hypotension and/or directly, due to the action of bradykinin on a tissue (adrenals), antagonizing its effect.

Marciniak et al. (36) have shown that histamine fails to promote edema in the dog forelimb when administered systemically into the left ventricle in concentrations which would exceed the brachial artery blood concentrations achieved by local infusion. These studies indicate that histamine not only fails to promote edema, but rather causes net extravascular fluid reabsorption. This is evidenced by a decrease in forelimb weight, which is only partially attributable to a reduction in intravascular blood volume. Marciniak et al. (35) have also shown that when histamine and norepinephrine are infused simultaneously by local infusion, forelimb weight, lymph flow and lymph total protein fails to increase.

This antagonism could be due to a shunting of blood from nutritional to non-nutritional channels, a direct blockade of histamine on the microvascular membrane by norepinephrine, or a combination of both. The antagonistic effect of norepinephrine could also explain the differential effects of locally and systemically administered histamine on fluid flux. Locally administered histamine (4 or 64 mcg/min, I.A.) fails to alter systemic arterial pressure or will minimally decrease it after edema develops, whereas systemically administered histamine (400 to 800 mcg/min) causes a fall in blood pressure. The hypotension would act as a stimulus for sympathoadrenal discharge with the resulting catecholamine release.

Since bradykinin is similar to histamine by causing marked hypotension upon systemic administration and promotes edema formation upon local administration, it is possible that the catecholamines may also antagonize the edemogenic effects of bradykinin. This study

attempt to determine the mechanism of the route dependent differential action of bradykinin on fluid filtration. The possible role of destruction of bradykinin in the blood and antagonism of the microvascular actions of bradykinin by catecholamines will be investigated. This will be accomplished by infusing bradykinin upstream and downstream to the pulmonary circulation, by infusing bradykinin locally during systemic hypotension, and by simultaneously infusing bradykinin and catecholamines locally into canine forelimbs while monitoring lymph flow, lymph protein concentration and/or forelimb weight. Since very little data is found in the literature, relevant to the effect of systemically administered bradykinin on fluid and protein efflux and edema formation, the effects of systemically administered bradykinin on these parameters will also be thoroughly investigated.

METHODS

Mongrel dogs of either sex, weighing approximately 20 kilograms were anesthetized with sodium pentobarbitol (30 mgm/kg) and respirated by positive pressure ventilation (Harvard Respiration Pump, Harvard Apparatus Co., Inc., Millis, Maryland). After surgery, ten thousand U.S.P. units of sodium heparin were administered intravenously to prevent blood coagulation.

The collateral-free, innervated forelimb, perfused at natural or at constant inflow, was used as the test organ for studying the effects of bradykinin and norepinephrine on extravascular fluid volume and hemodynamic parameters (21). Bradykinin was infused alone (0.8 mcg/min) or infused at this dose simultaneously with norepinephrine (4 mcg/min) into the brachial artery.

The surgical procedure consisted of sectioning the skin circumferentially about 5 cm above the elbow of the right forelimb with electrocautery. The brachial artery, the brachial and cephalic veins, and the forelimb nerves (median, musculocutaneous, radial and ulnar) were isolated and coated with an inert silicone spray to prevent drying. The muscles and remaining connective tissue were then sectioned with electrocautery. The humerus was cut, and the ends of the marrow cavity were packed with bone wax. Therefore, blood entered the

limb

chia

cann

lene

a 90

tubi

same

voir

ble

ties

anim

medi

al a

prec

dom

isol

fic

bed

the

All

sue

tin

to

by

limb only through the brachial artery and exited only through the brachial and cephalic veins.

The brachial and cephalic veins were partially transected and cannulated at the level of the elbow with short sections of polyethylene tubing (PE-320). The sections of tubing were 20 cm in length with a 90^{o} angle about 3 cm from the end. The terminal 3 cm angle of the tubing was inserted into the veins. The cannulas were secured at the same level as the veins, and the outflows were directed into a reservoir. The reservoir was maintained at a constant volume, via a variable speed Holter pump (Model RE-161, Extracorporeal Medical Specialties, King of Prussia, Penn.), which continually returned blood to the animal, via a cannulated jugular vein. In these experiments, the median cubital vein, which is the major anastomosis between the brachial and cephalic veins, was ligated. Thus, brachial venous outflow was predominantly from muscle, whereas cephalic venous outflow was predominantly from skin. Although this procedure does not completely isolate the skin and skeletal muscle, the amount of separation is sufficient to permit comparison of resistance changes in the two parallel beds (39). Blood flow (ml/min) was measured by timed collections from the brachial and cephalic venous outflows into graduated cylinders. All blood flows were converted from ml/min to ml/min/100 grams of tissue, based on the total weight of the forelimb after the experiment.

Brachial and cephalic vein pressures were monitored by inserting PE-60 polyethylene tubing into side branching vessels, located 3 to 5 cm distal to the elbow. Systemic arterial pressure was measured by inserting PE-240 polyethylene tubing into the common carotid artery.

This
sort
stre
with
the
ever

(Mod
to a
Palo

smal
was
loca
more
the
an
of

brac
the
art
brac
dle
pre
bra

This cannula was inserted in an upstream direction into the arch of the aorta. Pressure in a skin small vein was measured by cannulating upstream one of the small surface veins on the dorsal side of the paw with PE-60 tubing. The femoral vein was cannulated in each animal for the administration of heparin, sodium pentobarbitol and saline, whenever they were required.

All pressures were monitored with Statham pressure transducers (Model P23Gb, Statham Instruments, Inc., Oknard, California), connected to a recording Sanborn oscillograph (7700 series, Hewlett-Packard Co., Palo Alto, California).

In the experiments utilizing naturally perfused forelimbs, a small side branch of the brachial artery above the level of the elbow was isolated and cannulated upstream with PE-50 polyethylene tubing for local (intra-arterial) infusions of bradykinin. When bradykinin and norepinephrine were infused simultaneously, two small side branches of the brachial artery were cannulated. These cannulas were inserted in an upstream direction, so that the tip was located at the bifurcation of the side branch off the brachial artery.

In experiments using forelimbs perfused at constant flow, the brachial artery was isolated, tied off and transected about 5 cm above the elbow. Blood was obtained from a cannula inserted into the femoral artery and pumped at a constant controlled flow into the transected brachial artery. A Sigmamotor pump (Model T68H, Sigmamotor Inc., Middleport, New York) was used to keep the inflow constant. Perfusion pressure was measured by a cannula inserted into a side branch of the brachial artery distal to the site of inflow and was set during the

control period, 5 to 10 mmHg below aortic pressure. Local (intra-arterial) administration of drugs was by direct infusion into the pump circuit behind the Sigmamotor pump.

When surgery was completed, the forelimb was suspended on a wiremesh platform attached to a strain guage balance. The output from the balance was amplified and recorded on the Sanborn oscillograph, which thus monitored changes in forelimb weight throughout the experiment. The system was calibrated by adding known weights to the platform. The addition of a 2 gram weight caused a pen deflection of 10 to 20 mm on the chart paper. Vascular resistances were calculated as follows:

total skin resistance = Pa - Plsv/Fs/100 gms of forelimb,

total muscle resistance = Pa - Plmv/Fm/100 gms of forelimb,

skin large vein resistance = Pssv - Plsv/Fs/100 gms of forelimb,

where Pa = systemic arterial pressure, Plsv = cephalic vein pressure, Fs/100 gms = cephalic flow per 100 grams of forelimb, Plmv = brachial vein pressure, Fm/100 gms = brachial flow per 100 grams of forelimb, and Pssv = skin small vein pressure.

In the lymph studies, intact canine forelimbs perfused either naturally or at constant flow were used to collect lymph and measure lymph protein concentration. In the right forelimb, small incisions using electrocautery were made superficial to the brachial artery, the cephalic vein (above the elbow) and the second superficial dorsal metacarpal vein. A small incision was also made over the femoral triangle. A lymph vessel in the area of the cephalic vein was isolated and cannulated with PE-10 polyethylene tubing about 10 cm in length and

beveled at the cannulating end. All other lymph vessels in this area, which drain primarily the forelimb skin and paw, were tied off (40). Lymph was collected at 10 minute intervals in miniature 0.3 ml graduated cylinders, constructed from plastic pipettes. Lymph total protein concentration was measured by the spectrophotometric method of Waddell (56) on a Beckman DB Spectrophotometer (Model 24, Beckman Instruments, Inc., Fullerton, California). Local infusion of the drugs was accomplished by the same routes of administration used in the previous studies. Bradykinin (0.8 mcg/min or 10 mcg/min) was infused alone or infused (0.8 mcg/min) simultaneously with norepinephrine (4 mcg/min) intra-arterially.

The drugs used in these experiments were bradykinin (Shwartz/Mann, Division of Becton, Dickinson and Company) and levarterenol bitartrate (norepinephrine; Winthrop Laboratories, Special Chemical Dept.) in solutions of isotonic saline. They were administered intra-arterially or intravenously at a volume delivery rate of 0.2 ml/min with a Harvard Apparatus infusion/withdrawal pump.

In two experimental series, bradykinin (0.8 mcg/min or 10 mcg/min, I.A.) was infused locally for 60 minutes into forelimbs perfused at constant inflow, following hemorrhagic induced hypotension. Hemorrhagic hypotension was produced by removing the necessary amount of blood (via a PE-360 polyethylene catheter inserted into the femoral artery), to lower and maintain blood pressure at approximately 45 mmHg for 60 minutes. Lymph flow was also monitored in these experiments.

In one series of experiments, systemic bradykinin administration (140 mcg/min for 30 minutes, followed by 280 mcg/min for 30

minutes) was accomplished by infusing intravenously via a catheter inserted into the femoral vein up to the inferior vena cava.

In another experimental series, PE-240 polyethylene tubing was inserted down the right common carotid artery into the left ventricle of the heart. Initially, the catheter was connected to a pressure transducer and successful placement was confirmed by a typical left ventricular pressure tracing. Bradykinin (140 mcg/min for 30 minutes, followed by 280 mcg/min for 30 minutes) was then administered by infusion into this catheter.

Arterial blood samples (5 ml) were withdrawn from the cannula monitoring systemic arterial blood pressure. Samples were taken during a control period, followed by collections at 30 minute intervals throughout the experiment. Total plasma protein concentrations in grams per cent and hematocrits were determined from these samples.

All data were statistically analyzed by Analysis of Variance (Randomized Complete Block Design), and the means were compared to control by the Least Significant Difference Test (53).

RESULTS

Table 1

In naturally perfused forelimbs, intravenously infused bradykinin (140 to 280 mcg/min) produced a minimal increase in both lymph flow rate and lymph total protein concentration. Plasma protein concentration was not changed, while the hematocrits were increased significantly. Skin small vein pressure did not change, and systemic arterial pressure decreased moderately, although transiently returning near control levels by the end of the infusion period.

Table 2

In forelimbs perfused at constant inflow, systemic infusions of bradykinin into the left ventricle resulted in moderate reductions in perfusion pressure and systemic arterial pressure. Lymph flow rate and lymph total protein concentration were moderately increased. Plasma protein concentration was unchanged, and the hematocrit ratios were elevated. Skin small vein pressure minimally decreased.

Table 3

Hypotension induced by hemorrhage markedly decreased systemic aortic pressures. Hemorrhagic hypotension produced no effect upon

skin small vein pressure, lymph flow rate, lymph total protein concentration and hematocrit ratio. Perfusion pressure was markedly elevated, while plasma protein concentration decreased minimally. The local infusion of bradykinin (0.8 or 10 mcg/min, I.A.) initiated at minute 60 failed to produce any significant alterations of skin small vein pressure. Aortic pressure increased minimally, while perfusion pressure decreased substantially, relative to minute 60. Lymph flow rate slightly increased; however, the increase was not significant until the last 20 minutes of the infusion of the higher dose of bradykinin (10 mcg/min). Lymph total protein concentration was elevated minimally with the higher dosage of bradykinin and did not change with the lower dosage. Hematocrit was essentially not affected further by the local infusion of bradykinin.

Table 4

Table 4 shows the effects of bradykinin infused alone (0.8 mcg/min, I.A.) and in combination with norepinephrine (4 mcg base/min, I.A.) in naturally perfused forelimbs on weight, vascular pressures, resistances and blood flows. Bradykinin infused into the brachial artery slightly decreased systemic aortic blood pressure only during the latter 20 minutes of the infusion period, whereas blood pressure slightly increased with the concurrent infusion of bradykinin and norepinephrine. Forelimb weight moderately increased with bradykinin, yet decreased and slowly waned to control with the concurrent infusion. Skin small vein, cephalic vein and brachial vein pressures and the cephalic and brachial venous outflows increased during the first 5 to 10 minutes of

the bradykinin infusion period, and then slowly declined back to the control levels throughout the remainder of the experiment. With the simultaneous infusion, the vein pressures increased moderately and were maintained during the infusion period except for the cephalic vein pressure which did not change; both outflow rates decreased. Total skin and muscle resistances decreased within the first 5 minutes of the bradykinin infusion and then slowly attenuated back to control. No change was observed in skin large vein resistance with bradykinin infused alone. All resistances were markedly increased with the concurrent infusion of bradykinin and norepinephrine.

Table 5

Table 5 shows the effects of bradykinin infused alone (0.8 mcg/min, I.A.) and in combination with norepinephrine (4 mcg base/min, I.A.) in forelimbs perfused at constant inflow on forelimb weight, vascular pressures, resistances and blood flows. Systemic aortic pressure failed to change while perfusion pressure markedly decreased and then slowly increased to a level above control with the infusion of bradykinin into the brachial artery. The simultaneous infusion of bradykinin and norepinephrine increased systemic aortic pressure and markedly increased perfusion pressure. Forelimb weight increased with both the single and concurrent infusions; however, the concurrent infusion produced a considerably greater augmentation of this weight. Skin small vein, cephalic vein and brachial vein pressures together with the cephalic and venous outflows did not change with bradykinin infused alone. The vein pressures markedly increased with the concurrent

infusion, while the cephalic venous outflow decreased and the brachial venous outflow increased. Total skin and muscle resistances decreased within the first 5 minutes and then slowly waned back to control levels with bradykinin infused alone; no change was observed in skin large vein resistance. With the simultaneous infusion, total skin and skin large vein resistances increased, whereas total muscle resistance did not change.

Table 6

In naturally perfused forelimbs, local infusion of bradykinin (0.8 mcg/min, I.A.) produced no effect upon aortic pressure. When infused in combination with norepinephrine (4 mcg base/min, I.A.), aortic pressure was moderately increased. Skin small vein pressures were markedly increased with both infusions, although the augmentation was stronger with the concurrent infusion. Bradykinin alone caused marked increases in lymph flow and lymph protein concentration, but with the simultaneous infusion of norepinephrine, no change was observed in either of these parameters. During the simultaneous infusion of bradykinin and norepinephrine, a moderate increase in arterial hematocrit was observed and no change occurred in plasma protein concentration.

In forelimbs perfused at constant inflow, bradykinin (0.8 mcg/min, I.A.) did not change systemic aortic pressure or skin small vein pressure. Perfusion pressure decreased significantly over the first 20 minutes of the infusion but then increased, reaching levels significantly above control, during the latter 10 minutes of infusion. Bradykinin infused simultaneously with norepinephrine (4 mcg base/min,

I.A.) produced marked increases in all vascular pressures. Bradykinin infused alone increased lymph flow rate and lymph total protein concentration; however, only a very slight increase in lymph flow and no change in lymph total protein concentration was observed during the combination infusion. Plasma protein concentration did not change with either mode of infusion, while arterial hematocrits increased slightly only in the combined infusion of the drugs.

Table 1.--Effects of bradykinin infused intravenously (vena cava) into naturally perfused forelimbs for 60 minutes on lymph flow, protein concentration of lymph and plasma, vascular pressures and hematocrit (n=6).

	Control		Infusion Period					
			B140			B280		
Time (minutes)	-10	0	10	20	30	40	50	60
Systemic Arterial Blood Pressure (mm Hg)	112	112	98*	101*	102*	101*	102*	104†
Skin Small Vein Pressure (mm Hg)	11	11	11	11	10	11	11	11
Lymph Flow Rate (ml/10 min)	.01	.01	.01	.01	.02	.03†	.04*	.05*
Lymph Total Protein (grams %)	2.5	2.5	2.6	2.8†	3.0*	3.1*	3.4*	3.3*
Plasma Protein (grams %)		5.5			5.2			5.5
Hematocrit		39			43*			44*

* = $p \leq 0.01$ relative to zero time.

† = $p \leq 0.05$ relative to zero time.

Table 2.--Effects of bradykinin infused into the left ventricle of the heart at constant inflow for 60 minutes on lymph flow, protein concentration of lymph and plasma, vascular pressures and hematocrit (n=6).

	Control		Infusion Period					
			B140			B280		
Time (minutes)	-10	0	10	20	30	40	50	60
Systemic Arterial Blood Pressure (mm Hg)	123	123	80*	81*	82*	78*	78*	79*
Perfusion Pressure (mm Hg)	116	116	90*	95*	99*	99*	107	109
Skin Small Vein Pressure (mm Hg)	13	13	12	11*	10*	10*	10*	11*
Lymph Flow Rate (ml/10 min)	.02	.02	.04	.06	.09†	.09†	.11*	.12*
Lymph Total Protein (grams %)	1.8	1.8	1.9	2.0	2.4*	2.8*	2.7*	3.0*
Plasma Protein (grams %)		4.5			4.6			4.5
Hematocrit		39			44*			46*

* = $p \leq 0.01$ relative to zero time.

† = $p \leq 0.05$ relative to zero time.

Table 3.--Effects of locally infused bradykinin (0.8 or 10 mcg/min, I.A.) for 60 minutes following 60 minutes of hypotension produced by hemorrhage to lower and maintain aortic pressure near 45 mm Hg. Constant inflow.

Time (minutes)		Control		Hemorrhage						Bradykinin Infusion					
		-10	0	10	20	30	40	50	60	70	80	90	100	110	120
Systemic Arterial Blood Pressure (mm Hg)	B0.8	119	120	49*	43*	47*	50*	47*	55*	59*	66*	68*	68*	67*	65*
	B10	117	118	45*	46*	47*	46*	46*	58*	74*Ω	68*	68*	69*	66*	65*
Perfusion Pressure (mm Hg)	B0.8	110	111	165*	154*	166*	176*	183*	182*	125ω	130ω	126ω	133ω	137ω†	143ω*
	B10	108	109	149*	164*	161*	162*	182*	188*	102ω	117ω	122ω	126ω	136ω†	138ω†
Skin Small Vein Pressure (mm Hg)	B0.8	12	11	8	9	9	10	10	12	11	13	12	15	15	19ω†
	B10	12	12	10	10	9	9	9	9	10	11	11	16	20Ω	23ω†
Lymph Flow Rate (ml/10 min)	B0.8	.02	.02	.02	.02	.03	.03	.02	.03	.03	.04	.06	.06	.07†	.07†
	B10	.01	.01	.01	.01	.02	.01	.02	.02	.02	.04	.05	.08*ω	.08*ω	.12*ω
Lymph Total Protein (grams %0	B0.8	1.8	2.0	2.1	2.2	2.1	2.2	2.3	2.3	2.1	2.1	2.1	2.0	1.9	2.1
	B10	2.3	2.2	2.4	2.5	2.5	2.6	2.4	2.4	2.3	2.4	2.5	2.7	2.8†	3.3*ω
Plasma Protein (grams %)	B0.8		4.3			4.0			3.9			3.7†			3.6†
	B10		5.2			4.8†			4.3*			4.6*			4.4*
Hematocrit	B0.8		38			42			48*			45†			45†
	B10		40			40			43			45*			45*

* = $p \leq 0.01$ relative to zero time.

† = $p \leq 0.05$ relative to zero time.

ω = $p \leq 0.01$ relative to 60 minutes.

Ω = $p \leq 0.05$ relative to 60 minutes.

Table 4.--Effects of bradykinin (0.8 mcg/min, I.A.) and bradykinin and norepinephrine (4 mcg/min, I.A.) infused locally into naturally perfused forelimbs on weight, blood flows, vascular resistances and vascular pressures (n=6).

Time (minutes)		Control		Infusion Period						
		-5	0	2	5	10	15	30	45	60
Change in Weight (grams)	B0.8	0	0	8*	10*	11*	12*	14*	17*	19*
	B0.8 + N4	0	0	-10*	-8*	-6*	-5†	-2†	1†	1†
Systemic Arterial Blood Pressure (mm Hg)	B0.8	138	138	137	138	139	138	133	132†	132†
	B0.8 + N4	105	105	111	112†	111	116*	116*	109	113*
Skin Small Vein Pressure (mm Hg)	B0.8	12	12	25*	21*	18†	16	13	13	13
	B0.8 + N4	10	10	31*	33*	32*	33*	29*	29*	25*
Cephalic Vein Pressure (mm Hg)	B0.8	5	5	7†	7†	5	5	5	4	5
	B0.8 + N4	4	5	5	6	6	6	5	4	4
Brachial Vein Pressure (mm Hg)	B0.8	9	8	14*	12*	11†	10	10	10	10
	B0.8 + N4	5	5	15†	17*	15†	14†	15†	17*	18*
Cephalic Venous Outflow (ml/min/100 grams)	B0.8	12	12	23*	21*	17*	14	14	12	12
	B0.8 + N4	10	10	3*	2*	2*	2*	1*	1*	2*
Brachial Venous Outflow (ml/min/100 grams)	B0.8	7	7	15*	12*	10	7	6	5	6
	B0.8 + N4	7	7	3*	3*	4†	3*	2*	1*	1*
Total Skin Resistance ($mm\ Hg \times min \times ml^{-1} \times 100\ g^{-1}$)	B0.8	12	11	7†	7†	9	12	12	12	13
	B0.8 + N4	11	11	248†	358*	310†	401*	307†	326*	277†

Table 4.--Continued.

Time (minutes)		Control		Infusion Period						
		-5	0	2	5	10	15	30	45	60
Total Muscle Resistance ($mm\ Hg_1 \times min \times ml^{-1} \times 100\ g^{-1}$)	BO.8	19	20	10*	12*	17	22	22	24	21
	BO.8 + N4	21	21	39	76	112*	111*	105†	103†	117*
Large Skin Vein Resistance ($mm\ Hg_1 \times min \times ml^{-1} \times 100\ g^{-1}$)	BO.8	1	1	1	1	2	1	1	1	1
	BO.8 + N4	1	1	42	100†	102†	101†	63	93†	74

* = $p \leq 0.01$ relative to zero time.

† = $p \leq 0.05$ relative to zero time.

Table 5.--Effects of bradykinin (0.8 mcg/min, I.A.) and bradykinin and norepinephrine (4 mcg/min, I.A.) infused locally into constantly perfused forelimbs on weight, blood flows, vascular resistances and vascular pressures (n=6).

Time (minutes)		Control		Infusion Period						
		-5	0	2	5	10	15	30	45	60
Change in Weight (grams)	B0.8	0	0	3	3	4	5	8†	11*	14*
	B0.8 + N4	0	0	-3	3	10	15	25†	41*	60*
Systemic Arterial Blood Pressure (mm Hg)	B0.8	123	125	125	123	122	122	121	121	121
	B0.8 + N4	117	117	144*	158*	156*	144*	146*	138*	135*
Perfusion Pressure (mm Hg)	B0.8	118	121	56*	79*	96†	103	122	138	153*
	B0.8 + N4	110	111	187*	210*	211*	217*	222*	233*	242*
Skin Small Vein Pressure (mm Hg)	B0.8	14	14	13	13	14	14	15	15	14
	B0.8 + N4	12	12	39*	44*	40*	39*	35*	35*	37*
Cephalic Vein Pressure (mm Hg)	B0.8	5	4	4	4	4	4	4	4	4
	B0.8 + N4	6	7	16*	18*	18*	17*	15*	15*	16*
Brachial Vein Pressure (mm Hg)	B0.8	6	6	7	7	7	7	8	8	8
	B0.8 + N4	8	8	27*	30*	29*	26*	25*	26*	29*
Cephalic Venous Outflow (ml/min/100 grams)	B0.8	14	15	14	14	14	14	14	15	15
	B0.8 + N4	10	11	5*	6*	7*	6*	6*	6*	6*
Brachial Venous Outflow (ml/min/100 grams)	B0.8	12	12	13	13	13	13	13	13	12
	B0.8 + N4	9	8	14*	14*	13*	12*	12*	13*	13*

Table 5.--Continued.

Time (minutes)		Control		Infusion Period						
		-5	0	2	5	10	15	30	45	60
Total Skin Resistance ($mm\ Hg_l \times min \times ml^{-1} \times 100\ g^{-1}$)	B0.8	10	10	4*	5*	7†	8	9	10	11
	B0.8 + N4	12	12	37*	35*	31*	32*	35*	38*	42*
Total Muscle Resistance ($mm\ Hg_l \times min \times ml^{-1} \times 100\ g^{-1}$)	B0.8	14	14	5*	8*	9*	10†	10†	12	14
	B0.8 + N4	15	15	13	15	17	19	19	19	19
Skin Large Vein Resistance ($mm\ Hg_l \times min \times ml^{-1} \times 100\ g^{-1}$)	B0.8	1	1	1	1	1	1	1	1	1
	B0.8 + N4	1	1	6*	5*	4*	4*	4*	4*	4*

* = $p \leq 0.01$ relative to zero time. † = $p \leq 0.05$ relative to zero time.

Table 6.--Effects of bradykinin alone or infused concurrently with norepinephrine base intra-arterially into the forelimb for 60 minutes on lymph flow, protein concentration of lymph and plasma, vascular pressures and hematocrit (n=6).

Time (minutes)			Control		Infusion Period					
			-10	0	10	20	30	40	50	60
Systemic Arterial Blood Pressure (mm Hg)	NF	B0.8	104	105	105	106	107	107	104	101
		B0.8 + N4	107	108	138*	138*	138*	137*	136*	135*
	CF	B0.8	120	122	123	124	125	125	127†	125
		B0.8 + N4	113	113	136*	130*	126*	124*	125*	123*
Perfusion Pressure (mm Hg)	CF	B0.8	115	117	88*	98*	107	113	124	128†
		B0.8 + N4	109	108	157*	163*	170*	173*	176*	178*
Skin Small Vein Pressure (mm Hg)	NF	B0.8	10	10	16*	15*	14*	13*	13*	13*
		B0.8 + N4	9	9	25*	26*	27*	26*	23*	25*
	CF	B0.8	11	11	10	10	9	10	10	9
		B0.8 + N4	12	11	19*	18*	19*	19*	16†	16†
Lymph Flow Rate (ml/10 min)	NF	B0.8	.02	.02	.12	.42*	.46*	.45*	.47*	.44*
		B0.8 + N4	.01	.01	.02*	.01	.01	.01	.01	.01
	CF	B0.8	.01	.01	.04	.09†	.13*	.13*	.15*	.14*
		B0.8 + N4	.01	.01	.02	.03*	.03*	.03*	.03*	.03*

Table 6.--Continued.

Time (minutes)			Control -10	Control 0	Infusion Period 10	20	30	40	50	60
Lymph Total Protein (grams %)	NF	BO.8	2.2	2.2	3.7*	4.2*	4.0*	4.1*	4.4*	4.0*
		BO.8 + N4	2.0	2.1	2.5	2.4	2.3	2.2	2.2	2.2
	CF	BO.8	1.9	2.0	2.3	2.4	2.9*	3.3*	3.2*	3.2*
		BO.8 + N4	1.9	1.9	2.0	2.1	2.2	2.2	2.3	2.4†
Plasma Protein (grams %)	NF	BO.8		5.0						
		BO.8 + N4		4.4			4.5			4.7
	CF	BO.8		4.1			4.2			3.9
		BO.8 + N4		4.9			5.3			4.8
Hematocrit	NF	BO.8								
		BO.8 + N4		37			42*			44*
	CF	BO.8		38			39			40
		BO.8 + N4		39			42†			44*

* = $p \leq 0.01$ relative to zero time.

† = $p \leq 0.05$ relative to zero time.

DISCUSSION

These data suggest that the edemogenic action of bradykinin in the canine forelimb is route dependent. When bradykinin is administered systemically (140 to 280 mcg/min, i.v.) into naturally perfused forelimbs, flow rate and protein concentration of the lymph increased slightly, as compared to local bradykinin (0.8 mcg/min) and failed to produce visible or tactile signs of edema (Table 1). One could easily explain these results by the fact that bradykinin is destroyed in the pulmonary circulation. However, although it is well documented in the literature that bradykinin is destroyed in the lungs by kininases (1, 17), it is possible to introduce a large enough quantity of bradykinin intravenously to exceed the saturation point of the kininases in the lungs, or infuse bradykinin downstream to the lung, to bypass pulmonary inactivation. Therefore, bradykinin was infused (140 to 280 mcg/min) into the left ventricle of the heart in constantly perfused forelimbs (Table 2). Minimal increases in lymph flow and lymph total protein concentration were also observed, as compared to local bradykinin (0.8 mcg/min); however, left ventricular infusions increased these parameters slightly more than the intravenous infusions. This might suggest that at these systemic dosages of bradykinin, pulmonary inactivation plays a very minor role in its destruction.

there

blood

bed.

brad

tota

cons

a on

fore

brad

tors

be e

prot

at

str

nin

not

of

tri

car

pla

cre

ar

br

in

With the possibility of pulmonary inactivation eliminated, there still existed the feasibility that factors within the arterial blood may destroy bradykinin before it reached the studied vascular bed. Since in forelimbs perfused at constant inflow, locally infused bradykinin (0.8 mcg/min) exhibited increases in lymph flow and lymph total protein concentration, this possibility was expelled. During constant perfusion, locally administered bradykinin must traverse over a one to two meter length of polyethylene tubing before reaching the forelimb. This distance is greater or equal to any distance that bradykinin would travel during a left ventricular infusion. If factors inherent in the blood were inactivating bradykinin, it would not be expected to observe the marked increases in flow rate and total protein concentration of the lymph as is noted with the local infusions at constant inflow. This does not imply that bradykinin is not destroyed in the plasma. As noted previously, the half-life of bradykinin in plasma is about 15 seconds; however, this degradation rate is not sufficient to account for the route dependent differential action of bradykinin.

Since the differential actions of local and systemic (left ventricle only) bradykinin on lymph flow, protein efflux and fluid fluxes cannot be explained by involvement of the lung or inactivation in the plasma, it seems likely that they result from different actions on microvascular pressure, permeability to plasma proteins and/or surface area. An obvious difference between local and systemic infusions of bradykinin is the marked hypotension observed only with the systemic infusion (140 to 280 mcg/min). The decreased systemic arterial blood

pressure would induce a sympathoadrenal discharge, releasing catecholamines that may effectively antagonize the effects of bradykinin. Therefore, it was decided to determine if another type of hypotension known to elicit catecholamine release, could antagonize changes in lymph flow and protein concentration produced with bradykinin in forelimbs perfused at constant inflow. Hypotension was induced for 60 minutes with hemorrhage prior to initiating a local infusion of bradykinin (0.8 or 10 mcg/min, I.A.) for an additional 60 minutes (Table 3). Lymph flow was slightly increased with the lower dosage and minimally increased with the higher dosage. The increase observed with the lower dosage is most likely due to a rise in microvascular pressure, since skin small vein pressure rose, and lymph protein concentration did not change. However, with the higher dosage, lymph protein concentration increased slightly during the latter ten minutes of the infusion, suggesting that the increased lymph flow seen with the higher dosage of bradykinin resulted from both an increase in the microvascular pressure (inferred from increased skin small vein pressure) and a decrease in the transmural colloid osmotic pressure. Interestingly, this increase is not nearly as great as is observed with locally infused bradykinin alone. The antagonism is not peculiar to bradykinin hypotension, but is apparently related to the sympathoadrenal discharge.

To test the hypothesis that the catecholamines are antagonistic to the action of bradykinin in the microvasculature, experiments were performed to compare differences in forelimb weight changes, hemodynamics, lymph flow and lymph total protein concentration between local bradykinin infusions alone, and simultaneously with norepinephrine. The dosage of bradykinin used was 0.8 mcg/min, while the

dosage for norepinephrine was 4 mcg base/min.

In forelimbs perfused at natural flow, bradykinin (0.8 mcg/min) infused locally into the brachial artery for 60 minutes markedly augmented lymph flow, lymph protein concentration and forelimb weight (Tables 4 & 6). The weight gain represents increased extravascular fluid, since all segmental vascular resistances were constant or increasing from the two minute point onward. A constant or increasing vascular resistance suggests that vessel caliber was either constant or decreasing. Therefore, vascular volume changes cannot account for the increases in forelimb weight. Lymph total protein increased by an increase in microvascular permeability to protein; however, the mechanism for this direct effect remains speculative (32). When norepinephrine was infused simultaneously, the changes in lymph flow, lymph total protein concentration and forelimb weight were completely prevented. All segmental resistances increased, indicating a decrease in vessel caliber and reduced blood flow to the forelimb.

To determine the possible contributions of reduced forelimb blood flow in the naturally perfused forelimbs during the simultaneous infusions of bradykinin and norepinephrine, the experiments were repeated, using forelimbs perfused at constant inflow (Tables 5 & 6). Under this condition, the local intra-arterial infusion of bradykinin for 60 minutes increased lymph flow, lymph protein concentration and forelimb weight. Since vascular resistances were constant or rising (minute 5 onward), increased weight is due to increased extravascular fluid. The simultaneous infusion of norepinephrine into the brachial artery essentially prevented these changes in lymph flow and total protein concentration. The forelimb weight increased more with the

simultaneous infusion of norepinephrine and because no change was observed in the lymph total protein concentration, it must be ascribed to the rise in microvascular pressure (inferred from the increase in skin small vein pressure), attributable to the intense norepinephrine vasoconstriction (total skin and skin large vein resistances increased). Since lymph flow rate increased only slightly as compared to the forelimb weight increase (60 gms), the weight gain might be attributable to an augmentation of intracellular fluid volume. This effect is observed in exercise where increases in organ weight are due primarily to rises in intracellular water content (25). Another possibility for this discrepancy is that lymphatic drainage may have been obstructed, thereby accumulating extracellular fluid. However, this possibility seems quite remote, since there is no reason to suspect lymphatic blockage. Thus norepinephrine infused locally into the brachial artery prevents the marked protein efflux by bradykinin independent of reduced forelimb blood flow.

For the doses of bradykinin and norepinephrine used, a shift in blood flow occurs in the constantly perfused forelimbs. Blood flow increases in the brachial vein, which drains largely muscle tissue and is reduced in the cephalic vein, which drains largely the skin. This suggests that norepinephrine causes a shunting of blood flow from skin to muscle.

The antagonism of the bradykinin induced protein efflux by norepinephrine could be due to a blocking of the actions of bradykinin on the microvascular membrane, a shunting of blood flow from nutritional to non-nutritional channels, or a combination of both. Additional experimentation is needed to resolve these questions.

APPENDICES

APPENDIX

This appendix lists, in the form of tables, all the individual observations for the experiments performed in this study. Also listed are the means, standard error of the mean, and statistical significance.

The data in the appendix tables corresponds to the mean values in Tables 1-6 as follows:

Table Number	Appendix Table Number
1	A1
2	A2
3	A3, A4
4	A5, A6
5	A7, A8
6	A9, A10, A11, A12

Table A1.--Effects of bradykinin infused intravenously (vena cava) into naturally perfused forelimbs for 60 minutes on lymph flow, protein concentration of lymph and plasma, vascular pressures and hematocrit (n=6).

	Control		Infusion Period					
			B140			B280		
Time (minutes)	-10	0	10	20	30	40	50	60
Systemic Arterial Blood Pressure (mm Hg)	110	110	85	95	100	100	102	102
	120	120	105	120	125	125	125	130
	133	133	120	123	120	105	107	117
	95	92	80	75	75	85	85	87
	112	115	100	100	97	100	95	95
	100	100	95	95	95	92	95	92
means	112	112	98*	101*	102*	101*	102*	104†
standard error	±6	±6	±6	±7	±7	±6	±6	±7
Skin Small Vein Pressure (mm Hg)	11	11	12	13	13	14	14	14
	13	13	12	13	13	14	14	14
	12	12	13	13	13	13	13	12
	13	11	11	10	9	11	9	10
	8	8	9	8	8	9	8	8
	9	8	7	6	4	5	5	5
means	11	11	11	11	10	11	11	11
standard error	±1	±1	±1	±1	±2	±1	±2	±2

Table A1.--Continued.

	Control		Infusion Period					
			B140			B280		
Time (minutes)	-10	0	10	20	30	40	50	60
Lymph Flow Rate (ml/10 min)	.01	.01	.01	.02	.01	.03	.04	.09
	.01	.01	.01	.01	.01	.04	.09	.08
	.01	.01	.02	.02	.04	.06	.06	.07
	.01	.01	.01	.01	.01	.02	.02	.02
	.01	.01	.01	.01	.01	.02	.04	.04
	.01	.01	.01	.01	.01	.01	.01	.01
means	.01	.01	.01	.01	.02	.03†	.04*	.05*
standard error	±0	±0	±0	±0	±.01	±.01	±.01	±.01
Lymph Total Protein (grams%)	2.5	2.5	2.6	2.8	3.0	2.9	3.0	3.0
	2.0	1.9	1.9	2.0	2.6	2.4	3.0	2.7
	3.2	3.2	3.6	3.8	4.1	3.9	3.9	4.0
	1.5	1.5	1.7	1.9	2.0	2.6	2.7	2.9
	2.0	1.9	2.2	2.3	2.4	3.0	3.5	3.5
	3.6	3.7	3.7	3.8	3.8	3.8	4.0	3.9
means	2.5	2.5	2.6	2.8†	3.0*	3.1*	3.4*	3.3*
standard error	±.3	±.4	±.4	±.4	±.3	±.3	±.2	±.2

Table A1.--Continued.

Time (minutes)	Control		Infusion Period					
			B140			B280		
	-10	0	10	20	30	40	50	60
Plasma Protein (grams %)		5.4			5.6			5.0
		6.0			6.3			6.2
		6.1			3.9			5.9
		5.4			5.1			5.3
		5.2			5.3			5.2
		5.1			5.1			5.2
means		5.5			5.2			5.5
standard error		±.2			±.3			±.2
Hematocrit		41			42			40
		43			49			53
		38			43			43
		39			41			42
		39			45			48
		33			35			36
means		39			43			44
standard error		±1			±2*			±3*

* = $p \leq 0.01$ relative to zero time.

† = $p \leq 0.05$ relative to zero time.

Table A2.--Effects of bradykinin infused into the left ventricle of the heart at constant inflow for 60 minutes on lymph flow, protein concentration of lymph and plasma, vascular pressures and hematocrit (n=6).

	Control		Infusion Period					
			B140			B280		
Time (minutes)	-10	0	10	20	30	40	50	60
Systemic Arterial Blood Pressure (mm Hg)	130	130	85	85	85	82	85	85
	105	105	85	90	90	90	90	90
	102	120	80	80	82	80	80	87
	122	122	62	70	70	70	70	70
	130	135	65	65	62	50	50	55
	130	127	105	95	100	95	92	85
means	123	123	80*	81*	82*	78*	78*	79*
standard error	±4	±4	±6	±5	±6	±7	±6	±6
Perfusion Pressure (mm Hg)	123	123	110	107	97	95	115	120
	100	100	77	90	90	90	95	100
	115	110	75	85	100	100	110	110
	110	110	110	110	105	125	125	125
	125	130	75	85	100	85	95	100
	120	120	95	90	100	100	100	100
means	116	116	90*	95*	99*	99*	107	109
standard error	±4	±4	±7	±5	±2	±6	±5	±5

Table A2.--Continued.

	Control		Infusion Period					
			B140			B280		
Time (minutes)	-10	0	10	20	30	40	50	60
Skin Small Vein Pressure (mm Hg)	10	10	10	9	9	9	8	8
	12	12	12	10	8	10	10	10
	12	12	12	12	12	11	11	11
	18	18	13	13	11	10	13	14
	12	12	12	12	12	11	11	12
	11	11	11	10	10	10	9	9
means	13	13	12	11*	10*	10*	10*	11*
standard error	±1	±1	±0	±1	±1	±0	±1	±1
Lymph Flow Rate (ml/10 min)	.01	.01	.01	.01	.01	.01	.01	.01
	.02	.01	.02	.16	.18	.17	.21	.21
	.01	.01	.03	.04	.09	.10	.13	.15
	.05	.05	.10	.06	.09	.06	.04	.04
	.01	.02	.03	.08	.18	.20	.26	.28
	.01	.01	.01	.01	.01	.01	.01	.02
means	.02	.02	.04	.06	.09†	.09†	.11*	.12*
standard error	±0	±0	±.01	±.02	±.03	±.03	±.04	±.05

Table A2.--Continued.

Time (minutes)	Control		Infusion Period					
			B140			B280		
	-10	0	10	20	30	40	50	60
Lymph Total Protein (grams %)	1.9	1.9	1.9	2.0	2.3	2.7	3.0	2.9
	1.5	1.3	1.6	1.9	2.1	2.9	2.9	3.5
	1.6	2.0	2.3	2.2	2.9	2.5	2.2	2.3
	1.7	1.7	1.6	1.6	2.3	2.6	2.2	2.6
	1.8	1.8	2.1	2.0	2.5	3.1	3.3	4.1
	2.0	2.0	2.1	2.1	2.5	2.7	2.5	2.8
means	1.8	1.8	1.9	2.0	2.4*	2.8*	2.7*	3.0*
standard error	±.1	±.1	±.1	±.1	±.1	±.1	±.2	±.3
Plasma Protein (grams %)		4.5			4.8			4.6
		4.6			4.9			4.8
		3.1			3.7			4.2
		4.9			4.6			4.2
		4.6			4.6			4.3
		5.0			5.2			4.9
means		4.5			4.6			4.5
standard error		±.3			±.2			±.1

Table A2.--Continued.

	Control		Infusion Period					
			B140			B280		
Time (minutes)	-10	0	10	20	30	40	50	60
Hematocrit		36			39			40
		36			45			48
		43			47			49
		41			45			45
		38			43			47
		38			43			46
means		39			44*			46*
standard error		±1			±1			±1

* = $p \leq 0.01$ relative to zero time.

† = $p \leq 0.05$ relative to zero time.

Table A3.--Effects of locally infused bradykinin (0.8 mcg/min, I.A.) at constant inflow for 60 minutes, following 60 minutes of hypotension produced by hemorrhage to lower and maintain aortic pressure near 45 mm Hg.

	Control		Hemorrhage						Infusion Period					
Time (minutes)	-10	0	10	20	30	40	50	60	70	80	90	100	110	120
Systemic Arterial	150	150	55	50	52	47	45	47	47	55	97	105	110	110
Blood Pressure	130	135	52	42	40	52	40	55	62	70	70	67	52	45
(mm Hg)	107	105	45	32	42	45	50	62	65	77	62	62	65	65
	115	115	45	42	45	45	50	50	50	45	40	40	45	45
	102	102	50	47	52	60	45	70	70	75	72	67	67	60
	107	110	47	47	50	52	52	50	57	75	67	67	65	62
means	119	120	49*	43*	47*	50*	47*	55*	59*	66*	68*	68*	67*	65*
standard error	±8	±8	±2	±3	±2	±2	±2	±4	±4	±5	±8	±9	±9	±10
Perfusion Pressure	140	140	185	185	185	200	205	200	135	120	95	110	115	125
(mm Hg)	120	125	200	200	200	195	210	215	140	160	177	200	225	250
	97	105	150	100	140	150	170	175	125	140	140	150	150	155
	105	105	175	150	175	190	190	175	120	125	110	100	100	102
	95	95	140	140	155	160	170	170	110	115	120	120	115	115
	100	100	140	150	140	160	155	155	120	122	115	120	115	112
means	110	111	165*	154*	166*	176*	183*	182*	125ω	130ω	126ω	133ω	137ω†	143ω*
standard error	±7	±7	±10	±14	±10	±9	±9	±9	±5	±7	±12	±15	±19	±23
Skin Small Vein	12	12	10	11	11	14	12	12	11	13	12	15	17	18
Pressure	19	14	11	13	13	17	19	27	20	30	27	40	43	62
(mm Hg)	12	10	6	7	6	7	6	7	7	8	8	7	7	8
	10	10	8	8	7	7	8	8	8	8	9	9	9	9
	10	10	8	7	8	8	9	10	10	10	10	10	9	9
	10	10	6	7	7	7	5	5	7	7	6	6	7	10
means	12	11	8	9	9	10	10	12	11	13	12	15	15	19†Ω
standard error	±1	±1	±1	±1	±1	±2	±2	±3	±2	±4	±3	±5	±6	±9

Table A3.--Continued.

Time (minutes)	Control		Hemorrhage						Infusion Period					
	-10	0	10	20	30	40	50	60	70	80	90	100	110	120
Lymph Flow Rate (ml/10 min)	.02	.02	.01	.02	.02	.02	.02	.02	.01	.02	.01	.01	.02	.01
	.04	.04	.04	.06	.05	.07	.04	.08	.08	.12	.22	.21	.27	.26
	.01	.01	.02	.02	.02	.02	.02	.03	.03	.06	.10	.09	.12	.09
	.05	.05	.04	.02	.06	.03	.02	.02	.01	.01	.01	.03	.01	.01
	.01	.01	.01	.01	.01	.01	.01	.01	.01	.01	.01	.01	.01	.01
	.01	.01	.01	.01	.01	.01	.01	.01	.01	.01	.01	.01	.01	.01
means	.02	.02	.02	.02	.03	.03	.02	.03	.03	.04	.06	.06	.07†	.07†
standard error	±.01	±.01	±.01	±.01	±.01	±.01	±.00	±.01	±.01	±.02	±.04	±.03	±.04	±.04
Lymph Total Protein (grams %)	1.5	1.6	1.6	1.6	1.7	1.6	1.7	1.8	1.8	1.9	1.7	2.0	1.9	1.7
	1.6	2.1	2.1	1.7	1.9	2.6	2.5	3.7	2.2	1.9	1.9	1.5	1.2	1.0
	2.0	2.1	2.2	2.1	2.0	2.3	2.3	2.2	2.1	2.4	2.2	2.0	2.2	2.2
	3.1	3.7	3.5	4.0	3.3	3.3	3.1	2.9	3.0	3.1	3.0	2.7	2.2	2.5
	1.2	1.3	1.4	1.8	2.0	2.0	1.7	1.7	1.5	1.4	2.2	1.7	1.7	2.1
	1.5	1.4	1.8	1.7	1.7	1.6	1.8	1.9	2.0	1.9	1.9	2.1	2.1	3.0
means	1.8	2.0	2.1	2.2	2.1	2.2	2.3	2.3	2.1	2.1	2.1	2.0	1.9	2.1
standard error	±.3	±.4	±.3	±.4	±.3	±.3	±.3	±.3	±.2	±.2	±.2	±.2	±.2	±.4
Plasma Protein (grams %)		4.1			4.2			4.3			2.4			2.3
		4.8			3.9			4.1			3.8			3.6
		4.8			4.5			4.7			4.8			4.7
		5.1			4.4			3.1			4.0			4.0
		2.9			3.1			3.5			3.4			3.0
		4.4			3.7			3.6			3.7			3.7
means		4.3			4.0			3.9			3.7†			3.6†
standard error		±.3			±.2			±.2			±.3			±.3
Hematocrit		41			43			50			33			32
		38			41			40			40			42
		39			43			52			54			56
		35			42			46			42			40
		35			40			51			53			51
		39			41			48			50			49
means		38			42			48*			45†			45†
standard error		±1			±1			±2			±3			±3

* = $p \leq 0.01$ relative to zero time.

† = $p \leq 0.05$ relative to zero time.

ω = $p \leq 0.01$ relative to 60 minutes.

Ω = $p \leq 0.05$ relative to 60 minutes.

Table A4.--Effects of locally infused bradykinin (10 mcg/min, I.A.) at constant inflow for 60 minutes, following 60 minutes of hypotension produced by hemorrhage to lower and maintain aortic pressure near 45 mm Hg.

	Control		Hemorrhage						Infusion Period					
Time (minutes)	-10	0	10	20	30	40	50	60	70	80	90	100	110	120
Systemic Arterial	125	125	52	47	47	55	55	65	75	75	80	80	77	72
Blood Pressure	120	120	50	45	62	56	42	70	90	90	85	85	75	75
(mm Hg)	120	120	45	50	45	45	50	65	65	65	57	57	47	45
	122	125	47	47	47	50	45	45	42	40	40	40	35	32
	110	110	35	35	40	40	40	65	90	70	74	73	74	73
	105	107	42	50	50	40	45	40	80	65	70	80	85	90
means	117	118	45*	46*	47*	46*	46*	58*	74*Ω	68*	68*	69*	66*	65*
standard error	±3.2	±3.1	±2.5	±2.3	±1.7	±2.4	±2.3	±5.1	±7.4	±6.7	±6.8	±7.1	±8.1	±8.8
Perfusion Pressure	115	117	190	220	200	185	195	195	125	150	165	160	180	170
(mm Hg)	110	110	120	160	165	200	215	235	140	160	175	185	210	240
	110	110	135	145	140	140	185	185	75	90	85	90	95	95
	117	120	185	175	175	160	160	155	75	92	92	97	105	100
	103	103	145	130	120	120	150	160	80	85	90	95	95	95
	95	97	120	155	165	165	185	195	115	125	125	130	130	130
means	108	109	149*	164*	161*	162*	182*	188*	102ω	117ω	122ω	126ω	136ω†	138ω†
standard error	±3.3	±3.5	±12.8	±12.8	±11.4	±11.9	±9.7	±11.8	±11.7	±13.4	±16.3	±16.1	±19.8	±23.6
Skin Small Vein	11	11	9	9	9	9	9	9	10	10	11	12	12	13
Pressure	12	14	12	15	14	13	11	12	17	20	25	40	60	80
(mm Hg)	9	9	6	5	6	6	7	7	6	7	6	7	7	6
	12	12	7	7	7	6	7	7	7	7	7	9	10	10
	17	16	15	14	12	12	10	9	11	11	10	22	24	21
	12	11	8	8	8	8	8	7	10	9	9	9	9	9
means	12	12	10	10	9	9	9	9	10	11	11	16	20Ω	23†ω
standard error	±1.1	±1.0	±1.4	±1.6	±1.3	±1.2	±0.7	±0.8	±1.6	±2.0	±2.8	±5.2	±8.3	±11.6

Table A4.--Continued.

Time (minutes)	Control		Hemorrhage						Infusion Period					
	-10	0	10	20	30	40	50	60	70	80	90	100	110	120
Lymph Flow Rate (ml/10 min)	.01	.01	.01	.01	.01	.01	.02	.02	.02	.05	.06	.07	.05	.05
	.01	.01	.01	.01	.02	.01	.01	.01	.02	.05	.04	.18	.18	.36
	.01	.01	.01	.02	.02	.02	.03	.03	.03	.07	.11	.13	.12	.11
	.01	.01	.01	.01	.01	.01	.01	.01	.01	.01	.01	.02	.02	.03
	.02	.02	.02	.02	.02	.02	.02	.02	.03	.03	.04	.04	.08	.11
	.02	.01	.01	.01	.01	.01	.01	.02	.01	.01	.02	.02	.03	.03
means	.01	.01	.01	.01	.02	.01	.02	.02	.02	.04	.05	.08ω*	.08ω*	.12ω*
standard error	±0	±0	±0	±0	±0	±0	±.01	±.01	±.01	±.01	±.01	±.03	±.02	±.05
Lymph Total Protein (grams %)	3.5	3.5	3.4	3.5	3.6	3.5	2.9	2.4	2.4	2.0	2.3	3.0	2.5	2.0
	3.2	2.9	3.1	3.1	3.3	3.4	3.4	3.3	3.2	3.5	3.6	3.7	4.4	5.6
	2.5	2.3	2.7	3.0	2.8	2.7	2.8	2.8	2.6	2.6	2.6	3.4	3.6	4.8
	1.2	1.5	2.1	1.8	1.4	1.6	1.7	1.9	1.7	1.9	1.9	1.9	1.9	1.7
	1.4	1.2	1.2	1.2	1.8	2.0	1.8	1.7	1.7	1.8	1.5	1.6	1.7	2.6
	1.9	1.9	2.0	2.3	2.3	2.4	2.2	2.2	2.3	2.4	2.9	2.7	2.7	3.1
means	2.3	2.2	2.4	2.5	2.5	2.6	2.4	2.4	2.3	2.4	2.5	2.7	2.8†	3.3*ω
standard error	±.4	±.4	±.3	±.4	±.4	±.3	±.3	±.2	±.2	±.3	±.3	±.3	±.4	±.6
Plasma Protein (grams %)		4.9			4.3			4.1			5.0			4.2
		6.3			6.4			5.0			5.6			5.5
		4.8			4.6			4.4			4.7			4.0
		4.1			3.4			3.8			2.6			2.7
		6.0			5.2			4.6			4.7			4.8
		5.3			5.0			5.1			4.9			5.1
means		5.2			4.8†			4.3*			4.6*			4.4*
standard error		±.3			±.4			±.3			±.4			±.4
Hematocrit		37			36			42			47			48
		39			39			45			47			50
		45			46			51			50			50
		35			35			37			35			34
		44			44			46			48			48
		37			37			38			40			42
means		40			40			43			45*			45*
standard error		±1.7			±1.8			±2.2			±2.4			±2.6

* = $p \leq 0.01$ relative to zero time.

† = $p \leq 0.05$ relative to zero time.

ω = $p \leq 0.01$ relative to 60 minutes.

Ω = $p \leq 0.05$ relative to 60 minutes.

Table A5.--Effects of bradykinin (0.8 mcg/min, I.A.) infused locally into naturally perfused forelimbs on weight, blood flows, vascular resistances and vascular pressures (n=6).

Time (minutes)	Control		Infusion Period						
	-5	0	2	5	10	15	30	45	60
Change in Weight (grams)	0	0	8	14	14	14	16	17	20
	0	0	12	14	16	18	24	28	30
	0	0	2	3	4	4	8	11	16
	0	-1	5	5	5	5	10	14	17
	0	-1	14	14	14	13	13	13	13
	0	0	8	9	9	14	13	16	18
means	0	0	8*	10*	11*	12*	14*	17*	19*
standard error	±0	±0	±2	±2	±2	±2	±2	±2	±2
Systemic Arterial Blood Pressure (mm Hg)	120	120	118	120	121	120	122	118	120
	150	155	155	155	155	150	150	150	147
	135	135	135	135	135	135	130	125	125
	140	140	140	140	145	150	140	140	140
	150	150	150	150	150	150	150	150	150
	130	127	125	125	125	125	105	105	105
means	138	138	137	138	139	138	133	132†	132†
standard error	±5	±5	±6	±6	±6	±6	±7	±7	±7

Table A5.--Continued.

Time (minutes)	Control		Infusion Period						
	-5	0	2	5	10	15	30	45	60
Skin Small Vein Pressure (mm Hg)	8	8	16	16	16	15	10	9	11
	15	15	30	28	25	24	15	13	13
	15	15	15	14	13	11	13	13	17
	11	11	18	15	15	16	20	20	18
	15	15	50	35	30	20	11	11	11
	8	7	18	18	11	12	9	9	9
means	12	12	25*	21*	18†	16	13	13	13
standard error	±1	±2	±6	±3	±3	±2	±2	±2	±1
Cephalic Vein Pressure (mm Hg)	2	2	5	6	5	3	4	4	4
	5	5	6	7	7	6	5	5	5
	11	11	10	10	8	7	9	8	10
	2	2	5	4	3	2	2	2	2
	2	2	5	5	2	2	2	2	2
	5	5	11	11	7	7	5	5	5
means	5	5	7†	7†	5	5	5	4	5
standard error	±1	±1	±1	±1	±1	±1	±1	±1	±1

Table A5.--Continued.

Time (minutes)	Control		Infusion Period						
	-5	0	2	5	10	15	30	45	60
Brachial Vein Pressure (mm Hg)	6	6	10	10	10	9	7	7	6
	10	10	15	15	14	12	12	9	10
	11	11	13	14	15	15	17	15	15
	7	6	11	9	10	10	10	11	14
	7	7	20	11	5	5	5	5	5
	10	9	17	15	11	11	10	10	10
means	9	8	14*	12*	11†	10	10	10	10
standard error	±1	±1	±2	±1	±1	±1	±2	±1	±2
Cephalic Venous Outflow (ml/min/100 grams)	11	11	24	24	23	12	9	11	16
	14	15	23	24	23	21	17	17	10
	10	12	15	15	14	12	15	13	15
	14	14	22	20	16	14	15	12	12
	9	9	23	16	7	6	5	6	5
	12	11	29	29	19	21	20	14	16
means	12	12	23*	21*	17*	14	14	12	12
standard error	±1	±1	±2	±2	±2	±2	±2	±1	±2

Table A5.--Continued.

Time (minutes)	Control		Infusion Period						
	-5	0	2	5	10	15	30	45	60
Brachial Venous Outflow (ml/min/100 grams)	6	5	20	17	18	13	6	5	6
	6	6	13	11	10	8	8	5	5
	7	7	7	6	4	3	4	4	6
	7	8	14	11	9	8	8	8	8
	9	9	25	16	7	5	5	5	5
	6	5	10	8	9	7	4	5	5
means	7	7	15*	12*	10	7	6	5	6
standard error	±1	±1	±3	±2	±2	±1	±1	±1	±1
Total Skin Resistance ($mm\ Hg_1 \times min \times ml^{-1} \times 100\ g^{-1}$)	11	11	5	5	5	10	13	10	7
	10	10	10	6	6	7	9	9	14
	12	10	8	8	9	11	8	9	8
	10	10	6	7	9	11	9	12	12
	16	16	6	9	21	25	30	25	30
	10	11	4	4	6	6	5	7	6
means	12	11	7†	7†	9	12	12	12	13
standard error	±1	±1	±1	±1	±2	±3	±4	±3	±4

Table A5.--Continued.

Time (minutes)	Control		Infusion Period						
	-5	0	2	5	10	15	30	45	60
Total Muscle Resistance	19	23	5	6	6	9	19	22	19
(mm Hg × min × ml^{-1} ×	23	24	11	13	14	17	17	28	27
100 g^{-1})	18	18	17	20	30	40	28	28	18
	19	17	9	12	15	18	16	16	16
	16	16	5	9	21	29	29	29	29
	20	24	11	14	13	16	24	19	19
means	19	20	10*	12*	17	22	22	24	21
standard error	±1	±2	±2	±2	±3	±5	±2	±2	±2
Skin Large Vein Resistance	1	1	1	1	1	1	1	1	1
(mm Hg × min × ml^{-1} ×	1	1	2	1	1	2	1	1	1
100 g^{-1})	0.4	0.4	0.4	0.3	0.3	0.3	0.2	0.3	0.5
	1	2	1	1	1	1	2	2	1
	2	2	2	3	6	4	2	2	2
	0.3	0.2	0.4	0.5	0.4	0.5	0.4	0.4	0.4
means	1	1	1	1	2	1	1	1	1
standard error	±0	±0	±0	±0	±1	±0	±0	±0	±0

* = $p \leq 0.01$ relative to zero time. † = $p \leq 0.05$ relative to zero time.

Table A6.--Effects of bradykinin (0.8 mcg/min, I.A.) and norepinephrine (4 mcg/min, I.A.) infused locally into naturally perfused forelimbs on weight, blood flows, vascular resistances and vascular pressures (n=6).

Time (minutes)	Control		Infusion Period						
	-5	0	2	5	10	15	30	45	60
Change in Weight (grams)	0	1	-5	4	6	7	12	12	13
	0	0	-13	-10	-6	-6	-6	2	2
	0	1	-10	-9	-8	-7	-3	-1	-1
	0	-1	-10	-15	-12	-12	-6	-1	0
	0	1	-10	-12	-10	-10	-9	-7	-6
	0	0	-10	-8	-4	-2	-2	0	0
means	0	0	-10*	-8*	-6*	-5†	-2†	1†	1†
standard error	±0	±0	±1	±3	±3	±3	±3	±3	±3
Systemic Arterial Blood Pressure (mm Hg)	115	115	125	125	120	120	115	115	113
	100	98	100	103	103	115	97	105	105
	75	75	85	85	90	100	95	85	90
	120	125	130	135	125	130	150	130	125
	95	95	100	100	100	100	110	100	120
	125	120	125	125	130	130	128	120	125
means	105	105	111	112†	111	116*	116*	109	113†
standard error	±8	±8	±7	±8	±6	±6	±8	±7	±6

Table A6.--Continued.

Time (minutes)	Control		Infusion Period						
	-5	0	2	5	10	15	30	45	60
Skin Small Vein Pressure (mm Hg)	12	12	30	44	50	44	30	36	36
	10	10	27	40	24	33	24	35	23
	15	13	45	28	29	50	50	33	25
	5	6	18	13	20	25	28	20	16
	12	12	27	20	32	20	20	30	35
	7	7	40	50	35	25	21	21	13
means	10	10	31*	33*	32*	33*	29*	29*	25*
standard error	±1	±1	±4	±6	±4	±5	±5	±3	±4
Cephalic Vein Pressure (mm Hg)	8	8	2	4	4	4	10	4	4
	6	6	0	1	6	8	1	3	5
	3	4	5	1	5	8	7	6	6
	2	2	0	0	2	2	2	2	2
	1	1	0	0	0	0	0	0	0
	6	6	23	30	30	14	8	8	6
means	4	5	5	6	6	6	5	4	4
standard error	±1	±1	±4	±5	±3	±2	±2	±1	±1

Table A6.--Continued.

Time (minutes)	Control		Infusion Period						
	-5	0	2	5	10	15	30	45	60
Brachial Vein Pressure (mm Hg)	4	4	10	21	14	13	8	10	10
	6	6	7	20	14	15	20	25	30
	4	3	6	3	6	5	5	5	5
	4	4	9	10	25	21	22	27	25
	1	1	10	5	5	4	12	13	20
	9	9	45	45	25	25	21	20	16
means	5	5	15†	17*	15†	14†	15†	17*	18*
standard error	±1	±1	±6	±6	±4	±3	±3	±4	±4
Cephalic Venous Outflow (ml/min/100 grams)	8	8	0.1	0.1	0.1	0.1	0.1	0.1	0.1
	13	13	1	1	1	1	0.3	1	1
	5	6	3	2	2	2	2	1	1
	10	10	7	1	0.4	0.4	1	1	1
	12	13	1	0.2	0.4	0.2	0.4	0.2	1
	11	12	5	8	7	6	4	3	5
means	10	10	3*	2*	2*	2*	1*	1*	2*
standard error	±1	±1	±1	±1	±1	±1	±1	±0	±1

Table A6.--Continued.

Time (minutes)	Control		Infusion Period						
	-5	0	2	5	10	15	30	45	60
Brachial Venous Outflow (ml/min/100 grams)	10	10	4	3	2	1	2	2	1
	9	9	3	2	5	4	1	1	1
	2	2	2	1	3	3	1	1	1
	3	3	2	2	0.3	0.4	1	1	1
	7	8	2	0.4	0.4	0.4	1	1	1
	11	12	6	11	15	9	5	2	1
means	7	7	3*	3*	4†	3*	2*	1*	1*
standard error	±2	±2	±1	±2	±3	±1	±1	±0	±0
Total Muscle Resistance ($mm\ Hg \times min \times ml^{-1} \times 100\ g^{-1}$)	11	11	27	35	46	82	63	62	86
	10	10	36	44	20	24	154	80	83
	39	33	34	59	27	37	64	160	170
	41	47	71	74	333	273	183	147	167
	13	12	50	238	238	240	140	124	111
	11	10	13	7	7	10	24	44	84
means	21	21	39	76	112*	111*	105†	103†	117*
standard error	±6	±6	±8	±34	±57	±47	±27	±19	±17

Table A6.--Continued.

Time (minutes)	Control		Infusion Period						
	-5	0	2	5	10	15	30	45	60
Total Skin Resistance	14	14	1230	1210	1160	1160	1050	1110	1090
(mm Hg × min × ml^{-1} ×	7	7	100	146	81	357	320	146	111
100 g^{-1})	15	13	28	56	43	51	44	66	93
	12	13	19	225	308	320	123	98	103
	8	7	91	500	250	500	275	500	240
	11	10	19	11	15	20	32	34	22
means	11	11	248†	358*	310†	401*	307†	326*	277†
standard error	±1	±1	±197	±184	±176	±170	±156	±171	±165
Skin Large Vein Resistance	1	1	180	400	460	400	200	320	320
(mm Hg × min × ml^{-1} ×	0.3	0.3	27	56	15	21	77	46	20
100 g^{-1})	2	2	14	18	12	23	22	23	21
	0.3	0.4	3	22	45	58	22	14	12
	1	1	25	100	80	100	50	150	70
	0.1	0.1	3	2	2	2	4	4	1
means	1	1	42	100†	102†	101†	63	93†	74
standard error	±0	±0	±28	±62	±72	±62	±29	±50	±50

* = $p \leq 0.01$ relative to zero time. † = $p \leq 0.05$ relative to zero time.

Table A7.--Effects of bradykinin (0.8 mcg/min, I.A.) infused locally into constantly perfused forelimbs on weight, blood flows, vascular resistances and vascular pressures (n=6).

Time (minutes)	Control		Infusion Period						
	-5	0	2	5	10	15	30	45	60
Change in Weight (grams)	0	0	3	4	6	6	7	9	9
	0	0	3	2	0	-2	-4	-6	-6
	0	0	3	3	5	6	9	14	19
	0	-2	2	1	0	0	2	4	4
	0	-1	3	4	5	8	12	19	22
	0	1	2	5	9	11	19	27	36
means	0	0	3	3	4	5	8†	11*	14*
standard error	±0	±0	±0	±1	±1	±2	±3	±5	±6
Systemic Arterial Blood Pressure (mm Hg)	125	125	125	125	120	117	117	119	118
	135	140	140	140	145	145	145	145	140
	100	100	100	100	100	100	100	100	100
	100	100	100	90	90	90	85	80	75
	125	130	130	130	130	140	140	140	150
	155	155	155	150	145	140	140	140	140
means	123	125	125	123	122	122	121	121	121
standard error	±9	±9	±9	±9	±9	±10	±10	±11	±12

Table A7.--Continued.

Time (minutes)	Control		Infusion Period						
	-5	0	2	5	10	15	30	45	60
Perfusion Pressure (mm Hg)	125	125	50	65	75	75	95	125	145
	100	105	50	75	86	90	110	125	155
	140	140	55	75	95	100	145	175	195
	100	100	35	50	65	65	65	75	75
	112	120	45	55	76	87	95	110	125
	130	134	100	155	180	200	220	220	220
means	118	121	56*	79*	96†	103	122	138	153*
standard error	±7	±6	±9	±16	±17	±20	±22	±21	±21
Skin Small Vein Pressure (mm Hg)	12	12	12	11	11	11	11	11	11
	12	11	10	10	10	10	9	9	9
	15	15	15	15	15	15	12	12	12
	10	10	8	7	7	6	8	7	7
	15	14	10	11	13	14	14	17	15
	20	20	22	25	25	30	35	35	32
means	14	14	13	13	14	14	15	15	14
standard error	±1	±1	±2	±3	±3	±3	±4	±4	±4

Table A7.--Continued.

Time (minutes)	Control		Infusion Period						
	-5	0	2	5	10	15	30	45	60
Cephalic Vein Pressure (mm Hg)	5	5	5	5	5	4	5	4	5
	2	2	2	2	2	2	2	2	2
	3	3	3	3	3	3	3	3	3
	3	3	2	2	2	2	2	2	2
	9	8	7	7	8	9	8	9	7
	5	5	3	3	3	2	2	2	4
means	5	4	4	4	4	4	4	4	4
standard error	±1	±1	±1	±1	±1	±1	±1	±1	±1
Brachial Vein Pressure (mm Hg)	5	5	5	6	5	5	6	5	6
	7	7	7	7	7	7	7	7	7
	5	5	5	5	5	5	5	5	5
	3	3	2	2	2	2	2	2	2
	5	4	5	5	5	5	5	5	5
	12	12	15	17	20	20	25	25	25
means	6	6	7	7	7	7	8	8	8
standard error	±1	±1	±2	±2	±3	±3	±3	±3	±3

Table A7.--Continued.

Time (minutes)	Control		Infusion Period						
	-5	0	2	5	10	15	30	45	60
Cephalic Venous Outflow (ml/min/100 grams)	15	15	14	14	14	14	14	13	14
	9	10	10	10	10	10	10	11	10
	6	6	6	6	8	8	9	9	10
	22	22	18	19	18	18	18	19	18
	13	16	12	14	14	14	14	15	15
	21	23	22	22	21	20	18	22	22
means	14	15	14	14	14	14	14	15	15
standard error	±3	±3	±3	±3	±2	±2	±2	±2	±2
Brachial Venous Outflow (ml/min/100 grams)	15	15	15	14	15	15	15	15	15
	11	11	11	11	11	11	11	11	11
	30	29	30	30	29	29	27	28	27
	3	4	6	5	6	5	6	5	6
	9	8	10	9	8	9	8	9	8
	6	6	7	7	8	8	11	9	7
means	12	12	13	13	13	13	13	13	12
standard error	±4	±4	±4	±4	±3	±4	±3	±3	±3

Table A7.--Continued.

Time (minutes)	Control		Infusion Period						
	-5	0	2	5	10	15	30	45	60
Total Skin Resistance	8	8	3	4	5	5	6	9	10
(mm Hg × min × ml^{-1} ×	11	10	5	7	8	9	11	11	15
100 g^{-1})	23	23	7	7	12	12	16	19	19
	4	4	2	3	4	4	4	4	4
	8	7	3	3	5	6	6	7	8
	6	6	4	7	8	10	12	10	10
means	10	10	4*	5*	7†	8	9	10	11
standard error	±3	±3	±1	±1	±1	±1	±2	±2	±2
Total Muscle Resistance	8	8	3	4	5	5	6	8	9
(mm Hg × min × ml^{-1} ×	8	9	4	6	7	8	9	11	13
100 g^{-1})	5	5	2	2	3	3	5	6	7
	32	24	6	10	11	13	11	15	12
	12	15	4	6	9	9	11	12	15
	20	20	12	20	20	23	18	22	28
means	14	14	5*	8*	9*	10†	10†	12	14
standard error	±4	±3	±1	±3	±2	±3	±2	±2	±3

Table A7.--Continued.

Time (minutes)	Control		Infusion Period						
	-5	0	2	5	10	15	30	45	60
Skin Large Vein Resistance	0.5	0.5	0.5	0.4	0.4	0.5	0.4	0.5	0.4
(mm Hg_1 × min × ml^{-1} ×	1	1	1	1	1	1	1	1	1
100 g^{-1})	2	2	2	2	2	2	1	1	1
	0.3	0.3	0.3	0.3	0.3	0.2	0.3	0.3	0.3
	0.5	0.4	0.3	0.3	0.4	0.4	0.4	0.5	0.5
	1	1	1	1	1	2	2	2	1
means	1	1	1	1	1	1	1	1	1
standard error	±0	±0	±0	±0	±0	±0	±0	±0	±0

* = $p \leq 0.01$ relative to zero time. † = $p \leq 0.05$ relative to zero time.

Table A8.--Effects of bradykinin (0.8 mcg/min, I.A.) and norepinephrine (4 mcg/min, I.A.) infused locally into constantly perfused forelimbs on weight, blood flows, vascular resistances and vascular pressures (n=6).

	Control		Infusion Period						
Time (minutes)	-5	0	2	5	10	15	30	45	60
Change in Weight (grams)	0	0	6	8	11	14	18	26	30
	1	0	-20	-16	-12	-8	0	7	15
	0	0	-4	4	10	16	26	39	59
	0	2	2	15	29	39	63	103	163
	0	0	-3	2	11	14	21	27	31
	0	0	0	7	11	15	24	43	61
means	0	0	-3	3	10	15	25†	41*	60*
standard error	±0	±0	±4	±4	±5	±6	±8	±13	±22
Systemic Arterial Blood Pressure (mm Hg)	148	152	152	155	155	155	160	160	155
	140	138	175	190	183	155	170	150	150
	100	102	110	125	150	125	120	110	110
	125	127	160	180	165	165	150	150	135
	85	85	120	140	130	125	125	120	140
	105	100	144	160	150	140	150	140	130
means	117	117	144*	158*	156*	144*	146*	138*	135*
standard error	±10	±10	±10	±10	±7	±7	±8	±8	±8

Table A8.--Continued.

Time (minutes)	Control		Infusion Period						
	-5	0	2	5	10	15	30	45	60
Perfusion Pressure (mm Hg)	130	130	170	179	188	188	190	190	190
	112	113	187	220	225	235	250	260	260
	95	97	155	175	180	180	185	205	240
	120	121	250	280	280	280	280	290	290
	80	80	130	155	155	170	185	210	220
	123	125	230	248	240	250	240	240	250
means	110	111	187*	210*	211*	217*	222*	233*	242*
standard error	±8	±8	±19	±20	±19	±18	±17	±15	±14
Skin Small Vein Pressure (mm Hg)	7	7	30	35	40	37	35	33	32
	11	11	20	29	26	25	23	22	22
	11	10	55	63	52	52	50	52	60
	17	19	44	48	49	45	43	43	45
	10	10	40	43	33	34	28	31	28
	15	16	45	46	40	40	30	31	35
means	12	12	39*	44*	40*	39*	35*	35*	37*
standard error	±1	±2	±5	±5	±4	±4	±4	±4	±6

Table A8.--Continued.

Time (minutes)	Control		Infusion Period						
	-5	0	2	5	10	15	30	45	60
Cephalic Vein Pressure (mm Hg)	2	3	6	7	6	7	7	5	6
	8	8	17	22	20	17	15	15	15
	4	3	4	8	12	13	12	14	15
	11	12	30	30	30	29	25	23	23
	4	4	7	10	12	9	9	8	8
	9	9	29	30	30	26	22	24	27
means	6	7	16*	18*	18*	17*	15*	15*	16*
standard error	±1	±2	±5	±4	±4	±4	±3	±3	±3
Brachial Vein Pressure (mm Hg)	5	5	21	21	21	13	17	17	19
	9	9	23	25	25	22	21	23	25
	7	8	30	40	36	30	25	25	34
	13	14	35	35	35	35	33	34	34
	4	4	13	13	11	11	11	11	14
	9	8	40	45	45	45	45	45	50
means	8	8	27*	30*	29*	26*	25*	26*	29*
standard error	±1	±1	±4	±5	±5	±5	±5	±5	±5

Table A8.--Continued.

Time (minutes)	Control		Infusion Period						
	-5	0	2	5	10	15	30	45	60
Cephalic Venous Outflow (ml/min/100 grams)	6	7	4	5	5	5	5	6	5
	17	17	6	6	7	7	8	8	7
	14	14	3	6	8	8	6	6	8
	10	10	5	5	6	6	6	6	6
	5	5	4	4	5	5	5	4	3
	10	10	8	8	8	7	6	6	6
means	10	11	5*	6*	7*	6*	6*	6*	6*
standard error	±2	±2	±1	±1	±1	±0	±0	±1	±1
Brachial Venous Outflow (ml/min/100 grams)	6	6	7	7	6	5	5	6	7
	14	13	26	25	25	23	22	22	22
	3	3	13	11	10	9	10	10	8
	10	10	14	15	14	14	13	13	14
	7	7	10	9	7	8	8	9	10
	11	11	14	14	14	14	16	15	15
means	9	8	14*	14*	13*	12*	12*	13*	13*
standard error	±2	±1	±3	±3	±3	±3	±2	±2	±2

Table A8.--Continued.

Time (minutes)	Control		Infusion Period						
	-5	0	2	5	10	15	30	45	60
Total Skin Resistance	21	18	41	34	36	36	37	31	37
(mm Hg × min × ml^{-1} ×	6	6	28	33	29	31	29	31	35
100 g^{-1})	7	7	50	29	21	21	29	32	28
	11	11	44	50	42	42	43	45	45
	15	15	31	36	29	32	35	51	71
	11	12	25	27	26	32	36	36	37
means	12	12	37*	35*	31*	32*	35*	38*	42*
standard error	±2	±2	±4	±3	±3	±3	±2	±3	±6
Total Muscle Resistance	21	21	21	23	28	35	35	29	24
(mm Hg × min × ml^{-1} ×	7	8	6	8	8	9	10	11	11
100 g^{-1})	29	30	10	12	14	17	16	18	26
	11	11	15	16	18	18	19	20	18
	11	11	12	16	21	20	22	22	21
	10	11	14	15	14	15	12	13	13
means	15	15	13	15	17	19	19	19	19
standard error	±3	±3	±2	±2	±3	±4	±4	±3	±2

Table A8.--Continued.

Time (minutes)	Control		Infusion Period						
	-5	0	2	5	10	15	30	45	60
Skin Large Vein Resistance	1	1	6	6	7	6	6	5	5
(mm Hg × min × ml^{-1} ×	0.2	0.2	1	1	1	1	1	1	1
100 g^{-1})	1	1	17	9	5	5	6	6	6
	1	1	3	4	3	3	3	3	4
	1	1	8	8	4	5	4	6	7
	1	1	2	2	1	2	1	1	1
means	1	1	6*	5*	4*	4*	4*	4*	4*
standard error	±0	±0	±2	±1	±1	±1	±1	±1	±1

* = $p \leq 0.01$ relative to zero time.

† = $p \leq 0.05$ relative to zero time.

Table A9.--Effects of bradykinin (0.8 mcg/min) infused intra-arterially into the forelimb at natural inflow for 60 minutes on lymph flow, protein concentration of lymph and plasma, vascular pressures and hematocrit (n=6).

Time (minutes)	Control		Infusion Period					
	-10	0	10	20	30	40	50	60
Systemic Arterial Blood Pressure (mm Hg)	130	130	133	131	130	130	130	125
	105	105	107	115	118	110	115	110
	80	80	85	85	85	87	87	90
	79	78	78	80	78	78	80	82
	130	135	125	125	127	130	107	93
	100	100	100	100	102	105	105	105
means	104	105	105	106	107	107	104	101
standard error	±9	±10	±9	±9	±9	±9	±8	±6
Skin Small Vein Pressure (mm Hg)	10	10	18	16	14	13	13	13
	13	13	21	20	19	18	18	18
	10	10	14	12	11	11	11	12
	8	8	14	14	12	13	14	15
	12	12	15	15	15	13	12	12
	9	9	11	10	11	10	9	9
means	10	10	16*	15*	14*	13*	13*	13*
standard error	±1	±1	±1	±1	±1	±1	±1	±1

Table A9.--Continued.

Time (minutes)	Control		Infusion Period					
	-10	0	10	20	30	40	50	60
Lymph Flow Rate (ml/10 min)	.02	.02	.23	.74	.76	.67	.64	.59
	.03	.03	.36	.76	.71	.73	.71	.73
	.01	.01	.04	.57	.83	.83	.90	.84
	.01	.01	.01	.13	.09	.07	.16	.11
	.01	.01	.06	.19	.24	.26	.23	.20
	.02	.02	.01	.10	.14	.16	.16	.15
means	.02	.02	.12	.42*	.46*	.45*	.47*	.44*
standard error	±.00	±.00	±.06	±.13	±.14	±.13	±.13	±.13
Lymph Total Protein (grams %)	1.8	1.8	3.7	3.9	4.1	4.1	4.1	4.0
	2.2	2.0	3.9	3.9	4.0	4.0	4.0	4.1
	1.9	1.9	3.7	3.7	3.7	3.9	3.7	3.6
	2.5	2.7	3.9	4.3	4.3	4.7	4.7	4.2
	3.0	3.0	4.9	4.9	4.7	4.9	4.9	4.7
	1.8	2.0	2.3	4.2	3.4	3.2	4.9	3.3
means	2.2	2.2	3.7*	4.2*	4.0*	4.1*	4.4*	4.0*
standard error	±.2	±.2	±.3	±.2	±.2	±.2	±.2	±.2

Table A9.--Continued.

Time (minutes)	Control		Infusion Period					
	-10	0	10	20	30	40	50	60
Plasma Protein (grams %)		4.3						
		4.7						
		5.5						
		5.7						
		5.9						
		4.1			4.2			4.2
means		5.0						
standard error		±.3						
Hematocrit								
		38			37			38
means								
standard error								

* = $p \leq 0.01$ relative to zero time.

† = $p \leq 0.05$ relative to zero time.

Table A10.--Effects of bradykinin (0.8 mcg/min) and norepinephrine base (4 mcg/min) infused intra-arterially at natural inflow for 60 minutes on lymph flow, protein concentration of lymph and plasma, vascular pressures and hematocrit (n=6).

Time (minutes)	Control		Infusion Period					
	-10	0	10	20	30	40	50	60
Systemic Arterial Blood Pressure (mm Hg)	117	117	135	130	125	125	125	125
	120	120	125	135	135	135	135	135
	95	95	130	125	125	120	125	115
	107	105	145	135	135	132	127	125
	100	105	130	145	145	145	145	150
	100	105	160	155	160	162	160	157
means	107	108	138*	138*	138*	137*	136*	135*
standard error	±4	±4	±5	±4	±5	±6	±6	±7
Skin Small Vein Pressure (mm Hg)	8	7	21	27	20	20	12	21
	9	9	22	21	19	18	18	19
	9	9	28	24	23	25	29	30
	10	10	20	23	23	15	14	14
	11	11	27	30	45	40	35	37
	9	9	29	30	32	36	27	31
means	9	9	25*	26*	27*	26*	23*	25*
standard error	±0	±1	±2	±2	±4	±4	±4	±4

Table A10.--Continued.

Time (minutes)	Control		Infusion Period					
	-10	0	10	20	30	40	50	60
Lymph Flow Rate (ml/10 min)	.01	.01	.02	.01	.01	.02	.01	.01
	.01	.01	.01	.01	.01	.01	.01	.01
	.02	.01	.04	.01	.01	.01	.01	.01
	.02	.03	.04	.02	.01	.01	.01	.01
	.02	.02	.02	.01	.01	.01	.01	.01
	.01	.01	.01	.01	.01	.01	.01	.01
means	.01	.01	.02*	.01	.01	.01	.01	.01
standard error	±0	±0	±0	±0	±0	±0	±0	±0
Lymph Total Protein (grams %)	1.7	1.7	2.1	2.4	2.8	2.4	2.6	2.5
	1.4	1.7	1.5	1.4	1.3	1.4	1.3	1.3
	2.9	2.7	4.9	3.2	3.1	3.0	2.9	2.9
	2.4	2.4	2.2	2.6	2.4	2.5	2.4	2.2
	2.1	2.3	2.1	2.7	2.8	2.4	2.3	2.0
	1.6	1.8	2.2	2.0	1.5	1.7	1.8	2.0
means	2.0	2.1	2.5	2.4	2.3	2.2	2.2	2.2
standard error	±.2	±.2	±.5	±.3	±.3	±.2	±.2	±.2

Table A10.--Continued.

Time (minutes)	Control		Infusion Period					
	-10	0	10	20	30	40	50	60
Plasma Protein (grams %)		4.5			4.2			4.8
		4.6			4.1			3.9
		5.5			4.7			6.0
		4.1			4.9			4.2
		3.9			4.5			4.8
		3.8			4.6			4.8
means		4.4			4.5			4.7
standard error		±.3			±.1			±.3
Hematocrit		36			39			39
		36			42			43
		38			44			47
		37			43			43
		33			38			40
		42			48			50
means		37			42*			44*
standard error		±1			±1			±2

* = $p \leq 0.01$ relative to zero time.

† = $p \leq 0.05$ relative to zero time.

Table A11.--Effects of bradykinin (0.8 mcg/min) infused intra-arterially into the forelimb at constant inflow for 60 minutes on lymph flow, protein concentration of lymph and plasma, vascular pressures and hematocrit (n=6).

Time (minutes)	Control		Infusion Period					
	-10	0	10	20	30	40	50	60
Systemic Arterial Blood Pressure (mm Hg)	107	115	120	125	125	125	125	120
	125	125	130	125	125	125	130	125
	135	140	140	140	140	140	140	140
	125	125	125	125	125	125	130	125
	125	125	125	125	127	130	130	130
	100	100	100	105	105	105	105	110
means	120	122	123	124	125	125	127†	125
standard error	±5	±6	±6	±5	±5	±5	±5	±4
Perfusion Pressure (mm Hg)	105	110	95	90	100	110	110	112
	120	120	95	95	105	95	125	120
	130	135	105	125	140	160	175	185
	115	115	85	95	100	105	107	105
	125	125	90	100	110	125	130	140
	95	95	70	80	85	85	95	105
means	115	117	88*	98*	107	113	124	128†
standard error	±6	±7	±5	±7	±8	±11	±13	±13

Table A11.--Continued.

Time (minutes)	Control		Infusion Period					
	-10	0	10	20	30	40	50	60
Skin Small Vein Pressure (mm Hg)	7	7	7	7	6	7	7	6
	14	14	12	11	11	11	11	11
	10	11	10	9	9	9	10	10
	13	12	11	12	11	11	11	11
	5	5	5	5	5	6	5	5
	14	14	14	14	14	14	14	13
means	11	11	10	10	9	10	10	9
standard error	±1	±2	±1	±1	±1	±1	±1	±1
Lymph Flow Rate (ml/10 min)	.01	.01	.01	.02	.06	.06	.07	.06
	.01	.01	.01	.01	.07	.06	.10	.08
	.01	.01	.02	.07	.09	.08	.09	.09
	.01	.01	.01	.07	.13	.13	.16	.13
	.01	.02	.15	.29	.37	.33	.37	.34
	.02	.01	.02	.06	.08	.11	.12	.14
means	.01	.01	.04	.09†	.13*	.13*	.15*	.14*
standard error	±0	±0	±.02	±.04	±.05	±.05	±.05	±.05

Table A11.--Continued.

Time (minutes)	Control		Infusion Period					
	-10	0	10	20	30	40	50	60
Lymph Total Protein (grams %)	2.2	2.5	2.6	2.4	2.5	2.7	2.8	2.8
	1.4	1.4	2.0	1.4	3.0	3.0	3.0	3.1
	1.9	1.9	2.0	2.3	2.9	3.6	3.4	3.1
	1.9	2.0	2.0	1.9	2.8	2.9	3.3	3.2
	2.4	2.3	2.7	2.9	2.8	3.1	3.2	3.3
	1.5	1.4	2.3	3.3	3.6	4.2	3.7	3.4
means	1.9	2.0	2.0	2.4	2.9*	3.3*	3.2*	3.2*
standard error	±.2	±.2	±.2	±.3	±.3	±.4	±.3	±.3
Plasma Protein (grams %)		3.5			3.8			3.9
		4.4			4.0			3.7
		4.1			4.3			4.2
		4.4			4.4			3.4
		3.8			3.8			3.7
		4.6			4.8			4.5
means		4.1			4.2			3.9
standard error		±.2			±.2			±.1

Table A11.--Continued.

Time (minutes)	Control		Infusion Period					
	-10	0	10	20	30	40	50	60
Hematocrit		43			44			44
		32			34			34
		39			42			44
		34			35			36
		36			37			39
		42			42			44
means		38			39			40
standard error		±1			±1			±1

* = $p \leq 0.01$ relative to zero time.

† = $p \leq 0.05$ relative to zero time.

Table A12.--Effects of bradykinin (0.8 mcg/min) and norepinephrine base (4 mcg/min) infused intra-arterially at constant inflow for 60 minutes on lymph flow, protein concentration of lymph and plasma, vascular pressures and hematocrit (n=6).

Time (minutes)	Control		Infusion Period					
	-10	0	10	20	30	40	50	60
Systemic Arterial Blood Pressure (mm Hg)	105	105	107	105	105	100	95	90
	117	118	130	135	125	130	135	135
	100	100	130	120	115	107	110	110
	107	105	130	120	115	115	117	117
	130	130	165	155	152	152	150	152
	120	120	155	145	145	140	140	135
means	113	113	136*	130*	126*	124*	125*	123*
standard error	±6	±6	±5	±6	±6	±7	±7	±7
Perfusion Pressure (mm Hg)	105	105	150	155	175	175	175	165
	110	110	150	165	165	170	175	180
	90	90	125	135	145	140	145	145
	107	105	145	140	135	145	155	160
	125	125	185	175	185	200	195	205
	115	115	185	210	215	210	210	210
means	109	108	157*	163*	170*	173*	176*	178*
standard error	±6	±6	±7	±8	±8	±7	±7	±7

Table A12.--Continued.

Time (minutes)	Control		Infusion Period					
	-10	0	10	20	30	40	50	60
Lymph Total Protein (grams %)	1.9	2.0	2.0	2.3	2.4	2.2	2.2	2.3
	1.2	1.3	1.9	1.5	2.3	2.5	3.0	2.9
	1.4	1.7	1.8	1.6	2.1	1.9	2.1	2.3
	1.9	1.9	1.9	2.1	1.7	1.9	1.9	2.4
	2.6	2.6	2.2	2.2	2.1	2.0	1.9	2.1
	2.1	1.8	2.0	2.9	2.8	2.7	2.5	2.4
means	1.9	1.9	2.0	2.1	2.2	2.2	2.3	2.4†
standard error	±.4	±.4	±.5	±.5	±.6	±.5	±.6	±.6
Plasma Protein (grams %)		5.3			5.0			5.1
		5.2			7.9			4.4
		4.1			3.8			4.2
		4.4			4.6			4.8
		4.5			4.9			5.1
		5.7			5.4			5.4
means		4.9			5.3			4.8
standard error		±.4			±.7			±.5

Table A12.--Continued.

Time (minutes)	Control		Infusion Period					
	-10	0	10	20	30	40	50	60
Skin Small Vein Pressure (mm Hg)	13	11	18	19	17	16	16	16
	10	10	12	13	12	12	12	12
	12	12	24	20	18	17	17	16
	9	9	20	22	35	31	16	16
	13	11	17	17	19	21	20	20
	12	12	20	19	18	17	17	17
means	12	11	19*	18*	19*	19*	16†	16†
standard error	±1	±1	±2	±1	±2	±2	±2	±2
Lymph Flow Rate (ml/10 min)	.01	.01	.03	.05	.05	.03	.03	.03
	.02	.02	.02	.03	.05	.05	.04	.04
	.01	.01	.02	.01	.01	.02	.02	.03
	.01	.01	.01	.01	.01	.01	.01	.01
	.01	.01	.02	.03	.03	.03	.04	.06
	.02	.02	.03	.04	.04	.04	.03	.03
means	.01	.01	.02	.03*	.03*	.03*	.03*	.03*
standard error	±0	±0	±0	±0	±.01	±.01	±.01	±.01

Table A12.--Continued.

Time (minutes)	Control		Infusion Period					
	-10	0	10	20	30	40	50	60
Hematocrit		39			40			40
		34			35			36
		37			39			41
		44			51			54
		40			41			41
		41			48			50
means		39			42†			44*
standard error		±2			±2			±3

* = $p \leq 0.01$ relative to zero time.

† = $p \leq 0.05$ relative to zero time.

BIBLIOGRAPHY

BIBLIOGRAPHY

1. Alabaster, V. A. and Y. S. Bakhle. Converting enzyme and bradykininase in the lung. Cir. Res. Supp. II. 31:72-84, 1972.

2. Allwood, M. J. and G. P. Lewis. Bradykinin and forearm blood flow. J. Physiol. 170:571-581, 1964.

3. Appelgren, L. and D. H. Lewis. Capillary permeability-surface area product (PS) of Renkin in human skeletal muscle. Acta. Med. Scand. 184:281-282, 1968.

4. Berne, R. M. and M. N. Levy. Cardiovascular Physiology. The C. V. Mosby Co., Saint Louis, 1972.

5. Boissonnas, R. A., J. Pless and E. Sandrin. The synthesis of bradykinin and of related peptides. Ann. N.Y. Acad. Sci. 104:5, 1963.

6. Carter, R. D., W. L. Joyner and E. M. Renkin. Effects of histamine and some other substances on molecular selectivity of the capillary wall to plasma proteins and dextran. Microvas. Res. 7:31-48, 1974.

7. Clough, G., P. A. Fraser and L. H. Smoje. Compliance measurement in single capillaries of the cat mesentery. J. Physiol. 240:9P, 1974.

8. Daugherty, R. N., Jr., J. B. Scott, T. E. Emerson, Jr. and F. J. Haddy. Comparison of iv and ia infusion of vasoactive agents on dog forelimb blood flow. Amer. J. Physiol. 214:611-619, 1968.

9. Deyrup, I. J. Circulatory changes following the subcutaneous injection of histamine in dogs. Amer. J. Physiol. 142: 158-172, 1944.

10. Diana, J. N., R. Colantino and F. J. Haddy. Transcapillary fluid movement during vasopressin and bradykinin infusion. Amer. J. Physiol. 212:456, 1967.

11. Douglas, W. W. Polypeptides--Angiotensin, plasma kinins, and other vasoactive agents; prostaglandins. In: The Pharmacological Basis of Therapeutics, edited by L. S. Goodman and A. Gilman, MacMillan Publishing Co. Inc., New York, 1975.

12. Elliott, D. F., E. W. Horton and G. P. Lewis. Actions of pure bradykinin. J. Physiol. 153:473-480, 1960.

13. Elliott, D. F., G. P. Lewis and E. W. Horton. The isolation of bradykinin: A plasma kinin from ox blood. Biochem. J. 74:15, 1960.

14. Emerson, T. E., Jr. Changes of venous return and other hemodynamic parameters during bradykinin infusion. Amer. J. Physiol. 212:1455, 1967.

15. Erdös, E. G. and I. Miwa. Effect of endotoxin shock on the plasma kallikrein-kinin system of the rabbit. Fed. Proc. 27: 92-95, 1968.

16. Erspamer, V. and A. Glaesser. The action of eledoisin on the systemic arterial blood pressure of some experimental animals. Brit. J. Pharmacol. 20:516-527, 1963.

17. Friedli, B., G. Kent and P. M. Olley. Inactivation of bradykinin in the pulmonary vascular bed of newborn and fetal lambs. Cir. Res. 33:421-427, 1973.

18. Fung, Y. C., B. W. Zweifach and M. Intaglietta. Elastic environment of the capillary bed. Cir. Res. 19:441-461, 1966.

19. Gabbiani, G., M. C. Badonnel and G. Majno. Intra-arterial injections of histamine, serotonin, or bradykinin: a topographic study of vascular leakage. Proc. Soc. Exp. Biol. and Med. 135:447-452, 1970.

20. Garlick, D. G. and E. M. Renkin. Transport of large molecules from plasma to interstitial fluid and lymph in dogs. Amer. J. Physiol. 219:1595-1605, 1970.

21. Grega, G. J., R. L. Kline, D. E. Dobbins and F. J. Haddy. Mechanisms of edema formation by histamine administered locally into canine forelimbs. Amer. J. Physiol. 223:1165-1171, 1972.

22. Guyton, A. C. Medical Physiology. W. B. Saunders Company, Philadelphia, 1971.

23. Guyton, A. C., H. J. Granger and A. E. Taylor. Interstitial fluid pressure. Physiol. Rev. 51(3):527-563, 1971.

24. Haddy, F. J., T. E. Emerson, Jr., J. B. Scott and R. M. Daugherty, Jr. The effect of the kinins on the cardiovascular system. In: Handbook of Experimental Pharmacology, edited by E. G. Erdös, Springer Verlag, Berlin, 1970.

25. Haddy, F. J., J. B. Scott and G. J. Grega. Peripheral Circulation: fluid transfer across the microvascular membrane. Int. Rev. of Physiol. 9:63-109, 1976.

26. Harrison, D. C., W. L. Henry, B. Passo and H. A. Miller. Circulatory response to bradykinin before and after autonomic nervous system blockade. Amer. J. Physiol. 214:1035, 1968.

27. Innes, I. R. and M. Nickerson. Norepinephrine, epinephrine and the sympathomimetic amines. In: The Pharmacological Basis of Therapeutics, edited by L. S. Goodman and A. Gilman, MacMillan Publishing Co. Inc., New York, 1975.

28. Järhult, J. Comparative effects of angiotensin and noradrenaline on resistance, capacitance and precapillary sphincter vessels in cat skeletal muscle. Acta. Physiol. Scand. 81:315-324, 1971.

29. Joyner, W. L., R. D. Carter, G. S. Raizes and E. M. Renkin. Influence of histamine and some other substances on blood-lymph transport of plasma protein and dextran in the dog paw. Microvas. Res. 7:49-60, 1974.

30. Kahlson, G. and E. Rosengren. New approaches to the physiology of histamine. Physiol. Rev. 48:155-196, 1968.

31. Kline, R. L., D. P. Sak, F. J. Haddy and G. J. Grega. Pressure-dependent factors in edema formation in canine forelimbs. J. Phar. and Exper. Ther. 193:452-459, 1975.

32. Kline, R. L., J. B. Scott, F. J. Haddy and G. J. Grega. Mechanisms of edema formation in canine forelimbs by locally administered bradykinin. Amer. J. Physiol. 225:105, 1973.

33. Landis, E. M. and J. R. Pappenheimer. Exchange of substances through the capillary walls. Handbook of Physiology, Circulation, Section 2, Vol. 2, American Physiological Society, Washington, D.C., p. 961, 1963.

34. Lewis, G. P. Plasma kinins and other vasoactive compounds in acute inflammation. Ann. N.Y. Acad. Sci. 116:847, 1964.

35. Majno, G., S. M. Shea and M. Leventhal. Endothelial contraction induced by histamine-type mediators. J. Cell Biol. 42:647-672, 1969.

36. Marciniak, D. L., D. E. Dobbins, J. J. Maciejko, J. B. Scott, F. J. Haddy and G. J. Grega. Antagonism of histamine edema formation by norepinephrine. Amer. J. Physiol. (Submitted for publication).

37. Marciniak, D. L., D. E. Dobbins, J. J. Maciejko, J. B. Scott, F. J. Haddy and G. J. Grega. Effects of systemically infused histamine on transvascular fluid and protein transfer. Amer. J. Physiol. (Submitted for publication).

38. Mellander, S. and I. Nordenfelt. Comparative effects of dihydroergotamine and noradrenaline on resistance, exchange and capacitance functions in the peripheral circulation. Clin. Sci. 39:183-201, 1970.

39. Miller, M. E., G. C. Christensen and H. E. Evans. Anatomy of the Dog. W. B. Saunders Company, Philadelphia, p. 432, 1964.

40. Milnor, W. R. Capillaries and lymphatic vessels. In: Medical Physiology, edited by V. B. Mountcastle, The C. V. Mosby Company, Saint Louis, 1974.

41. Nies, A. S., R. P. Forsyth, H. E. Williams and K. L. Melmon. Contribution of kinins to endotoxin shock in unanesthetized rhesus monkeys. Cir. Res. 22:155-164, 1968.

42. Parratt, J. R. A comparison of the effects of the plasma kinins, bradykinin and kallidin, on myocardial blood flow and metabolism. Brit. J. Pharmacol. 22:34-46, 1964.

43. Pitts, R. F. Physiology of the Kidney and Body Fluids. Yearbook Medical Publishers, Incorporated, Chicago, 1974.

44. Renkin, E. M., R. D. Carter and W. L. Joyner. Mechanism of the sustained action of histamine and bradykinin on transport of large molecules across capillary walls in the dog paw. Microvas. Res. 7:49-60, 1974.

45. Renkin, E. M. and D. G. Garlick. Blood-lymph transport of macromolecules. Microvas. Res. 2:392-398, 1970.

46. Rocha e Silva, M. Chemical mediators of the acute inflammatory reaction. Ann. N.Y. Acad. Sci. 116:899, 1964.

47. Rocha e Silva, M., W. T. Beraldo and G. Rosenfeld. Bradykinin, a hypotensive and smooth muscle stimulating factor released from plasma globulin by snake venoms and by trypsin. Amer. J. Physiol. 156:261-273, 1949.

48. Rocha e Silva, M. and J. G. Leme. Chemical Mediators of the Acute Inflammatory Reaction. Pergamon Press, New York, 1972.

49. Rosenberg, J. G., R. C. Lillehei, J. Longerbeam and B. Zimmermann. Studies on hemorrhagic and endotoxin shock in relation to vasomotor changes and endogenous circulating epinephrine, norepinephrine and serotonin. Ann. Surg. 154:611-627, 1961.

50. Rowe, G. G., A. Afonso, C. A. Castillo, F. Lioy, J. E. Lugo and C. W. Crumpton. The systemic and coronary hemodynamic effects of synthetic bradykinin. Amer. Heart J. 65:656, 1963.

51. Rowley, D. A. Venous constriction as the cause of increased vascular permeability produced by 5-hydroxy-tryptamine, histamine, bradykinin and 48/80 in the rat. Brit. J. Path. 45:56, 1964.

52. Shirley, H. H., C. G. Wolfram, K. Wasserman and H. S. Mayerson. Capillary permeability to macromolecules: stretched pore phenomenon. Amer. J. Physiol. 190:198-193, 1957.

53. Sokal, R. R. and F. J. Rohlf. Biometry. Freeman, San Francisco, 1969.

54. Starling, E. H. On the absorption of fluid from the connective spaces. J. Physiol. (London) 19:312-326, 1896.

55. Vogel, R. and G. Zickgraf-Rüdel. Evaluation of the role of kinins in experimental pathological and clinical conditions; the therapeutic use of kallikrein inhibitor. In: Handbook of Experimental Pharmacology, edited by E. G. Erdös, Springer Verlag, New York, 1970.

56. Waddell, W. J. A simple ultraviolet spectrophotometric method for the determination of protein. J. Lab. Clin. Med. 48:311-314, 1956.

57. Werle, E. Discovery of the most important kallikreins and kallikrein inhibitors. In: Handbook of Experimental Pharmacology, edited by E. G. Erdös, Springer Verlag, New York, 1970.

58. Wiederheilm, C. A. The interstitial space and lymphatic pressures in the bat wing. In: Pulmonary Circulation and Interstitial Space, edited by A. P. Fishman and H. H. Hecht, University of Chicago Press, Chicago, 1969.

59. Wilhelm, D. L. Kinins in human disease. A. Rev. Med. 22:63-84, 1971.

60. Zachariea, H., S. Henningsen, J. Sondergaard and P. Wolf-Jurgensen. Plasma kinins in inflammation--relation to other mediators and leukocytes. Scand. J. Clin. Lab. Invest. Suppl. 107: 85-94, 1969.