Volume 1

The Physiology and Pharmacology of the Microcirculation

Edited by

NICHOLAS A. MORTILLARO

PHYSIOLOGIC AND PHARMACOLOGIC BASES OF DRUG THERAPY

Physiologic and Pharmacologic Bases of Drug Therapy

Series Editors

Stanley Greenberg
and
Thomas M. Glenn

Department of Pharmacology
College of Medicine
University of South Alabama
Mobile, Alabama

Gene C. Palmer (Editor) Neuropharmacology of Central Nervous System and Behavioral Disorders, 1981

R. Douglas Wilkerson (Editor) Cardiac Pharmacology, 1982

Nicholas A. Mortillaro (Editor) The Physiology and Pharmacology of the Microcirculation, Volume 1, 1983

The Physiology and Pharmacology of the Microcirculation

VOLUME 1

EDITED BY

Nicholas A. Mortillaro

Department of Physiology
College of Medicine
University of South Alabama
Mobile, Alabama

1983

Academic Press

A Subsidiary of Harcourt Brace Jovanovich, Publishers
New York London
Paris San Diego San Francisco São Paulo Sydney Tokyo Toronto

ACADEMIC PRESS, INC.
111 Fifth Avenue, New York, New York 10003

United Kingdom Edition published by
ACADEMIC PRESS, INC. (LONDON) LTD.
24/28 Oval Road, London NW1 7DX

Library of Congress Cataloging in Publication Data

Main entry under title:

The Physiology and pharmacology of the microcirculation.

(Physiologic and pharmacologic bases of drug therapy)
Includes index.
1. Microcirculation. 2. Capillaries--Effect of drugs on. I. Mortillaro, Nicholas A. II. Series.
QP106.6.P48 1982 612'.135 82-20562
ISBN 0-12-508301-7 (v.1)

PRINTED IN THE UNITED STATES OF AMERICA

83 84 85 86 9 8 7 6 5 4 3 2 1

To my wife Mildred, daughter Susan, and son Philip

2 Regulation of Vascular Smooth Muscle of the Microcirculation

Stan Greenberg
Frederick A. Curro
Toshiki P. Tanaka

THE PHYSIOLOGY AND PHARMACOLOGY OF THE MICROCIRCULATION, VOLUME 1

ISBN 0-12-508301-7

I. Introduction

The final common macromolecular factor regulating both arterial pressure and fluid homeostasis at the tissue level is the vascular smooth muscle (VSM) of the microcirculation. Although the VSM is modulated by the level of circulating humoral factors, the central and peripheral nervous systems, and endogenous local factors such as platelets, leukocytes, and mast cells, with their inherent vasoactive products, it is the ultimate capacity of the VSM to respond to these factors with either contraction or relaxation that determines the hemodynamic events resulting from these modulating factors. Despite the plethora of information on VSM function in large arteries and veins, little information is available on the pharmacologic and humoral responsiveness of the microcirculation (Mellander and Johansson, 1968; Vanhoutte, 1978; Kaley and Altura, 1978; Altura, 1978a,b, 1981; Bohr *et al.*, 1978). Information on microvascular mechanisms of contraction and relaxation is almost nonexistent, although recent studies have attempted to investigate receptor mechanisms in microvessels from brain (Estrada and Krause, 1982; Gerritsen, 1982) and other organs. The specific aims of this chapter are to provide the reader interested in VSM and the microcirculation with the current knowledge relating to (*a*) the ultrastructural characteristics of the VSM, (*b*) the innervation of the VSM of the microcirculation, (*c*) the role of the endothelium as a modulator of VSM function, (*d*) the structural and functional basis of VSM contraction and relaxation, and (*e*) the effects of vasoactive endogenous and pharmacologic substances on the VSM and the mechanisms by which these substances alter vascular resistance.

Although this chapter cannot be encyclopedic either in providing definitive answers to each of the problems or in covering the entire pharmacologic armamentarium of therapeutic modalities, it is hoped that the information presented will serve as a base from which young investigators in the field will initiate questions and the experimental techniques from which the answers will arise.

II. Vascular Smooth Muscle in the Microcirculation

A. Ultrastructural Characteristics of Vascular Smooth Muscle

Rhodin (1974, 1980) and Majno (1965) stressed that "microcirculation" is the collective name for the smallest components of the cardiovascular channels, the arterioles, capillaries, and venules, each with its own characteristic structure and function. Because each organ has a unique microcirculation, knowledge of the macroanatomy of the microcirculation of one vascular bed does not imply knowledge of the macroarchitectural arrangement of others (Rhodin, 1981). Thus, the generalized discussion in this chapter tries to encompass the major characteristics of most vascular beds, with individualities delineated in the discussions of specific microcirculations within this volume. Figure 1 shows the general architecture of the systemic vascular tree of a mammalian organism.

Arterioles are essentially small arteries [<0.5 mm outer diameter (OD)] with at least two layers of smooth muscle cells. The smallest arterioles contain one layer of smooth muscle cells or several cells scattered widely apart within the vessel wall (Rhodin, 1980, 1981). Both arterioles and precapillary sphincters contain an inner lining of endothelial cells, which maintain the fluid integrity of the vessel by means of "tight junctions." Myoendothelial junctions penetrate the connective tissue basal lamina and separate the smooth muscle from the endothelial cells in arterioles from many organs (Williamson *et al.*, 1969). These myoendothelial junctions appear to serve as a site for circulating humoral factors to reach the smooth muscle from the vascular space. Within the connective tissue of the arteriolar wall nonmyelinated nerve terminals impinge on the VSM cells to form the myoneural or neuroeffector junction. It is from the nodular terminal varicosities that neurotransmitter is released, diffuses across the synaptic cleft, and interacts with the appropriate receptors on the VSM cell to produce vasoconstriction or vasodilation.

The capillaries (5 μm OD) are tubes joined by flat endothelial cells and by junctional areas, predominantly tight junctions (Rhodin, 1980, 1981). These are discussed elsewhere in this volume. However, some capillaries are surrounded by precapillary cells called pericytes (Weibel, 1974), which have their own basal lamina. These pericytes may have the capacity to contract and therefore could alter capillary blood flow. The endothelial cells of the capillaries contain actin and perhaps myosin filaments and may exhibit active contraction. Simionescu *et al.* (1982) showed that endothelial cells from large veins contracted in response to histamine.

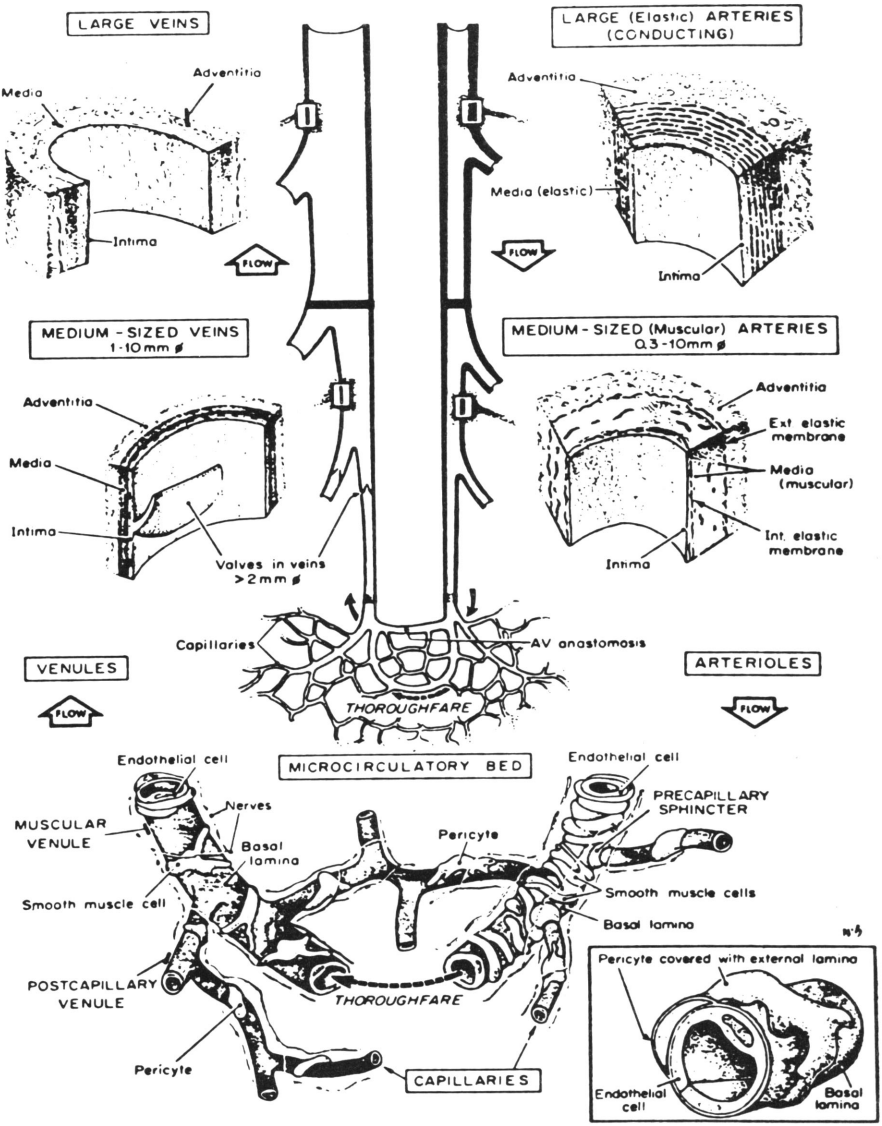

Fig. 1. Schematic drawing summarizing major characteristics of principal segments of blood vessels of mammals. (From Rhodin, 1980, with permission.)

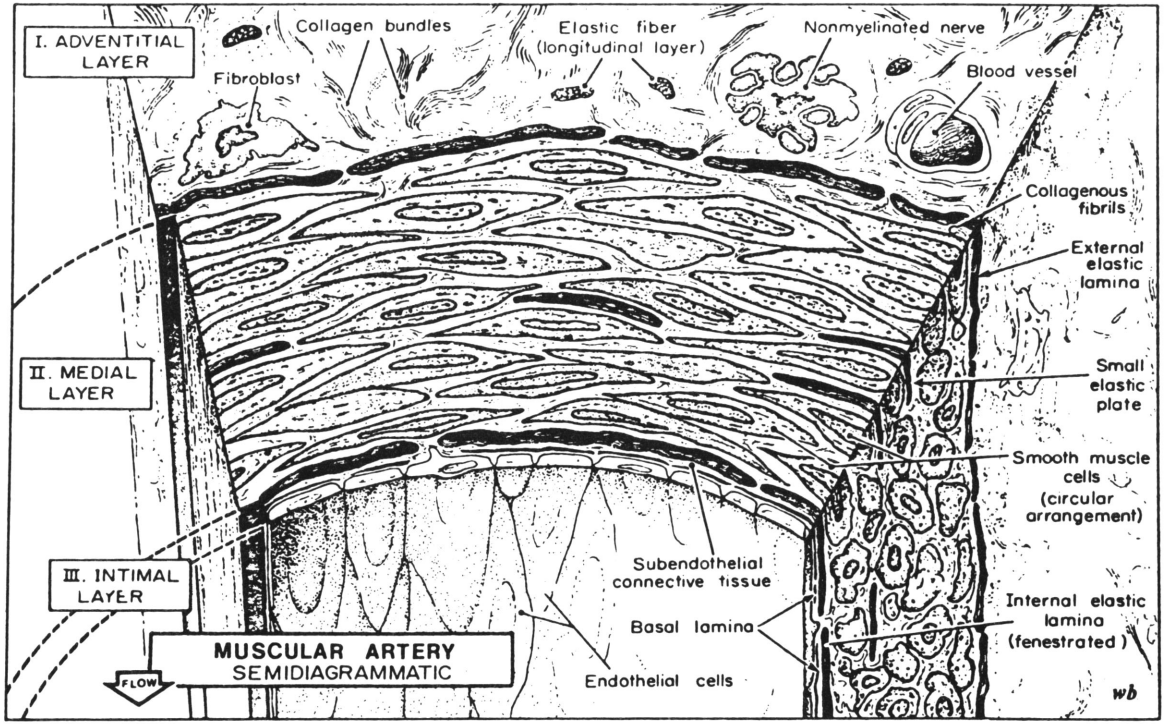

Fig. 2. Summary of the major components of the wall of a muscular artery. (From Rhodin, 1980, with permission.)

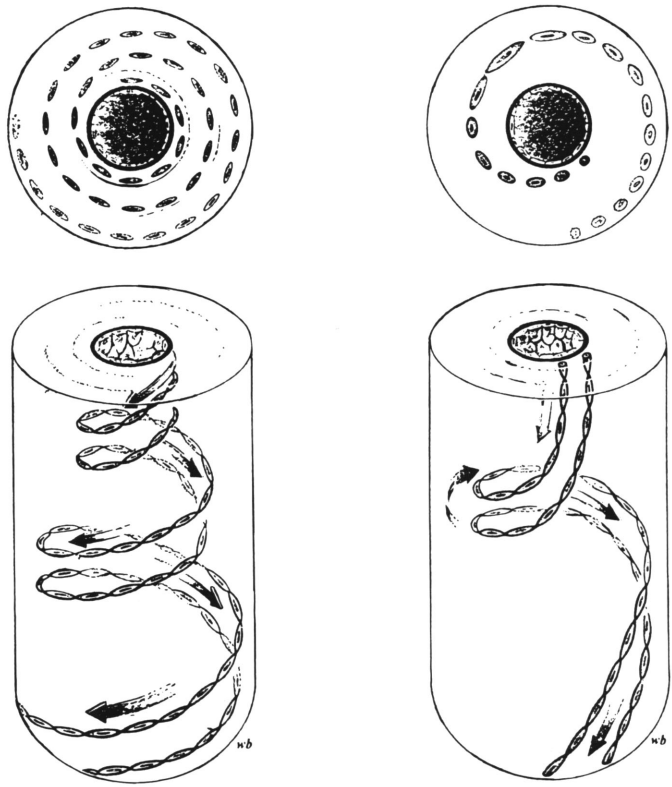

Fig. 3. Most likely arrangement of smooth muscle in most arterioles (left) and in some bovine vessels (right). (From Rhodin, 1980, with permission.)

These contractions, much slower than those of the VSM, may modulate microcirculatory VSM tone or, more likely, the opening and closing of "pores" in the endothelial spaces. According to Rhodin (1968) arterial capillaries lie closer to the arterioles than the venules and are endowed with a continuous endothelium. Venous capillaries are endowed with a fenestrated endothelium and lie closer to the venules than the arterioles. This arrangement would be consistent with a mechanism for hemodynamic mediation of fluid transfer.

Figures 4 and 5 show the five basic venules in the microcirculation. The endothelium of the venular capillaries is continuous with the endothelium of the venules up to the collecting veins. All endothelial cells have a continuous cytoplasm with a variable number of cytoplasmic vesicles (Rhodin, 1981). The tight junction of the venous endothelium is "looser" than that of the arteriolar and capillary endothelium, and the junctional regions

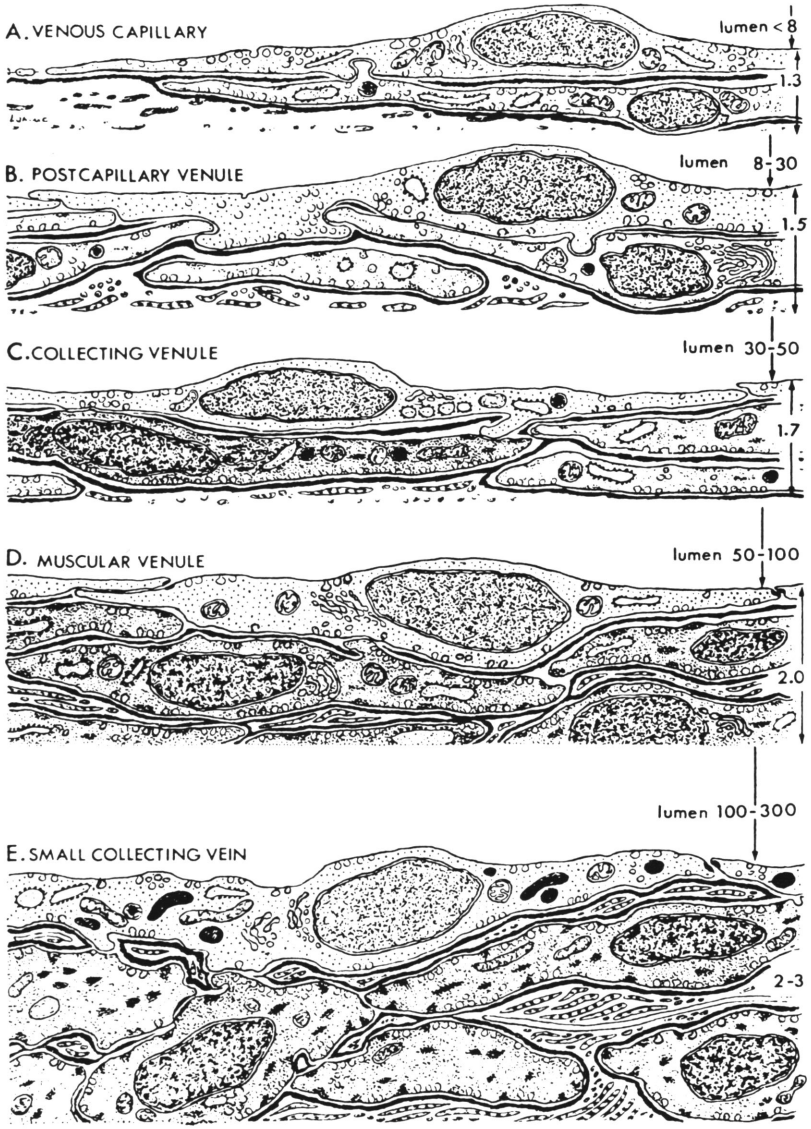

Fig. 4. Summary of transition of venous segments in microcirculation. Lumen measurements in micrometers. (From Rhodin, 1968, with permission.)

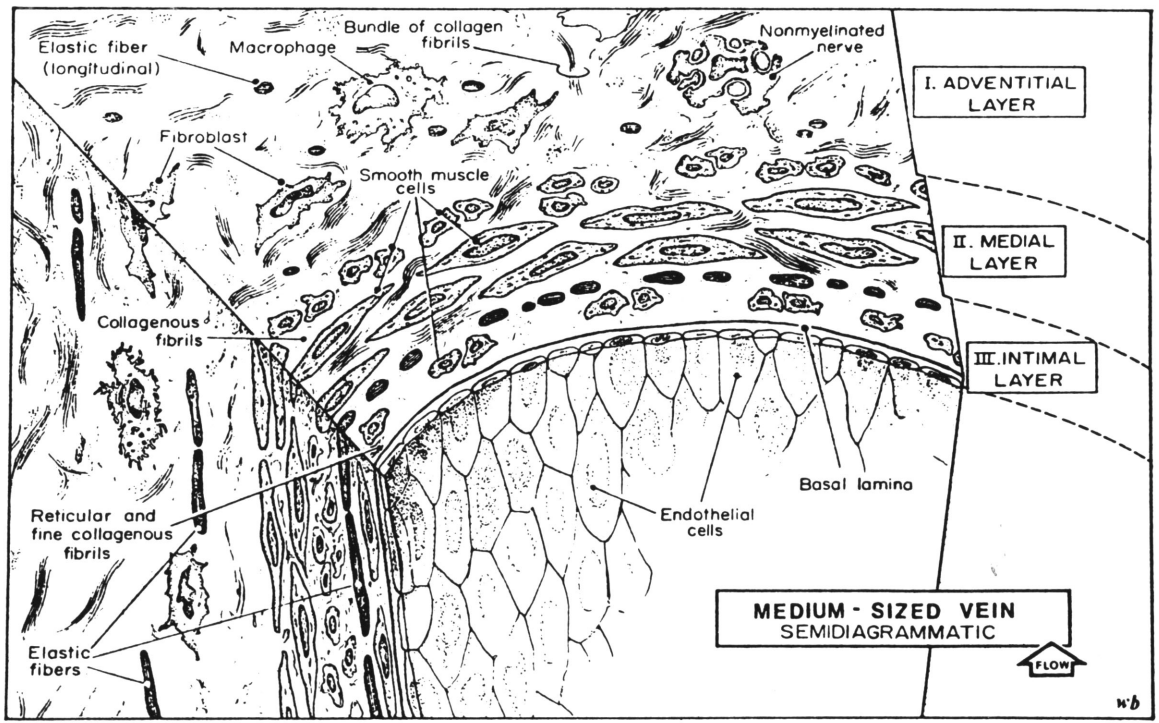

Fig. 5. Major components of wall of medium-sized vein. (From Rhodin, 1980, with permission.)

can open up the intercellular clefts when stimulated with histamine and 5-hydroxytryptamine (5-HT). According to Rhodin (1980, 1981) this precedes leukocyte migration into the portion of the vein in which the damage has occurred. A discontinuous layer of pericytes surrounds the postcapillary venules and then gives rise to layers of venous smooth muscle cells, which are of a lower number and density than those of the arterioles. The venules are also innervated primarily with an adrenergic innervation, and release of transmitter results in slower and less powerful contractions of the venules.

In small arterioles the muscle cells are elongated, fusiform with tapering ends, generally about 5 μm in diameter, and about 20 μm in length with an irregular profile, thus providing a high surface-to-volume ratio (Gabella, 1981). The cell membrane is trilaminar and of uniform thickness. Electron-dense bands of inpocketings, known as caveolae, surface vesicles, or plasmalemmal vesicles, are grouped in rows, two to four caveolae wide, parallel to the long axis of the cell. More than 33% of the plasma membrane at the cell surface forms caveolae, which are continuous with the extracellular space. Popescu (1974) suggested that these caveolae may be involved in the transmembrane transport of calcium ion across the plasma membrane. The cell membrane is coated by a basal lamina and is involved in the formation of cell-to-cell junctions (Gabella, 1981).

Bands of electron-dense material approximately 300–400 μm reinforce the cell membrane on the cytoplasmic side in VSM. These dense bands project into the cytoplasm in the form of wedge-shaped protrusions and are opposed to the fibrous material of VSM and the extracellular space. It has been suggested that these dense bands may serve as a mechanical link between the plasma membrane and the collagen fibrils of VSM. However, this remains questionable (Gabella, 1981). The sarcoplasmic reticulum of VSM is abundantly present beneath and parallel to the cell membrane, near the nuclear poles where Golgi and mitochondria are also present, and among the myofilaments. The function of the sarcoplasmic reticulum is still poorly understood. The granular endoplasmic reticulum is probably involved in protein synthesis, whereas the smooth sarcoplasmic reticulum is involved in the regulation of ion concentration, notably that of calcium. Mitochondria, the energy packets of the cell, are localized primarily in the regions of the cells near the poles of the nuclei, whereas some are scattered near the cellular membrane and sarcoplasmic reticulum. The Golgi apparatus is also usually situated in the region of the nuclear poles and is associated with the rough endoplasmic reticulum. The Golgi, as in all cells, is associated with protein synthesis in VSM. Although microtubules exist in VSM, no function has been assigned to them. Smooth muscle

cells may be associated with phagocytitic activity, and it is not incidental that VSM contains lysosomes.

The VSM contains thick, thin, and intermediate-sized filaments. The thick filaments may be helically arranged, as in skeletal muscle, and are found in hexameric units arranged in a helical conformation with a 72-nm periodicity. The cells of VSM also contain thin filaments of actin, usually arranged in hexagonal packing, which normally extend for several micrometers along the cell length and then branch and divide. The thin filaments also appear to be tropomyosin. The native tropomyosin can be separated into tropomyosin and leiotonin, which may serve the same function as troponin, in VSM. The intermediate filaments are approximately 10 nm in diameter and are localized in the proximity of dense bodies, the cell surface, and dense bands. Although the intermediate filaments do not appear to penetrate the dense bodies, it has been suggested that these filaments form the cytoskeleton of the smooth muscle cell, which gives support to the myofilaments. In this regard, the dense bodies of VSM, which run parallel to the myofilaments and are at times continuous with the dense bands, have insertions with thin filaments (Gabella, 1981; Ashton *et al.*, 1975). It has also been suggested that these dense bodies and dense bands correspond to the Z bands of skeletal muscle, thus making the entire VSM cell the sarcomeric unit of contraction. According to Gabella (1981), "The distribution of myofilaments and the occurrence of dense bands—instead of Z lines—probably accounts for the remarkable amount of shortening a smooth muscle cell can undergo. It may also account for the wide changes in shape of the cell transverse profile."

Smooth muscle cells are usually separated from each other by 100-nm gaps containing collagen and elastin fibrils and extracellular matrix material. However, regions of opposition of smooth muscle cells occur (Fig. 6 and 7). Tight junctions appear to exist in some VSM in the circle of Willis (Tani *et al.*, 1977). Nexuses or gap junctions exist in most VSM and are characteristic of the gap junctions found in most cells (for review see Henderson, 1975). The nexuses appear to be the site of electrical activity propagated from one cell to another. A typical smooth muscle cell is presented in Fig. 8.

B. Innervation of the Microcirculation

Drugs and humoral substances may affect microvascular smooth muscle either directly by altering the level of VSM tone, or indirectly by interfering with neurotransmission to the microcirculation. Table I summarizes

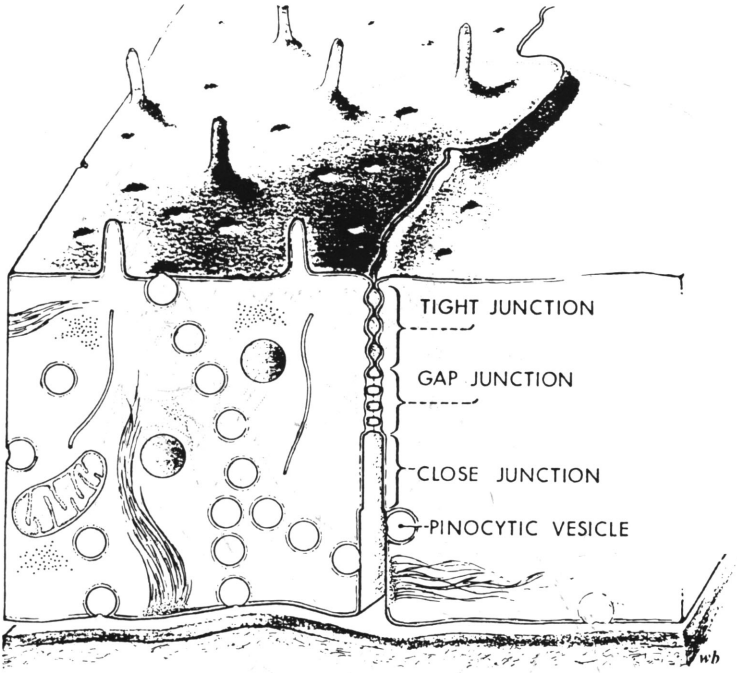

Fig. 6. Summary of general appearance of several types of cell junctions. Pinocytic vesicle: 500–600 Å. Junctions: tight (zonula occludens), punctate fusions; gap (macula communicans), gap = 20 Å, polygonal lattice of subunits; close, gap = 40 Å. Markers for molecules with ϕ (in Å) = 5 (ionic lanthanum); 20 (colloidal lanthanum); 17–20 (heme peptides); 25 × 34 × 42 (myoglobin); 50 (horseradish peroxidase); 45–200 (dextrans); 110 (ferritin). (From Rhodin, 1980, with permission.)

the density of innervation of the microcirculation as determined by histochemical fluorescence measurement of adrenergic nerve density and electron microscopic evidence of the presence of adrenergic nerves. The precapillary sphincters and collecting venules appear to be devoid of adrenergic innervation, whereas the small veins, arterioles, and terminal arterioles appear to be richly endowed with adrenergic varicosities. There is little evidence for a cholinergic innervation of the microcirculation, with the exception of the brain (D'Alecly and Rose, 1977; Heistad and Marcus, 1978) and the kidney. However, microvascular smooth muscle in the systemic and peripheral circulations appears to be endowed with both cholinergic and adrenergic receptors (Estrada and Krause, 1982; Altura, 1978a,b, 1981). Kadowitz and Hyman (1973) and Kadowitz *et al.* (1975a,b, 1976, 1981) have shown that the pulmonary circulation of many

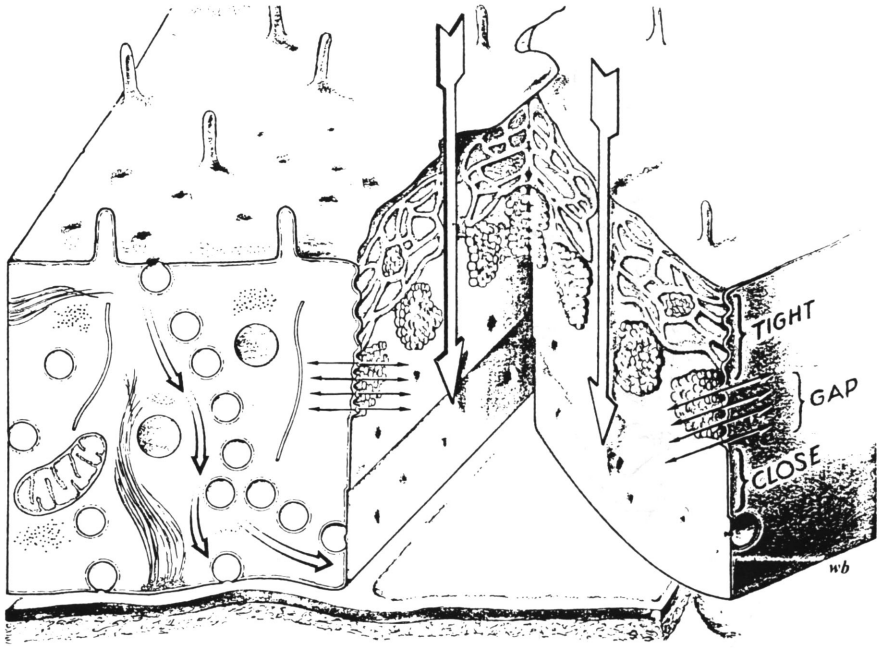

Fig. 7. Summary of general appearance of several types of cell junctions as seen from freeze-fracture segments. (From Rhodin, 1980, with permission.)

species responds to stimulation of both parasympathetic and adrenergic nerves and that the small blood vessels are innervated with both adrenergic and cholinergic varicosities. However, the size of the vessels at which the innervation disappears has not been determined. In contrast, Su *et al.* (1978) and Su and Bevan (1976) measured the contractile responses of small pulmonary arteries from rabbits to stimulation of adrenergic nerves and showed that the response to nerve stimulation disappears in vessels between 200 and 400 μm (OD). This would suggest that terminal arterioles of the pulmonary circulation may not be richly innervated with adrenergic nerve fibers.

Assuming the adrenergic innervation in the macrocirculation and that in the microcirculation are similar, then the factors that control the release of neurotransmitter should also be similar. Tables II and III summarize their purported mechanism of action. When such drugs as guanethidine, bretylium, and guanacline inhibit the release of norepinephrine (NE) from adrenergic nerves innervating the microcirculation, the more densely innervated microcirculatory components should exhibit the greatest decrease in VSM tone. Similar changes in the microcirculation should be ob-

Fig. 8. Typical smooth muscle cell from abdominal aorta of squirrel monkey. (From Rhodin, 1980, with permission.)

TABLE I
Density of Adrenergic Innervation in Splanchnic and Skeletal Muscle Microvessels[a]

Microvessel	Evidence[b]	
	Electron micrographic	Histochemical
Small muscular arteries	++++	++++
Arterioles	++++	+++
Terminal arterioles	++	++
Precapillary sphincters	±	−
Collecting venules	−	−
Muscular venules	±	±
Small veins	++	++

[a] From Altura (1978a,b) with permission.
[b] +, Degree of innervation; −, lack of innervation.

served with the α-adrenoceptor antagonists and agents such as reserpine, which prevents the retention of NE in the granular pool of NE, and 6-hydroxydopamine (6-OHDA), which causes degeneration of adrenergic nerves. When these compounds are administered, arterioles and terminal arterioles relax or dilate, whereas muscular venules do not exhibit an in-

TABLE II
Influence of Drugs that Affect Tissue Levels of Catecholamines on Reactivity of Mesenteric Arterioles and Muscular Venules to Exogenously Applied Constrictor Doses of Norepinephrine[a,b]

Drug	Dose	Microvessel and response to NE	
		Arterioles (20–28 μm)	Muscular venules (34–35 μm)
Procaine	10^{-4}–10^{-5} M[c]	Potentiated	No effect[e]
Cocaine	10^{-4}–10^{-5} M	Potentiated	No effect[e]
Bretylium	5–10 mg/kg[d]	Potentiated	No effect[e]
Guanethidine	5–10 mg/kg	Potentiated	No effect[e]
Reserpine	3–5 mg/kg	Potentiated	No effect[e]

[a] From Altura (1978a,b) with permission.
[b] ED_{30} doses of norepinephrine (NE) were applied topically before and after administration of the drugs.
[c] Procaine and cocaine were superfused on the mesenteric vessels for 30–45 min.
[d] Bretylium, guanethidine, and reserpine were administered acutely intravenously 20–45 min before a challenge to control ED_{30} doses of NE.
[e] Signifies that the constrictor responses to NE were equivalent to those before drug administration.

TABLE III

Effects of Inhibitors of Catecholamine Degradation on Responsiveness of Rat Mesenteric Arterioles and Venules to Exogenously Applied Norepinephrine[a,b]

Inhibitor[c]	Dose[d] (mg/kg)	Arterioles (21–28 μm)	Venules (36–45 μm)
MAO inhibitor			
Tranylcypromine	5–15	Potentiated	No effect[e]
Iproniazid	150–300	Potentiated	No effect[e]
COMT inhibitor			
Pyrogallol	150–300	Potentiated	Potentiated

[a] From Altura (1978a,b) with permission.

[b] ED_{30} doses of norepinephrine (NE) were applied topically before and after administration of the inhibitors.

[c] MAO, Monoamine oxidase; COMT, catechol *O*-methyltransferase.

[d] Inhibitors were administered 30–60 min before challenge with control ED_{30} doses of NE.

[e] Signifies that the constrictor responses to NE were equivalent to those before administration of the MAO inhibitors.

crease in diameter (Altura and Hershey, 1967; Altura, 1971a, 1978a,b, 1981). These findings provide pharmacologic evidence supporting the lack of innervation of venules in the microcirculation.

This conclusion is also supported by studies evaluating the effects of drugs that release NE from adrenergic nerves (indirectly acting sympathomimetic amines) on microvascular reactivity. Norepinephrine, epinephrine, and the selective α_1-receptor agonist phenylephrine act directly on VSM to elicit contraction, whereas tyramine, ephedrine, and amphetamine are indirectly acting sympathomimetic amines. The directly acting receptor antagonists contract intact rat venules, whereas the indirectly acting sympathomimetic amines are devoid of this activity (Altura, 1978b). All these findings support the conclusion that small venules (30–60 μm OD) probably receive little or no adrenergic innervation.

Substances other than NE may be released from adrenergic nerves innervating the microvascular smooth muscle. There is sufficient evidence that purines may be coreleased with other transmitters from nerves or sites innervating VSM. Thus, adenosine triphosphate (ATP) and other purines may function as modulators of neurotransmission in VSM (Su, 1977; DeMey *et al.*, 1979; Moylan and Westfall, 1979; Levitt and Westfall, 1982). The purines may function as inhibitors of neurotransmission (Su, 1977), are released upon stimulation of adrenergic nerves (Su, 1975; Burnstock, 1976), have their release facilitated by NE and inhibited by α_1-receptor antagonists, α_2-receptor antagonists, α_2-receptor agonists, and 6-

OHDA (Levitt and Westfall, 1982), and cause relaxation of blood vessels with high tone. The purines may be derived from the NE storage vesicle, which releases its content of ATP, binding protein, and neurotransmitter by exocytosis (Smith and Winkler, 1972). However, experiments of Levitt and Westfall (1982) in which they destroyed the adrenergic varicosities with 6-OHDA and inhibited NE release by approximately 80% and purine release by approximately 60% suggest that the purine may be released from sites other than the NE-containing vesicle of adrenergic nerves or from nonadrenergic nerves. Further studies are necessary to evaluate the site and mechanism of purine nucleotide release and the significance of purinergic nerves in the microcirculation.

C. Mechanisms of Inactivation of Norepinephrine

Five potential mechanisms are involved in the termination of action of NE released from adrenergic nerves. Interference with these mechanisms can alter the microvascular effects of NE. Diffusion of NE from its receptor site and transport across the vascular wall to the vascular space accounts for one mechanism. Blood flow to the microvasculature is an important determinant of the elimination of NE by this mechanism. The NE released from adrenergic nerves may be taken back up into the nerve terminal by a sodium-dependent, ouabain-inhibitable, energy-dependent, cocaine-inhibitable, saturable, stereospecific transport mechanism (uptake$_1$). Once taken back into the nerve terminal, the NE is primarily deaminated by monoamine oxidase (MAO) and subsequently methylated by catechol *O*-methyltransferase (COMT), the lesser neuronal metabolic mechanism. In contrast, in the event of inhibition of uptake$_1$ or when the concentration of NE is exceedingly high, NE is taken up into the VSM by a sodium-dependent, saturable, stereospecific, hydrocortisone- and cholesterol-inhibitable mechanism (uptake$_2$). Once taken up into the VSM, NE is primarily subjected to the action of COMT with some metabolism via MAO. Finally, the binding of NE to collagen, elastin, and the extracellular matrix may play a role in terminating the action of NE in VSM (for references see reviews by Bevan, 1982; Langer *et al.*, 1981; Smith and Winkler, 1972).

The importance of each of these pathways in the microcirculation has been indirectly assessed by the administration of inhibitors of some of these systems and the evaluation of their effects on the responses to exogenous administration of NE. As summarized in Table III, inhibition of uptake$_1$ and MAO enhances the responses of both arterioles and venules to NE. These data also support the conclusion that the arterioles, but not the

venules, are innervated with adrenergic nerves and that MAO is the primary mechanism of NE inactivation. In the venules, uptake$_2$ and COMT are primarily involved in terminating the action of NE.

D. Endothelium as a Site of Humoral Factor Inactivation and Production

The endothelium is no longer thought to be a simple barrier to diffusion of substances across the vascular wall but a site for the synthesis and degradation of hormones and the transport of drugs, an active mediator of antithrombotic activity, and an obligatory factor in active vasodilation. This discussion focuses on the role of the endothelium in metabolism and generation of vasoactive agents and its obligatory role in vasodilation.

Norepinephrine, 5-HT (serotonin), and other biogenic amines are taken up into endothelial cells by carrier-mediated, saturable transport mechanisms apparently distinct for each amine (Hughes *et al.*, 1969; Strum and Junod, 1972; Ryan and Ryan, 1981). Histamine and epinephrine appear to be refractory to endothelial-mediated uptake. Once taken up into the endothelial cells, NE and 5-HT are metabolized and some of the metabolites are released into the vascular space (Junod, 1972a,b; Shepro *et al.*, 1975). Thus, drugs that compete for these transport processes may enhance the concentration of 5-HT and NE available to interact with the smooth muscle, whereas drugs that stimulate the uptake of these substances into the endothelium may decrease the action of 5-HT and NE on the microvasculature. Because imipramine, iproniazide, hydrocortisone, cholesterol, chlorpromazine, and amphetamine inhibit the endothelial uptake of 5-HT and NE into endothelial cells, some of the adverse effects attributed to these drugs may result from their capacity to compete with these biogenic amines for binding sites along the endothelial cell surface (Junod, 1972a,b; Orton *et al.*, 1975; Ryan and Ryan, 1981).

Adenosine is taken up by endothelial cells. This action is inhibited by dipyridamole, a coronary and peripheral vasodilator. Pearson *et al.* (1978) and Ryan and Ryan (1981) have suggested that some of the effects of dipyridamole may be related to its capacity to inhibit the endothelial uptake of adenosine. The enzyme involved in the degradation of adenine nucleotides, 5′-nucleotidase, is abundant along the luminal surface of endothelial cells, primarily in association with caveolae and/or pinocytotic vesicles (Smith and Ryan, 1970, 1971; Ryan and Smith, 1971a,b). Adenosine diphosphate is degraded to AMP, which in turn is metabolized to adenosine and inosine. The adenosine is taken up into the endothelial cell and resynthesized to ADP and ATP. The significance of this endothelial nucleo-

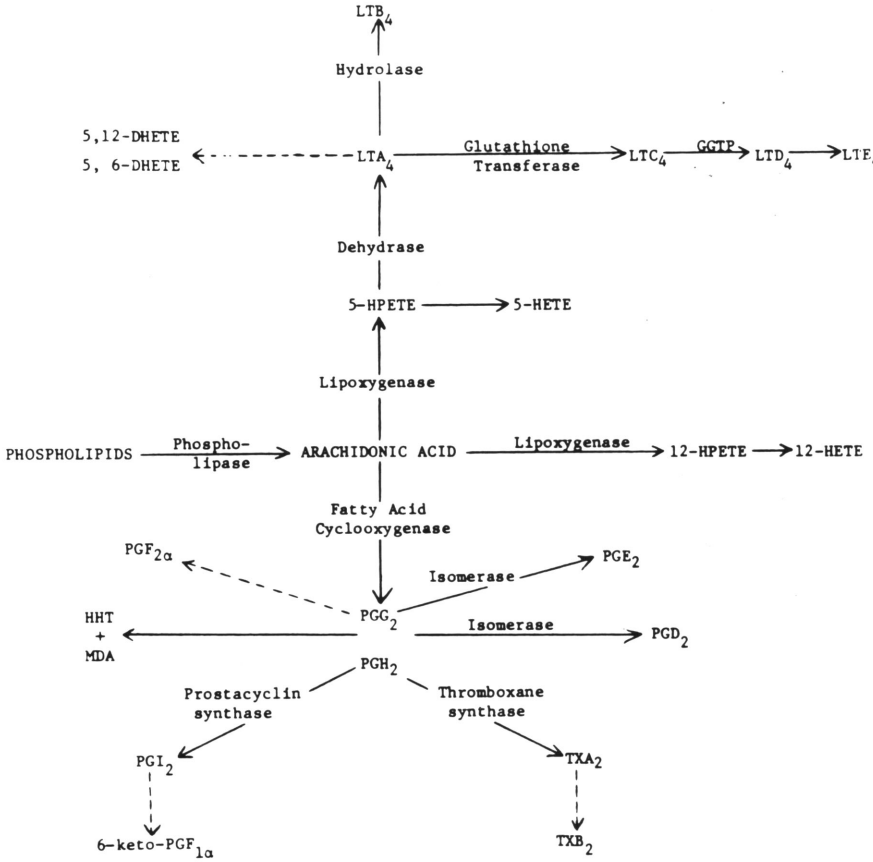

Fig. 9. Scheme of the major metabolic transformations of arachidonic acid. The enzymes responsible for the conversions are shown in between the arrows. The dashed arrows indicate nonenzymatic transformations. GGTP, γ-Glutamyl transpeptidase; HETE, hydroxy-5,-8,10,14-eicosatraenoic acid; HHT, hydroxyheptadecatetraenoic acid; HPETE, hydroperoxyeicosatetraenoic acid; LT, leukotriene; MDA, malonyldialdehyde; TX, thromboxane. (From Salmon, 1982. *In* Cardiovascular Pharmacology of the Prostaglandins. Copyright 1982, Raven Press, New York.)

tide metabolic mechanism is still hypothetical. However, circulating ADP is a stimulator of platelet aggregation. Endothelial inactivation of ADP and its production of the vasodilator antiaggregating agent adenosine may be a mechanism designed to prevent platelet aggregation. Moreover, inosine is believed to be protective of VSM under conditions of hypoxia (Bloom *et al.*, 1979). The significance of adenosine as a mediator of reactive hyperemia, at the level of the microcirculation, also becomes important in view of the capacity of adenosine to release a subsequent vasodila-

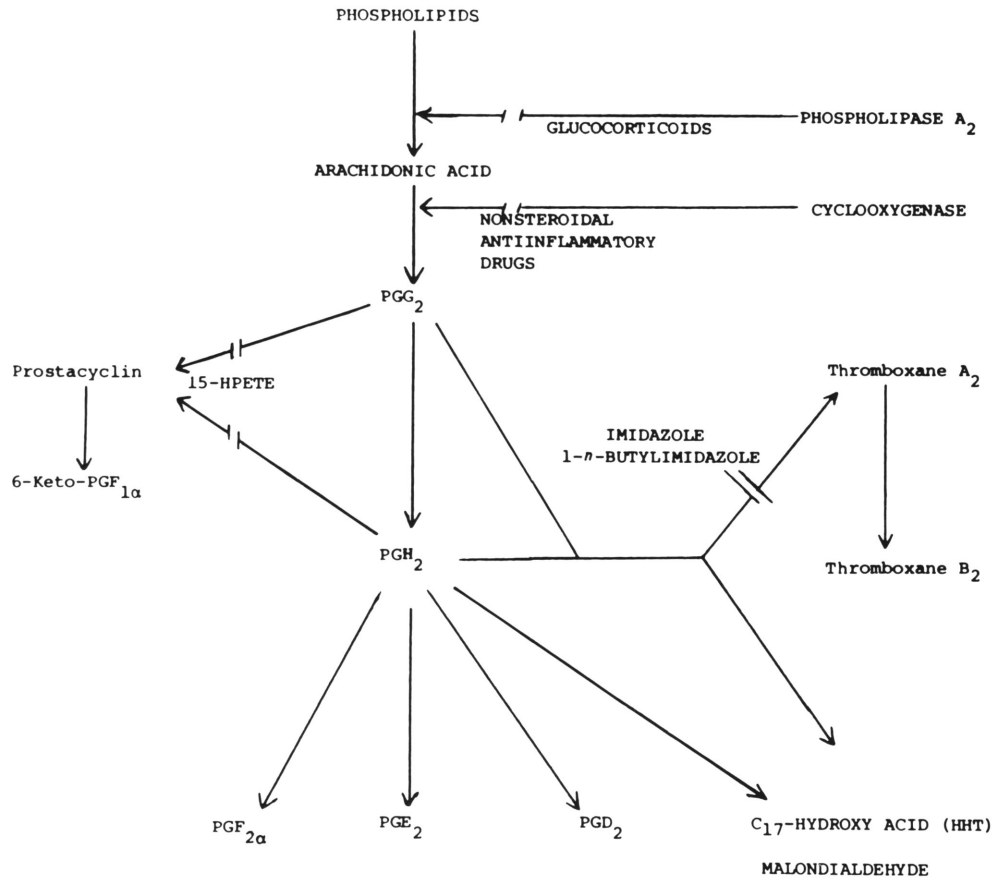

Fig. 10. Arachidonic acid metabolism by cyclooxygenase and sites of inhibitors. (From Salmon, 1982. *In* Cardiovascular Pharmacology of the Prostaglandins. Copyright 1982, Raven Press, New York.)

tor metabolite from the endothelium itself (see Section II,E). Thus, endothelial uptake of ADP may protect the VSM against platelet aggregation and aid in promoting VSM relaxation. Further studies are needed to define the significance of this system in the microcirculation.

Figures 9–12 summarize the synthetic and degradative mechanisms for prostaglandins (PG). The endothelium is a site of selective PGE_2 and prostacyclin (PGI) production. It appears that the larger arteries synthesize PGI, whereas the endothelium of the smaller arteries synthesizes PGE (Gerritsen, 1982). The endothelium is also a site for inactivation of PGE and PGF. Prostaglandins of this series are taken up by a probenecid-inhib-

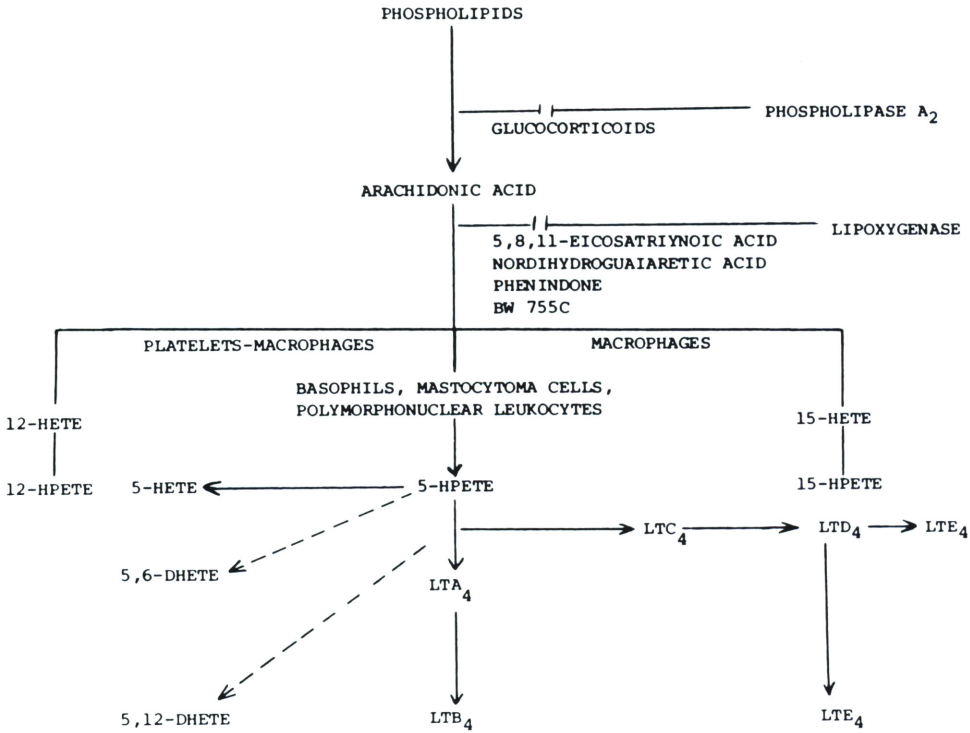

Fig. 11. Arachidonic acid metabolism by lipoxygenase. (From Salmon, 1982. *In* Cardiovascular Pharmacology of the Prostaglandins. Copyright 1982, Raven Press, New York.)

itable active uptake process (Bito and Baroody, 1975; Eling and Anderson, 1976) and metabolized primarily to the dihydro-15-ketoprostanoid derivative. Prostaglandins of the A, B, and I series, and perhaps thromboxanes, may not be taken up by the endothelium (Ryan and Ryan, 1980, 1981). The prostaglandin synthase appears to be a membrane-bound enzyme localized to the plasma membrane, whereas the enzymes responsible for degradation appear to be cytoplasmic (soluble). Endogenous prostanoids may be synthesized at the surface of the cell membrane and diffuse down a concentration gradient to the outside of the cell, where they act. The prostanoids that accumulate within the cell may be degraded within the cell of synthesis by the metabolizing enzyme system, accounting for the lack of preformed storage sites for prostanoids.

The functions of the endothelial cells are many. Although PGI and adenosine formed by the endothelium are vasodilator metabolites, the function of endothelial-derived PGI appears to be modulation of aggregation rather than vasodilation. Figure 13 is a model derived from Gorman

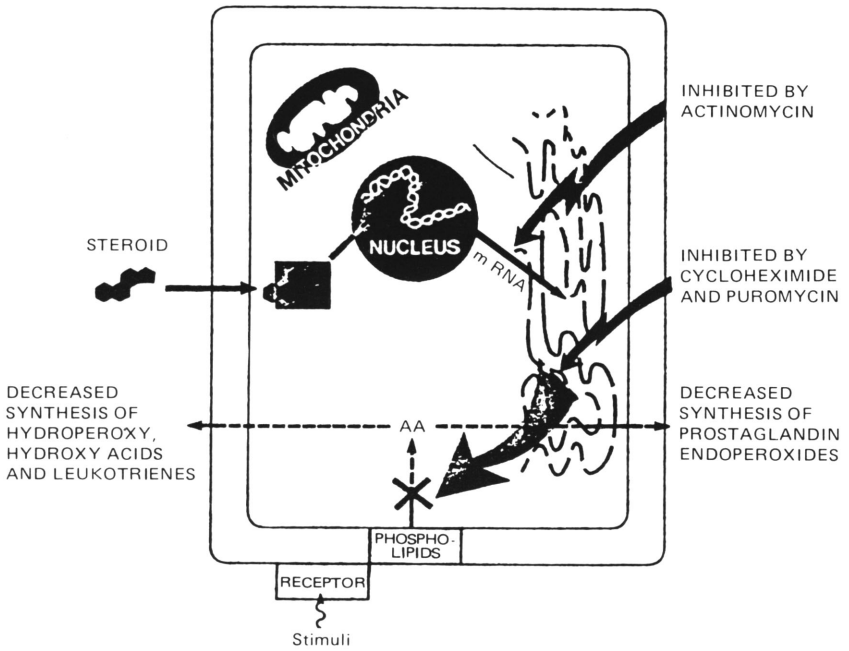

Fig. 12. Mechanism of inhibition of arachidonate metabolism. (From Salmon, 1982. *In* Cardiovascular Pharmacology of the Prostaglandins. Copyright 1982, Raven Press, New York.)

(1982) summarizing the interaction between the platelet and the vascular endothelium. It is believed that the interaction of the aggregating platelets with the endothelial wall and/or the release of lipid peroxides from the aggregating platelets results in action of prostacyclin synthase and elaboration of PGI. The PGI then acts on the platelet to stimulate an increase in cyclic AMP, which results in a decline in free calcium, inhibition of release of 5-HT and ADP, granule stabilization, and decreased release of thromboxane A_2 from the platelet. All of this decreases platelet adhesiveness and prevents aggregation and thrombus formation, if not platelet deposition on the endothelium. Indirectly, this action of the endothelium prevents the release of a potent coronary and cerebral vasoconstrictor, thromboxane A_2, and in this manner does protect the VSM from the deleterious constrictor influences of the circulating humoral factors.

In addition to the antithrombogenic effect of PGI, endothelial cells of veins and capillaries possess plasminogen activator (Todd, 1959, 1964). Moreover, endothelial cells bind thrombin, which rapidly suppresses the fibrinolytic activity of the endothelial cells (for references see Ryan and Ryan, 1981). Thrombin has also been reported to contract VSM (White, 1982). Thus, the endothelium of the microcirculation is both antithrombo-

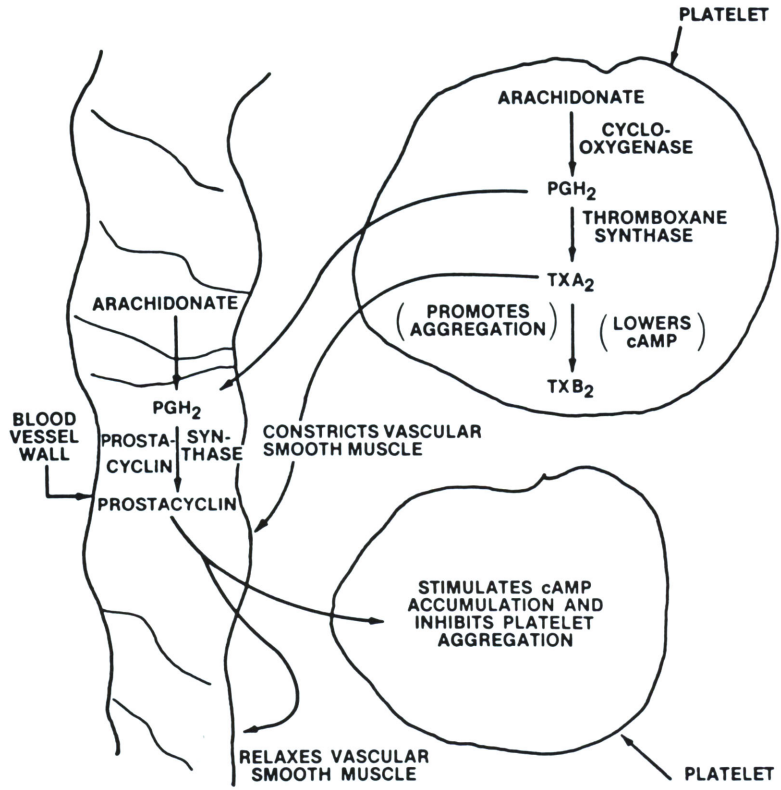

Fig. 13. Model for interaction of PGI_2 with platelets and endothelium. (From Gorman, 1982, with permission.)

genic and hemostatic. The significance of these activities and their relationship to the VSM remain to be explored.

The endothelium also modulates VSM function by acting on polypeptide hormones. The two most important of these act in Yin–Yang fashion to produce vasoconstriction and vasodilation, respectively. They are angiotensin and bradykinin. Endothelial cells contain peptidases, the most important of which is dipeptidyl carboxypeptidase [angiotensin-converting enzyme (ACE) or kinase II], which activates the vasoconstrictor decapeptide angiotensin I to the potent pressor octapeptide hormone angiotensin II and inactivates the nonapeptide vasodilator bradykinin to the pepta- and heptapeptides, which are relatively inactive (see Ryan and Ryan, 1977a,b, 1980). Thus, ACE can convert an active vasodepressor agent to a potent inactive metabolite and convert a prohormone to one of the most potent pressor agents in the circulation.

The ACE is localized along the external surface of the endothelial cells and some smooth muscles (e.g., uterus) and VSM (for references see Ryan and Ryan, 1977a,b, 1980), thus allowing conversion of these substances in the vascular space. The enzyme hydrolyzes the histidine–phenylalanine bond of angiotensin I and forms angiotensin II. The ACE both inactivates and degrades bradykinin by first hydrolyzing the proline–phenylalanine bond of the nonapeptide (inactivation) and then the serine–phenylalanine bond of the heptapeptide (degradation). The angiotensin II formed can then be acted on by endothelial aminopeptidase A to form the potent adrenal cortical stimulating hormone deaspartylangiotensin II (angiotensin III).

The rates of inactivation of bradykinin and activation of angiotensin II by the endothelium may play an important role in determining the microvascular pressures in such diverse diseases as hypertension and diabetes and in pregnancy, in which renin substrate is tremendously increased, providing a potential high concentration of angiotensin I, shock, and other conditions associated with altered endothelial activity. In view of this the finding of Stalcup *et al.* (1979a,b) and Stalcup (1982) that hypoxia inhibits ACE activity suggests that increased levels of vasodilator substances such as bradykinin may compromise the circulation, in the face of a decreased angiotensin II and III pressor influence, in neonatal and adult respiratory distress syndromes, not because of altered VSM function but rather because of endothelial mechanisms. The significance of the Stalcup experiments must await confirmation by independent investigations.

The brief summary provided herein shows that the endothelium of VSM has the potential for synthesizing and degrading humoral factors important in microvascular function. Disease-induced or drug-induced alterations of the vascular endothelium may modify microcirculatory VSM function as effectively as those interventions that alter the VSM directly. Evaluation of drug effects on the microcirculation must be interpreted in terms of the effects of the drugs or interventions on both the VSM and the endothelium. Whether there are therapeutic goals that can be achieved by interfering with or promoting the endothelial mechanisms involved in prostanoid, NE, 5-HT, and adenosine metabolism remains to be shown by future investigation.

E. Endothelium as a Mediator of Vascular Relaxation and Contraction

It is well known among physiologists and pharmacologists studying VSM that humoral substances such as bradykinin and acetylcholine (ACh) con-

tract VSM *in vitro*, despite the inherent vasodilator properties in the microcirculation (McGiff *et al.*, 1972; Needleman *et al.*, 1974; Furchgott, 1955; Bohr *et al.*, 1978; Vanhoutte, 1978; Somlyo and Somlyo, 1968). Vanhoutte *et al.* (1973) suggested that the vasodilator response of ACh may result from an inhibitory effect on adrenergic neurotransmitter release, whereas ACh directly contracts VSM. However, some investigators reported ACh-mediated relaxation of VSM *in vitro* (Jeliffe, 1962; Kuriyama and Suzuki, 1978; Lee *et al.*, 1978).

Serendipitously, Furchgott and Zawadski (1979, 1980a,b,c) and Furchgott *et al.* (1981) found that if the endothelial surface of the blood vessel were not in contact with the glass or wax surface of the material used to prepare helical strips of blood vessels, or if care were taken to avoid contact of the skin with the endothelial surface of vessels, or if rings, rather than helical strips, of VSM were prepared, these blood vessels relaxed in response to ACh and a whole series of vasodilator compounds that had previously been shown to contract VSM *in vitro*. Subsequent analyses demonstrated that rubbing the endothelial surface with wet filter paper or wooden dowels obliterated the endothelium and VSM relaxation *in vitro*. These observations have been confirmed by several investigators (Altura and Chand, 1981; DeMey and Vanhoutte, 1978, 1980a,b,c,d, 1981; Greenberg *et al.*, 1982a,b; Tanaka *et al.*, 1982a).

Although the characteristics of ACh-dependent relaxation appear to be mediated by distinct endothelial cholinergic muscarinic receptors that differ from those on the smooth muscle (see Section III,H) the responses to ATP, adenosine, bradykinin, the calcium ionophore A-23187, ADP, and substance P appear to be dependent on the presence of an intact endothelium for either full or partial expression of their relaxant action (Furchgott *et al.*, 1981; Greenberg *et al.*, 1982a,b; Altura and Chand, 1981). Utilizing a sandwich technique in which endothelial-incompetent VSM was mounted as a sandwich with endothelial-competent VSM oriented so that it could neither relax nor contract to vasoactive substances, administration of ACh and other substances produced relaxation in the endothelial-deficient VSM. This suggested to Furchgott and Zawadski (1980a,b,c,d) that ACh and other substances released a vasoactive substance from the endothelium that acted on the smooth muscle to promote relaxation of VSM.

To prostaglandinologists the most likely candidate for a mediator of endothelial-dependent relaxation of VSM is prostacyclin (Needleman *et al.*, 1976; Moncada *et al.*, 1976a,b). Furchgott *et al.* (1981) tested prostacyclin, cyclic GMP, bradykinin, and cyclic AMP as potential mediators of endothelial-induced relaxation of VSM *in vitro*. Each of these were ruled out as possible candidates because the magnitude of relaxation produced

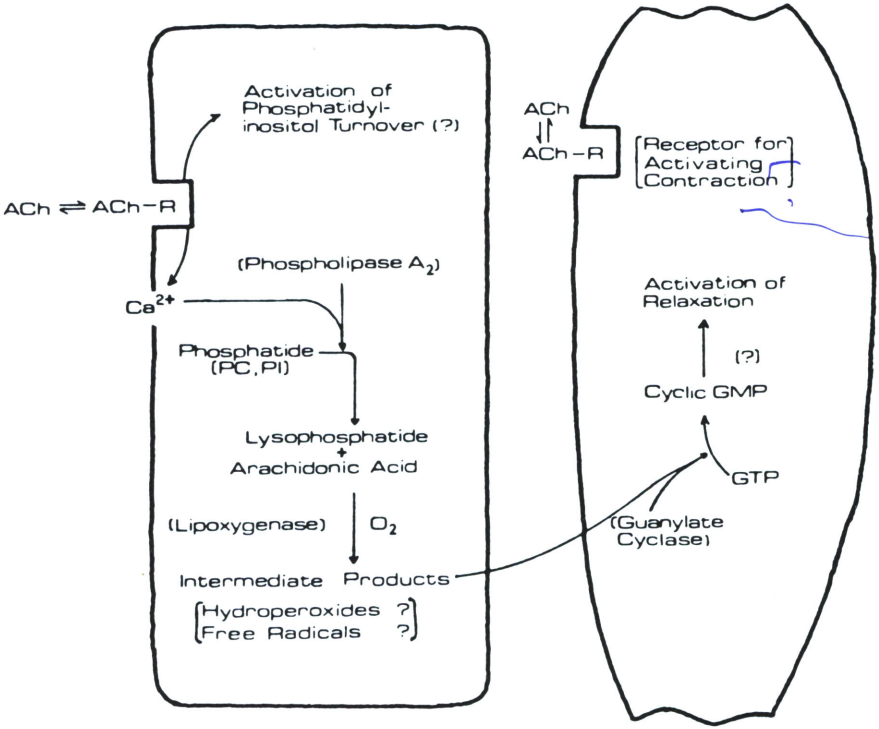

Fig. 14. Hypothetical scheme for ACh endothelial-dependent relaxation in endothelial (left) and smooth muscle (right) cells. PC, Phosphatidylcholine; PI, phosphatidylinositol. (From Furchgott *et al.*, 1981. *In* Vasodilation. Copyright 1981, Raven Press, New York.)

did not approach that of the stimulating substance. However, anoxia inhibited ACh-mediated relaxation, as did 5,8,11,14-eicosatetraynoic acid (ETYA), a lipoxygenase inhibitor. The inhibition of relaxation was selective for endothelial-mediated relaxation and not antagonistic for isoproterenol, nitroglycerin, AMP, or adenosine. The possibility that ACh increased intracellular calcium ion in the endothelium was considered. This resulted in an increased phospholipase A_2 activity and the activation of a lipoxygenase with the formation of either relaxant hydroperoxides or free radicals (Fig. 14). The intermediate lipoxygenase products then acted on the VSM cell to stimulate the accumulation of cyclic GMP (Hidaka and Asano, 1977; Hidaka *et al.*, 1979; Goldberg *et al.*, 1978; Schultz *et al.*, 1979; Spies *et al.*, 1980), which in some undefined manner relaxed the VSM. Although a more specific inhibitor of lipoxygenase BW-755-C, 3-amino-1-[*m*-(trifluoromethyl)phenyl]-2-pyrazoline, did not inhibit endothelial-mediated relaxation by ACh, the free-radical scavengers hydro-

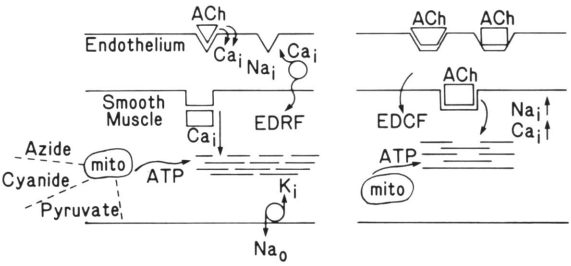

Fig. 15. Alternate mechanism for ACh-mediated relaxation (left) and contraction (right). EDCF, Endothelial-derived contractile factor; EDRF, endothelial-derived relaxation factor.

quinone and the antimalarial agent quinacrine inhibited endothelial-dependent ACh-mediated relaxation of VSM. Quinacrine inhibits phospholipase A_2 activity but may also inhibit the ACh receptor or the actions of ACh on calcium ion in the endothelium.

Although the hypothesis proposed by Furchgott *et al.* (1981) is attractive, there is some difficulty in reconciling the data and the interpretation. A finite time is required to inhibit lipoxygenase and phospholipase A_2 by these inhibitors. Their actions on VSM occur within 1 min. The concentration of inhibitor required to inhibit endothelial-dependent relaxation is 10–100 times that required for inhibition of lipoxygenase. The effects of the inhibitors are acutely reversible. ETYA acetylenates the lipoxygenase and irreversibly inhibits the enzyme. Finally, Greenberg *et al.* (1982b) showed that ETYA, hydroquinone, and other inhibitors, in low concentrations sufficient to inhibit lipoxygenase, did not inhibit bradykinin- or ACh-mediated relaxation of canine mesenteric arteries, but in high concentrations inhibited both ACh and NE responses in mesenteric arteries and veins even in the absence of endothelium (Greenberg *et al.*, 1982b; Greenberg and Kadowitz, 1982). The experiments of Greenberg *et al.* (1982b) showed that ACh endothelial-dependent relaxation was inhibited by ouabain in concentrations that did not affect the responses to NE or ACh responses in endothelial-deficient preparations. An alternative hypothesis is summarized in Fig. 15. The interaction of ACh, bradykinin, and other endothelial-dependent substances releases an unknown mediator from the endothelium. This substance acts on the VSM to stimulate an influx of potassium ion and hyperpolarizes the cell by stimulating an electrogenic sodium pump within the VSM cell membrane. As the membrane potential moves mostly toward the potassium equilibrium potential, the permeability of calcium ion is decreased. This results in a decreased calcium–calmodulin interaction and a decreased phosphorylation of the *p*-myosin light chain (see Section II,J) and relaxation of VSM. Alterna-

tively, several distinct mediators may exist in the endothelium, and the effects of the mediator on the VSM may be dependent on the type of mediator released from the endothelium, which is dependent in turn on the vasoactive relaxant substance. The nature of the endothelial mediator of VSM relaxation and the significance of endothelial-mediated relaxation to *in vivo* microcirculatory function remain to be defined.

The endothelium may be involved not only in VSM relaxation, but in contraction of the VSM as well. Endothelial destruction inhibits the contractile responses of porcine VSM to transmural nerve stimulation (Greenberg *et al.*, 1980) and ACh (Greenberg *et al.*, 1982a,b; Tanaka *et al.*, 1982b) in canine mesenteric and pulmonary veins. The magnitude of the change is only 20% of the maximal contractile response to ACh and approximately 50% of the response to nerve stimulation. This, at least for nerve stimulation, may have a pathophysiologic correlate in shock, in that pulmonary and systemic blood vessels obtained from shocked swine and dogs exhibit endothelial destruction and depression of the response to nerve stimulation (Greenberg *et al.*, 1980). It is possible that the effects of the endothelial mediator differ on arterial and venous smooth muscle or that the endothelial mediator differs when obtained from artery and vein. The nature of the factors obligatory for both endothelial-mediated relaxation and contraction remains to be elucidated.

F. Smooth Muscle as a Site of Production of Humoral Factors

Agonist-mediated contraction of VSM is attended by an increased release of prostaglandins, prostacyclin, and thromboxanes derived from the VSM (Salmon *et al.*, 1978; McGiff *et al.*, 1976; Needleman *et al.*, 1976; Hagen and White, 1978; Greenberg *et al.*, 1981; Greenberg and Kadowitz, 1982) as well as the endothelium. The mechanisms of synthesis and degradation are shown in Figs. 9–12. Local production of these prostanoids appears to be involved in VSM function and modulation of adrenergic neuroeffector transmission (see reviews by Kadowitz *et al.*, 1982a,b; Kadowitz and Hyman, 1982; Armstrong, 1982; Hedquist, 1977) because Marcus *et al.* (1982) showed that endothelial denudation of VSM resulted in smooth muscle production of PGI without any effect on platelet aggregation or thrombus formation.

The release of prostanoids from the VSM and the endothelium as well appears to be modified and/or regulated by many factors that affect either the biosynthesis or degradation of the prostanoids. Bradykinin, angiotensin II, and thrombin may activate phopholipase A_2 activity, thereby in-

creasing the release of arachidonic acid from phospholipid, or may promote the activity of phosphatidyl-inositol phosphodiesterase (Bell and Majerius, 1980; Bell *et al.*, 1980), increasing the synthesis of prostaglandins. β-Thromboglobulin and its precursor γ-thromboglobulin appear to inhibit PGE and PGI synthesis in aortic smooth muscle in culture (see Pearson, 1982). Low-density lipoproteins (LDL), if present for 60 min or more, inhibit PGI production whereas high-density lipoproteins counteract the effect of LDL but do not exert any effect on their own. The mechanism, although speculative, may be related to the capacity of LDL to bind to specific cell receptors, be taken up into the endothelium and perhaps smooth muscle, and destroy the cells (Brown and Goldstein, 1979; Evensen, 1979). Albumin can increase the transport of free fatty acids into the cell, thereby increasing substrate for PG cyclooxygenase and increasing or decreasing prostanoid synthesis.

Macrocortin, a 15,000-dalton protein, may be induced by nonsteroidal antiinflammatory agents acting on the cell. This protein may inhibit phospholipase A_2 activity, thereby decreasing prostanoid synthesis (Blackwell *et al.*, 1978, 1980). A plasma factor that may be a phospholipid bound to albumin has been found to contract the perfused coronary arteries of hearts *in situ* and stimulate $PGF_{2\alpha}$ synthesis. The action of this phospholipid is antagonized by indomethacin. This substance, which acts only when in free phospholipid form, stimulates both lipoxygenase and cyclooxygenase activity and promotes platelet aggregation as well as VSM contraction. The mechanism of this inhibitor remains obscure (Moretti *et al.*, 1976; Moretti and Abraham, 1978a,b; Moretti and Lin, 1980).

Normal human plasma appears to contain a substance, designated reciprocal coupling factor, that both enhances $PGF_{2\alpha}$ breakdown and inhibits $PGF_{2\alpha}$ synthesis. This factor, enhanced by sufinpyrazone, increases in diabetes and decreases in thyrotoxicosis and may be a natural antithrombotic substance (Hoult and Moore, 1982). Several factors exist in human plasma that stimulate PGI synthesis and possibly inhibit thromboxane synthase activity (see Pearson, 1982). These have not yet been characterized but may play a role in protecting the endothelium and VSM against thrombosis or vasoconstriction in such disease states as renal failure, in which their activity increases, and may play an adversary role in disease states in which they are diminished, such as hemolytic uremic failure and thrombocytopenic purpura (Pearson, 1982; Tables IV and V).

Thus, the VSM as well as the endothelium can synthesize prostanoids. The synthesis of these factors is not controlled solely by the VSM but is also subject to modulation by plasma factors of a diverse nature. Drug effects on the microvasculature, rather than being simply endothelial or VSM dependent, may also modify VSM function by altering the synthesis

TABLE IV
Some Biological Effects of Leukotrienes

Vasodilation: PGE_2/PGI_2
Edema: PGE_2/PGI_2; leukotrienes C_4, D_4, and E_4
Leukocyte migration (adhesion, chemotaxis): Leukotriene B_4
Contraction: Leukotrienes C_4, D_4, E_4

and/or activation of these plasma factors, which subsequently affects the synthesis and degradation of these prostanoids and, secondarily, VSM tone. The prudent investigator will now be wise to consider the hematologic effects of pharmacologic antagonists when evaluating microcirculatory function.

G. Mechanisms of Vascular Smooth Muscle Excitation

The poor longitudinal transmission of electrical activity and the large amount of connective tissue in blood vessels make the recording of electrical activity in VSM more difficult than in any other smooth muscle preparation. However, for the sake of completeness, the electrical activation of VSM is discussed here briefly. The electrical activation of VSM plays a role in mediating VSM contraction, although both the nature of this activity and the importance differ in different vessels and with different modes of contraction within the same VSM (Keatinge, 1979).

The resting membrane potential of VSM is mediated by the permeability of the cell membrane to potassium ion. The relatively greater permeability to sodium ion results in a higher resting membrane potential in VSM and other smooth muscles than in skeletal muscle or nerve. A coupled sodium–potassium transport exchange mechanism is responsible for maintaining the gradient of sodium and potassium ion across the VSM cell. The smooth muscle of the arterioles is generally electrically quiescent except at times of release of neurotransmitter from the adrenergic nerves innervating the VSM. Upon release of NE, the VSM becomes partially depolarized and fires bursts of action potentials. The rate of firing of these action potentials appears to be related to the magnitude of increase in mechanical tension (Speden, 1964; von Loh and Bohr, 1973). Several discharges of the nerves in succession are required to initiate or fire an action potential (Hirst, 1977; Holman and Suprenant, 1979). The action potential is followed by a brisk contraction. Both the action potentials and the subsequent contractions are conducted for only a limited distance and seldom involve more than one arteriole (Hirst, 1977).

TABLE V

Interactions between Blood Components and Prostaglandin Synthesis[a,b]

Blood component	Tissue or system tested	Outcome
Plasma, serum, Cohn fractions IV and V, especially IV-4, haptoglobin, and albumin	Seminal vesicle microsomes	PG synthesis ↓
	Isolated rat fundus strip preparation contracted with arachidonic acid	Prevents increase in tone
	Soybean lipoxygenase	Oxygen uptake ↓
Plasma or albumin	Platelet lipoxygenase	↓ PG, TXB_2, HETE synthesis
Plasma or serum (labile, nondialyzable factor)	Cultured bovine endothelial cells	↑ PGI_2, synthesis (bioassay or RIA)
Platelet-derived β-thromboglobulin	Cultured bovine endothelial cells	↓ PGI_2, synthesis (bioassay)
Plasma	"Exhausted" rat aorta rings	↑ PGI_2, release (bioassay)
Plasma or lipid extract of albumin	Perfused rabbit heart	PG-Dependent vasoconstriction ↑
	Cardiac or renal microsomes (rabbit)	↑ PG synthesis
	Platelets and platelet microsomes	↑ PG, TXB_2, HETE synthesis
	Platelet aggregation to arachidonate	Biphasic effect
Plasma or albumin	Platelet aggregation to arachidonate	↓ Aggregation and ↑ release of arachidonate metabolites
Plasma or factor bound to albumin from adult, but not fetus	Human thyroid adenoma/mononuclear cell coculture	↓ PGE yield
Serum, probably platelet-derived growth factor	Cultured 3T3 mouse fibroblasts	↑ Phospholipase A_2 activity and PGE_2, $PGF_{2\alpha}$ release
Serum (plasma less effective)	Cultured MC5-5 mouse fibroblasts	↑ PGE_2 release by RIA
Serum	Phagocytosing rat leukocytes	↓ PGE_2 release by bioassay
Haptoglobin and acute-phase sera	Arachidonate hypotension in rabbit	Agents protect (via ↓ PG synthesis?)
Albumin	Spontaneous decomposition of endoperoxide PHG_2	↑ PGD_2 formation at expense of PGE_2
Plasma, platelet rich or platelet poor	Spontaneous decomposition of prostacyclin	Plasma ↓ rate; platelets may convert PGI_2 to 6-keto-PGE_1

[a] From Hoult and Moore (1982). *In* Cardiovascular Pharmacology of the Prostaglandins. Copyright 1982, Raven Press, New York.

[b] Abbreviations: PG, prostaglandin; TXB_2, thromboxane B_2; HETE, 12L-hydroxy-5,8,10,14-eicosatetraenoic acid; PGI_2, prostacyclin; RIA, radioimmunoassay.

Sodium ion and, to a limited extent, calcium ion appear to carry the current in the smooth muscle cells of VSM (Keatinge, 1968a,b). The action potentials can be obtained in calcium-free solution, indicating that they are due primarily to the sodium current. However, unlike the sodium channels of nerve and skeletal muscle, tetrodotoxin cannot block the action potential or the entry of sodium into VSM. The action potentials of VSM can be blocked by verapamil, the calcium-channel-blocking agent, which also blocks these tetrodotoxin-resistant sodium channels in other smooth muscles (Golenhofen and Lammel, 1972). However, the action of verapamil is neither uniformly consistent nor specific. Specific, independent calcium channels also appear to exist in VSM, and these appear to be involved in the resting permeability of VSM to calcium ion. These channels seem to be distinct from those of the voltage-dependent calcium channel, in that they are not blocked by verapamil or D-600 (Weiss, 1981a,b; Vanhoutte, 1980).

Under special circumstances, such as anoxia, blood vessels exhibit widely conducted action potentials preceded by rhythmic changes in mechanical tension or pressure. This type of activity is also present under naturally occurring physiologic conditions in portal veins and some venules (Axelsson *et al.*, 1967; Funaki, 1961; Funaki and Bohr, 1964). Such contractions have a propulsive action, which is inefficient but of value in the dual circulation of the liver (Keatinge, 1979). Under normal conditions the high resting potassium permeability of most VSM, which increases on depolarization, prevents and opposes the development of regenerative action potentials (Mekata, 1971; Keatinge, 1978). Unlike the normal action potentials of VSM, these repetitive spike discharges of rhythmically active VSM appear to be mediated by a slowly inactivating calcium and magnesium current, which is independent of sodium ion (Keatinge, 1978). The significance of this unique magnesium channel remains speculative. However, Keatinge (1978, 1979) suggests that an increase in intracellular magnesium between spike discharges may aid in promoting VSM relaxation, thus contributing to the rhythmic fluctuations in VSM tone.

H. Sources of Activator Calcium in Vascular Smooth Muscle

The manner in which excitation of VSM by vasoactive agents initiates an increase or decrease in tension development remains an unresolved matter. Three major mechanisms exist to explain the sources of activator calcium in VSM. Humoral factors and agonists that either increase spike discharge or promote a maintained depolarization are believed to stimulate

the opening of voltage-dependent channels, which increase the entry of both sodium ion and calcium ion. Both the number of channels that are open and the time during which they remain open determine the relative increase of extracellular calcium ion into the cell. Entry of calcium ion in response to electrical activation of VSM seems to be an integral component of the response because the electrical activity fails in calcium-free media at a time when the intracellular concentration of calcium ion falls to a threshold level for contraction (Hinke, 1965; Keatinge, 1979). It has been suggested that this process of calcium entry triggers the release of intracellular calcium ion from sequestered sites and thereby raises the ionized concentration of calcium ion above 10^{-7} *M* to initiate the contractile process (Sigurdsson *et al.*, 1975), similar to a process that occurs in striated muscle. However, little evidence exists to support this hypothesis. The calcium ion that enters from extracellular or membrane-bound sites may be sufficient to interact with calmodulin, thereby activating the contractile proteins (Section II,J). The entry of activator calcium ion linked to changes in the membrane potential of VSM has been designated electrical–mechanical coupling (Somlyo and Somlyo, 1968, 1970; Bohr, 1973) or spike activation mechanisms (SAM) (Golenhofen *et al.*, 1973; Fig. 16).

Norepinephrine can contract VSM either with or without a concomitant change in membrane potential (Bohr *et al.*, 1978; Somlyo and Somlyo, 1968, 1970; Altura and Altura, 1977a,b; Altura, 1978a,b, 1981; Vanhoutte, 1978, 1980, 1981a,b; Weiss, 1981a,b). The contractile responses of VSM to low concentrations of NE are inhibited rapidly by withdrawal of extracellular calcium ion, whereas those to high concentrations are not inhibited (Weiss, 1977). Moreover, the responses to NE, some prostanoids, thromboxanes (Greenberg *et al.*, 1973a,b; Bevan *et al.*, 1973; Hudgins and Weiss, 1968; Greenberg, 1981a; McNamara *et al.*, 1980; Altura and Altura, 1977b), and other vasoactive substances are not antagonized by inhibitors of calcium influx into the cell, such as nifedipine, D-600, or verapamil (for references see Vanhoutte, 1980; Weiss, 1981a,b). However, the responses to these agonists are inhibited by metabolic inhibitors, anoxia, or a reduction in temperature. It is thought that these agonists increase membrane permeability to calcium ion by opening up receptor-linkage voltage-independent channels in the cell membrane, as well as by causing a release of intracellular calcium ion from sequestered sites within the VSM cell (Bohr *et al.*, 1978; Weiss, 1981a,b). Keatinge (1979) has suggested that the increase in membrane permeability of calcium ion and/or the release of intracellular sequestered calcium ion brought about by vasoactive agents acting through pharmacomechanical coupling (Somlyo and Somlyo, 1968) or spike-free activation mechanisms (SFAM) (Golenhofen *et*

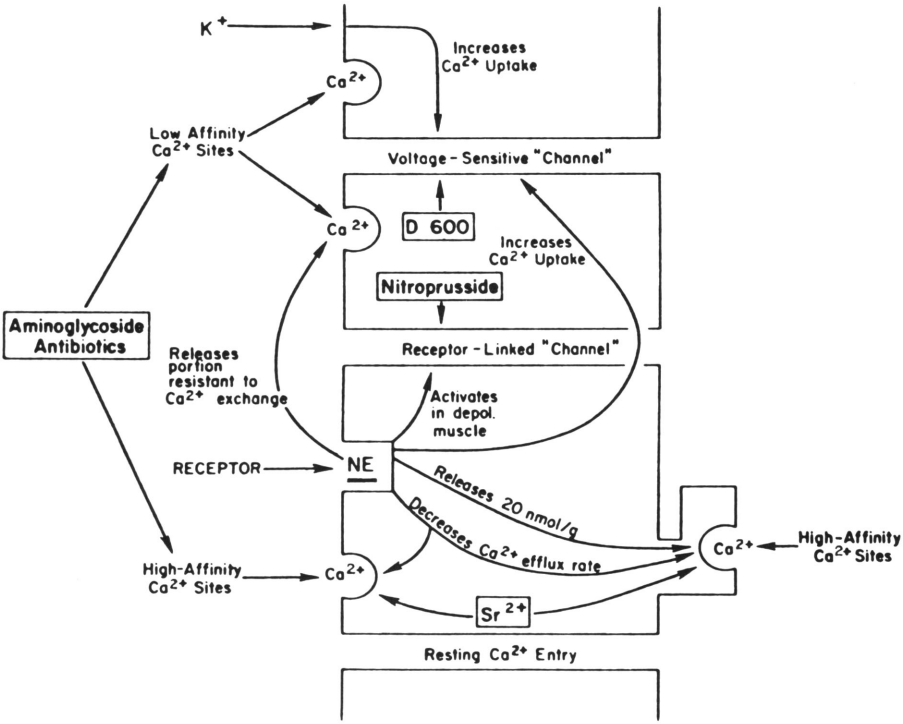

Fig. 16. Model for sites of action of different inhibitory agents on calcium uptake pathways and associated calcium-binding sites in vascular smooth muscle. (From Weiss, 1981, with permission.)

al., 1973) must do so by releasing or stimulating the synthesis of a chemical mediator or event, since the temperature sensitivity of the process indicates a chemical rather than a physical process. It is unlikely that the mediator is a prostanoid, despite the suggestion of Horrobin *et al.* (1976). These investigators showed that indomethacin inhibited the contractile responses of NE in rat VSM. In most preparations, indomethacin enhances the responses to NE (see Greenberg and Kadowitz, 1982). The chemical mediator, if one exists, remains unknown.

Vasodilators may bring about a decrease in VSM tone by (*a*) antagonizing or opposing the electrical changes produced by vasoconstrictor agonists, thereby decreasing the entry of extracellular calcium ion into the cell; (*b*) directly reducing calcium influx into the cell by promoting electrical changes opposite to, but independent of, those produced by vasoconstrictor agents; (*c*) directly increasing the efflux of calcium ion from the cell; or (*d*) promoting the sequestration of intracellular calcium ion by sar-

coplasmic reticulum, mitochondria, or other subcellular organelles (Vanhoutte and Leusen, 1981; Vanhoutte, 1978, 1981a,b). Other evidence also suggests that vasodilators may promote VSM relaxation either by decreasing the binding of calcium ion to the regulatory protein calmodulin (Stull and Sanford, 1981) or by increasing the rate of dephosphorylation of myosin light chains (Small and Sobieszek, 1977, 1980; Perry and Grand, 1979). In the latter instances, relaxation is promoted in the absence of a change in free ionized calcium ion.

In summary, extracellular, intracellular, and membrane-bound calcium ions may serve as sources of activator calcium ion in VSM. Whereas SAM or electrical–mechanical coupling mechanisms may be associated with the entry of calcium from the outside of the cell through voltage-dependent channels, SFAM or pharmacomechanical coupling appears to be related to the entry of calcium into the cell via voltage-independent channels and release of sequestered calcium ion from within the cell. Relaxation of VSM is associated with decreases in the entry and release of calcium from these sources but may also be associated with direct effects of vasodilator compounds on the contractile proteins or calmodulin. Although graded depolarization appears to be evident in the microcirculation smooth muscle cells (Chernukh and Timkina, 1976; Funaki and Bohr, 1964; Kumamoto, 1977), the pools of activator calcium involved in VSM contraction and relaxation remain to be defined.

I. Energy and Vascular Smooth Muscle Contraction

The VSM is unique in that it utilizes a large amount of energy for a long period of time to maintain arterial pressure, without undergoing fatigue. The VSM maintains the integrity of the arterial pressure with approximately 4% of the basal metabolic rate of energy production, whereas equivalent energy utilization by skeletal muscle would require twice the energy output provided by the total basal metabolic rate of the organism (Paul, 1980). This efficient utilization of energy occurs with VSM in which the basal pool of high-energy phosphates is 2–5 μmol per gram of blood vessel, a value of 10–20 times lower than that found in skeletal muscle. Since the basal rate of high-energy phosphate utilization in VSM is 1–3 μmol/g per minute preformed ATP cannot serve as a source of energy for the maintenance of VSM tone. Rather, newly synthesized ATP from oxidative metabolism and phosphocreatine via the Lohman reaction

$$\text{Phosphocreatine} + \text{ADP} \rightleftharpoons \text{ATP} + \text{creatine}$$

must subserve VSM tension development. Thus, in VSM intermediary metabolism is more important than in skeletal muscle, where brief con-

tractions are supported from preformed high-energy phosphate pools and the energy debt is repaid after the contraction is completed.

Steady-state oxygen consumption is coupled to isometric force development in VSM. Thus, the energy production by mitochondria should be limited by the amount of available ADP. It has been suggested that the limiting source of ADP in the VSM is derived from the hydrolysis of ATP via actomyosin ATPase activity (Nishiki *et al.*, 1978). However, because of the relatively low concentration of ADP in VSM this has not yet been verified experimentally. The major sources of substrate for energy production in VSM appear to be both exogenous glucose and preformed glycogen. However, VSM can utilize lipid substrates in both the presence and absence of glucose (Furchgott, 1966; Paul, 1980), because the enzymes for the conversion of lipids, proteins, and carbohydrates into ATP appear to exist in VSM and the respective substrates can support VSM contraction. The preferred substrate under physiologic conditions remains to be elucidated.

The VSM is unique among tissues in that approximately 90% of its glucose metabolism results in lactate formation (aerobic glycolysis) with approximately 30% of its ATP produced by this mechanism. Peterson and Paul (1974) and Gluck and Paul (1977) provided convincing evidence that the formation of lactate was increased by vasoconstrictor agents where the (K,Na)-ATPase was functional but that ouabain-induced contracture was associated with a decreased lactate production. It has been suggested that aerobic glycolysis provides the energy to support the sodium–potassium pump mechanism (Paul, 1980; Hellstrand and Paul, 1980, 1982) involved in maintaining the normal intracellular concentration of sodium and potassium ion (Table VIII, p. 108).

Although ATP is essential for both contraction and relaxation, relaxation appears to be more sensitive to inhibition by blockade of oxidative metabolism than contraction. DeMey and Vanhoutte (1978), Furchgott *et al.* (1981), and Greenberg *et al.* (1982b) have shown that hypoxia, azide, and cyanide abolish ACh-, adenosine-, and bradykinin-mediated relaxation of VSM in concentrations that do not inhibit contraction by NE. Although the metabolic bases for these events remain undefined, the data suggest that the increase in vascular resistance with hypoxia or hypoxic-induced vasoconstriction *in vitro* may result from abolition of vasodilator mechanisms, which then allow vasoconstrictor mechanisms to proceed unopposed. Further studies are necessary to elucidate the metabolic factors regulating VSM functions.

A discussion of the metabolic factors affecting VSM would be incomplete without an examination of the role of the cyclic nucleotides in VSM. The present state of knowledge concerning the role of cyclic AMP and cyclic GMP in VSM is, at best, controversial. Drugs that stimulate the

TABLE VI
Alterations in Activities of Enzymes of the Prostaglandin System in Pathophysiologic States[a,b]

Species, organ	Treatment	Prostaglandin synthesis (microsomes)	Prostaglandin metabolism (cytosol)
Rat kidney	Adrenalectomy	—	↑
	Renal hypertrophy	↓	—
	New Zealand hypertension	N/C	↓
	Renal artery clamp hypertension	↑	—
	Wistar–Okamoto hypertension	—	↓[c]
	Wistar–Okamoto hypertension	↑[d]	—
	Wistar–Okamoto hypertension	?[e]	↓
Rat lung	Pregnant	—	↑
	Parturition	—	↓
Guinea pig lung	Exposure to 100% O_2, 2 days	N/C	↓
Rabbit kidney	Ureter-obstructed hydronephrotic	↑[d]	—
	Renal vein constricted	↑[d]	—
Rabbit lung	Late pregnant/progesterone-treated	—	↑
	Parturition	—	↓

[a] From Hoult and Moore (1982). *In* Cardiovascular Pharmacology of the Prostaglandins. Copyright 1982, Raven Press, New York.
[b] Key: ↑, activity increased; ↓, activity decreased; N/C, no change in activity; —, not tested.
[c] Refers to the enzyme 9-hydroxyprostaglandin dehydrogenase. 15-Hydroxyprostaglandin dehydrogenase activity was normal.
[d] Synthesis from perfused organ.
[e] Assay was in crude homogenate: degradation interferes.

accumulation of cyclic AMP as well as cyclic GMP produce relaxation of VSM. However, increases in these nucleotides within the VSM cell may also be associated with contraction of the smooth muscle or no change in smooth muscle tone. The evidence leading to the hypothesis proposed for the action of cyclic nucleotides in VSM will be summarized here. For a detailed discussion of this topic the reader is referred to excellent reviews (Amer, 1977; Andersson *et al.*, 1972; Bar, 1974; Berridge, 1975; Diamond, 1978; Crain and Appleman, 1978; Goldberg *et al.*, 1973; Kukovetz *et al.*, 1981).

The original concept to emerge was that if cyclic AMP was associated with relaxation then cyclic GMP would oppose relaxation or initiate contraction. This concept was experimentally verified when it was shown that some vasoconstrictors, such as phenylephrine and $PGF_{2\alpha}$, increased cyclic GMP levels in bovine and canine arteries and veins (Dunham *et al.*, 1974; Kadowitz *et al.*, 1973, 1975a; Joiner *et al.*, 1975). This stimulation of cyclic GMP formation was indirect and not due to agonist-mediated ef-

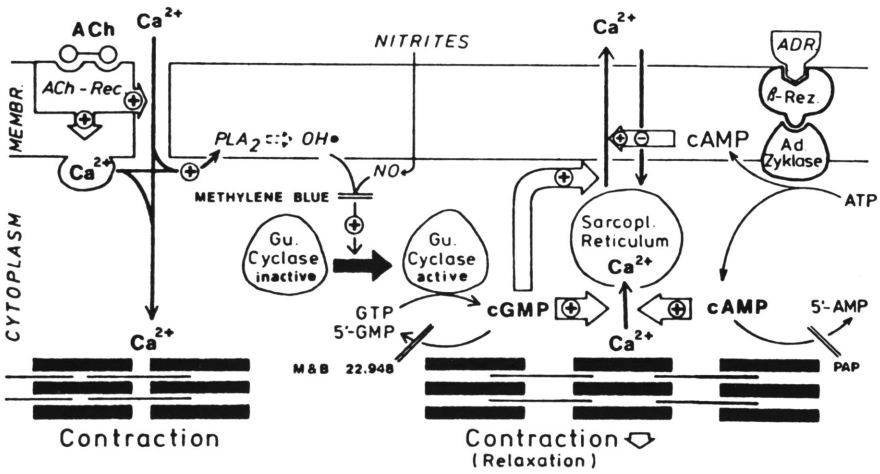

Fig. 17. Schematic representation of role of cyclic GMP and AMP in smooth muscle relaxation. Ad. Zyklase, Adenylate cyclase; ADR, adrenalin; β-Rez., β-receptor; Gu. cyclase, guanylate cyclase; PAP, papaverine; Sarcopl. Reticulum, sarcoplasmic reticulum. (From Kukovetz *et al.*, 1981. *In* Vasodilation. Copyright 1981, Raven Press, New York.)

fects on guanylate cyclase, but rather was a calcium-dependent event associated with an inhibition of phosphodiesterase activity. However, this concept was shattered when it was shown that nitrites produced relaxation in VSM, which was associated with an increase in cyclic GMP (Katsuki *et al.*, 1977). This effect was independent of calcium ion and occurred in broken-cell preparations. Methylene blue inhibited nitrate- and nitroprusside-induced relaxation and the accumulation of cyclic GMP (Kadowitz *et al.*, 1981; Gruetter *et al.*, 1979). Moreover, drugs that inhibited phosphodiesterase potentiated the relaxant effects of these vasodilators (Kukovetz *et al.*, 1979a,b). The reported effects of the nitroso compounds were not secondary to an increase in cellular calcium ion because (*a*) these compounds increased calcium efflux from VSM without increasing calcium influx (Zsoster *et al.*, 1977); (*b*) in the absence of extracellular calcium ion, nitrates and nitroprusside decreased calcium efflux from rabbit renal arteries and inhibited KCl-induced contraction, indicating that calcium sequestration was enhanced (Hester *et al.*, 1979); and (*c*) nitrites and nitroprusside do not directly inhibit calcium sequestration by sarcoplasmic reticulum from rabbit aorta (Thorens and Hausler, 1979). Thus, although the evidence is at best circuitous and circumstantial, it is possible that cyclic GMP could regulate VSM relaxation by stimulating calcium sequestration by sarcoplasmic reticulum or enhancing calcium extrusion from the VSM cell (Fig. 17).

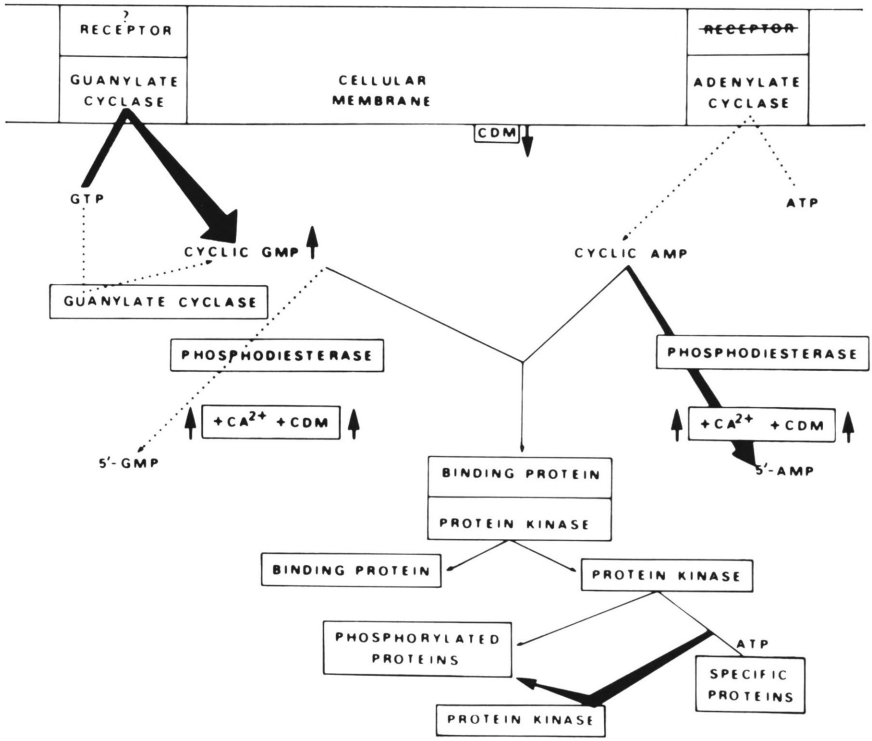

Fig. 18. Mechanism of cyclic nucleotide action. Specific proteins include histones, nonhistone chromosomal proteins, ribosomes, membrane proteins, and certain enzymes. CDM, Calmodulin. (From Criss and Kakiuchi, 1982, with permission.)

The evidence supporting a role and mechanism for cyclic AMP in VSM is firmer. Cyclic AMP stimulates calcium sequestration by subcellular organelles involved in reducing the free concentration of calcium ion, such as sarcoplasmic reticulum and mitochondria. Cyclic AMP accumulates in most VSM when β-receptor-mediated vasodilation occurs and when vasodilator phosphodiesterase inhibitors are employed. Cyclic AMP levels are decreased in conditions associated with increased tension development, such as hypertension (Amer, 1973, 1977; Amer *et al.*, 1974), whereas cyclic AMP-dependent protein kinase (Fig. 18) appears to be involved in the regulation of the relaxation component of contractile protein regulation (see below). Thus, it is possible that stimulation of adenylate kinase, via β-receptor-linked or independent mechanisms, or inhibition of phosphodiesterase results in an increase in cyclic AMP, enhanced calcium extrusion from the cell or sequestration within the subcellular orga-

nelles involved in calcium regulation, or both, and subsequent relaxation. Alternatively, the influx of calcium ion may be inhibited by cyclic AMP, which could result in a decrease in calcium binding to calmodulin and relaxation. Finally, cyclic AMP-dependent protein kinase appears to be involved in the inactivation of myosin light-chain kinase, which can promote relaxation even in the absence of changes in calcium ion.

The available data support the hypothesis that both cyclic AMP and cyclic GMP mediate relaxation of VSM, probably by decreasing the concentration of ionized calcium that can interact with calmodulin and possibly by phosphorylating myosin light-chain kinase or dephosphorylating myosin. P. J. Kadowitz (personal communication) has suggested that cyclic GMP may be involved in the action of pharmacologic agents, whereas cyclic AMP mediates the responses to physiologic mediators and modulators of VSM tone. Further studies are necessary to resolve this question.

J. Biochemical Mechanisms Regulating Vascular Smooth Muscle Contraction and Relaxation

The major contractile proteins in VSM are actin, myosin, tropomyosin, and leiotonin A and C (Adelstein and Klee, 1980; Stull and Sanford, 1981; Small and Sobieszek, 1980; Ebashi *et al.*, 1978). Leiotonin C, a calcium-binding protein, differing from both calmodulin and troponin C, may be involved in the regulation of the thin contractile filament, actin, whereas calmodulin appears to be involved in the phosphorylation of the thick filament, myosin. Troponin, present in cardiac and skeletal muscle, may not exist in VSM. Calcium regulation of VSM contraction differs from that for skeletal and cardiac muscles. In the latter muscle, calcium ion removes an inhibitory effect of troponin C on the interaction between actin and myosin. In VSM, calcium ion activates the inactive state of myosin (Fig. 19).

Actin is a globular protein (MW 43,000) that polymerizes at physiologic ionic strength into a double-helical filament. In addition to its structural role in muscle, actin activates ATP hydrolysis by myosin. Tropomyosin (MW 66,000) sits in the groove created by the two strands of actin. The actual function of tropomyosin in VSM is unknown, but tropomyosin enhances the capacity of actin to activate myosin ATPase activity (Small and Sobieszek, 1977, 1980). Leiotonin A and C may, but this is questionable (Ebashi *et al.*, 1978), take the place of troponin and interact with both tropomyosin and actin. Myosin is a hexameric protein consisting of two heavy chains (MW 200,000) and two pairs of light chains (MW 20,000 and 15,000). The 20,000-dalton light chain (hereafter referred to as *p*-myosin light chain) can undergo reversible, covalent phosphorylation and de-

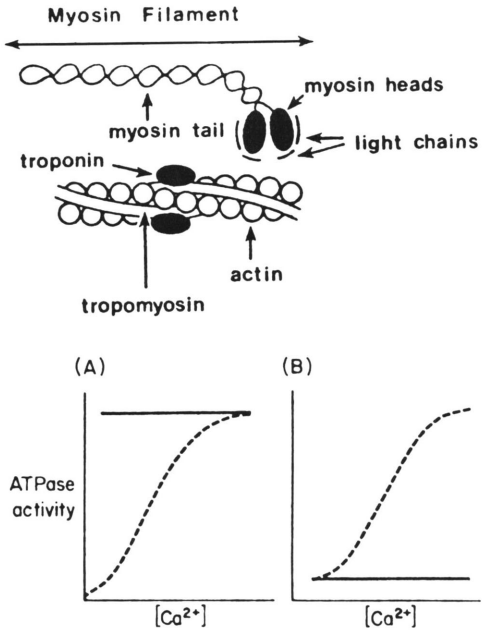

Fig. 19. Regulatory proteins and contractile elements of vascular smooth muscle. Lower panel: ATPase activity of calcium-independent (A) and calcium-regulated (B) actomyosin. Note the lower level of calcium-dependent activity in the absence of calcium (heavy black line). (From Triggle, 1981, with permission.)

phosphorylation, which is believed to be responsible for the velocity of shortening of smooth muscle. The enzymes responsible for myosin phosphorylation (protein kinases) are made up of two proteins: a calcium-binding small protein, known as calmodulin, and a larger protein, which contains most, if not all, of the catalytic activity (Dabrowksa *et al.*, 1978). The catalytic enzyme appears to be regulated by calmodulin.

When an agonist interacts with the cell membrane or intracellular organelles to increase the concentration of free, ionized calcium ion above 10^{-7} *M*, it is believed that four molecules of calcium bind to calmodulin (calcium–calmodulin). The calcium–calmodulin complex then binds to the inactive form of the myosin light-chain kinase to form the active complex calcium–calmodulin–myosin kinase (active). This active complex of calcium–calmodulin–myosin kinase catalyzes the phosphorylation of the *p*-myosin light chains, resulting in an active form of myosin susceptible to activation by actin. Myosin light-chain phosphatase, a calcium-independent enzyme, dephosphorylates *p*-myosin light chain, allowing the cycle to begin anew. It is the phosphorylation and dephosphorylation of the

myosin light chains that results in the interaction between actin and myosin and thereby the cyclic attachment and detachment of the myosin heads to the actin filament. The attachment of the myosin heads to the actin results in a change in the angle of the orientation of the actin–myosin, which allows the filaments to slide past each other, similar to the mechanism in skeletal and cardiac muscle. Adenosine triphosphate provides the energy for this process, and actin activation of myosin ATPase activity releases this energy (Adelstein and Klee, 1980).

Conti and Adelstein (1980) suggested that relaxation by agonists (e.g., epinephrine) results from stimulation of adenylate cyclase, which stimulates the formation of cyclic AMP from ATP. The increased cyclic AMP binds to the regulatory subunit of inactive protein kinase to expose the catalytic subunit of protein kinase. This catalytic subunit of protein kinase phosphorylates myosin light-chain kinase. Because the phosphorylated form of the myosin light-chain kinase is less capable of binding with calmodulin, the myosin light-chain kinase is, in effect, inactivated. A decreased myosin kinase activity results in a decreased phosphorylation of myosin. Since dephosphorylated or unphosphorylated myosin cannot interact with actin, VSM tension declines.

Thus, it appears that changes in the level of ionized calcium may regulate the binding of calmodulin to myosin kinase and the degree of activity of the myosin light-chain kinase through cyclic AMP-dependent protein kinase activity. Moreover, drugs that affect the interaction between calcium and calmodulin greatly affect VSM tone and thereby arterial pressure and resistance. It must be stated that the mechanism of contraction and relaxation summarized above is still speculative. Many questions relating to the concentrations and affinity of the kinases and the calcium–calmodulin interaction have not been answered. Moreover, the role of leiotonin A and C in VSM contraction and relaxation is unknown. Further studies are necessary to validate the proposed concept. However, at present, on the basis of the available evidence, it appears that the phosphorylation and dephosphorylation of myosin may be the most important factor regulating VSM contraction and relaxation (for further discussion see Adelstein, 1978; Adelstein and Eisenberg, 1980; Small and Sobieszek, 1980; Ebashi *et al.*, 1978).

K. Mechanisms of Vascular Smooth Muscle Contraction and Relaxation

The evidence presented above suggests that contraction of VSM may occur by four distinct mechanisms. Ions, such as potassium, and low concentrations of NE may induce changes in sodium permeability across the

cell membrane, decreasing the membrane potential and causing an influx of sodium and calcium ions through voltage-dependent calcium channels. The increased influx of calcium ion results in the binding of calcium to calmodulin, with the subsequent binding to and activation of *p*-myosin light-chain kinase. The complex of calcium–calmodulin and activated *p*-myosin light-chain kinase results in the phosphorylation of the light chain of myosin and the subsequent events that mediate contraction. The contractile response may be regulated or modulated by the simultaneous production of vasodilator substances such as prostacyclin or PGE_2. These modulators may act by increasing adenylate cyclase activity, with its increased production of cyclic AMP and cyclic AMP-dependent protein kinase, which then acts by affecting the dephosphorylation of myosin light-chain kinase. Similarly, high concentrations of NE or 5-HT may induce discreet changes in the voltage-independent calcium channels, increasing the concentration of intracellular calcium ion with its attendant effects on the biochemical events mediating contraction.

In contrast, vasoactive agents, such as thromboxane A_2 and the endoperoxide analog 9a,11a-epoxymethanoprostaglandin H_2, vasopressin, oxytocin, and other agents may act to promote an intracellular release of sequestered calcium ion from within the cell. This process may also involve the presence of a second mediator other than the cyclic nucleotide system, in that some of these agonists would appear to be impermeable to the cell (Ryan and Ryan, 1981). The biochemical processes subserving this mode of activation may differ from that of NE, in that (*a*) the responses to agonists that appear to release intracellular calcium ion are susceptible to inhibition by anoxia and metabolic inhibitors and (*b*) the responses are highly magnesium dependent (see discussion of individual agents, Section III). Drugs such as ouabain may contract VSM by inhibiting the transport (K,Na)-ATPase. Inhibition of this enzyme results in an accumulation of sodium ion in the cell, partially depolarizing the membrane and increasing calcium entry via voltage-dependent calcium and sodium channels, whereas intracellular sodium ion may inhibit calcium binding and the loss of potassium may cause a loss of magnesium ion and subsequent inhibition of relaxation. Finally, the possibility exists that drugs, or perhaps humoral agents, may contract VSM by (*a*) inhibiting the dephosphorylation of myosin light-chain kinase, (*b*) enhancing the binding of calcium to calmodulin, or (*c*) promoting the phosphorylation of the 20,000-dalton myosin light chain. Although these three mechanisms have not yet been demonstrated, they serve as potential models for drug-induced contraction of VSM.

The process of contraction is subserved by energy derived from the Embden–Myerhoff pathway. However, when this cycle is inhibited, en-

ergy (ATP) derived from the pentose phosphate shunt and anaerobic metabolism appears to be sufficient to maintain the integrity of the contractile process for many humoral agonists. The exceptions to this seem to be prostanoids, 5-HT, vasopressin, and oxytocin, as well as the contractile responses to ACh.

Relaxation of VSM may be mediated by several distinct and independent mechanisms. Calcium-blocking agents are believed to promote relaxation of VSM by inhibiting voltage-dependent and voltage-independent entry of calcium ion into the intracellular compartment of VSM, thereby decreasing the availability of ionized calcium to the calmodulin–leiotonin–actomyosin system of contractile and regulatory proteins (for references see Stull and Sanford, 1981). In addition, several calcium-channel-blocking agents also appear to decrease or prevent the mobilization of tightly bound or sequestered intracellular calcium ion, thereby contributing to the relaxant action of such drugs as dilitazem and nifedipine on VSM (Weiss, 1981a,b).

Nitroprusside, nitrites, and other drugs are believed to stimulate the conversion of GTP to cyclic GMP, which inhibits contraction of VSM by an as yet unknown mechanism (Goldberg *et al.*, 1978; Hidaka and Asano, 1977; Kadowitz *et al.*, 1982a,b). Acetylcholine, ATP, adenosine, and bradykinin may stimulate the formation of a hydroperoxide vasodilator material within the vascular endothelium (Furchgott and Zawadski, 1979, 1980a,b,c), which may then act on the smooth muscle to stimulate guanylate cyclase and promote relaxation (Furchgott *et al.*, 1981). The (sodium–potassium)-activated, magnesium-dependent ATPase pumping mechanism located in the cell membrane may also play a role in vasodilation (Haddy, 1978; Webb *et al.*, 1981; Bonaccorsi *et al.*, 1977; Anderson, 1976). Drugs that promote relaxation by this mechanism may stimulate the activity of the pumping mechanism, thereby promoting an accumulation of potassium ion within the cell, decreasing the sodium ion concentration, and promoting a hyperpolarization of the VSM cell (Bonaccorsi *et al.*, 1977; Jones, 1980; Limas and Cohn, 1974). Finally, as stated above, vasodilators may directly affect the binding of calcium ion to calmodulin, thereby decreasing the capacity of myosin kinase to phosphorylate myosin. Alternatively, vasodilators may directly affect the dephosphorylation of myosin light-chain kinase. Although the mechanisms of action of some humoral factors and pharmacologic modalities have been elucidated, it is unknown whether these mechanisms persist in the VSM of the microcirculation.

Energy is required for relaxation as well as contraction, and relaxation is therefore an active process. In many types of VSM, the vasodilator response to ACh, adenosine, or bradykinin is inhibited in solutions with low

pO_2 values (DeMey and Vanhoutte, 1978). Similarly, metabolic inhibitors such as azide and cyanide, in concentrations that do not affect VSM contraction, abolish relaxation of VSM in response to ACh and prostanoids (Greenberg *et al.*, 1973a,b, 1974a,b, 1982b). The mechanism may be related to the fact that relaxation is more dependent on ATP than contraction or that the inability of ATP-dependent calcium sequestration mechanisms to function overrides the signal provided by dephosphorylation of myosin (see Section II,I).

III. Humoral Factors Affecting Vascular Smooth Muscle

A. Catecholamines

The microcirculation of most species is endowed with receptors on the VSM for catecholamines. Adrenergic receptors have been classified into α- and β-adrenergic receptors (Alquist, 1948) and subclassified into α_1 and α_2 receptors as well as β_1 and β_2 adrenoceptors (see reviews by Bertelsen and Pettinger, 1977; Wikberg, 1979; Vanhoutte, 1982). The α_1 adrenoceptors may be presynaptic or postsynaptic. Primarily the latter are activated by epinephrine > norepinephrine > phenylephrine > clonidine and are inhibited by prazosin > phentolamine > yohimbine > tolazoline > clonidine. α_2-Adrenergic receptors appear to be found both on presynaptic and on postsynaptic sites of the adrenergic neuroeffector complex (primarily the former), are extrasynaptic in location on the postsynaptic side, are activated by clonidine > α-methylnorepinephrine > norepinephrine > epinephrine > phenylephrine, and are inhibited by yohimbine > phentolamine > tolazoline >>> prazosin. The β_2 receptors are located primarily on VSM and other smooth muscles as well as presynaptically, are activated by isoproterenol and terbutaline, and are inhibited by propranolol, pindolol, and other nonselective β-receptor blocking agents. So-called selective β_1 blocking agents inhibit VSM responses mediated by β_2 receptors if the concentration of blocking agent is high enough.

A gradient of sensitivity to the α sympathomimetic actions of the catecholamines exists in the microcirculation, that is, metarterioles > precapillary sphincters > terminal arterioles > muscular venules > collecting venules. The capacity of metarterioles and venules to respond to both NE and α-methylnorepinephrine suggests that the microcirculation is endowed with both α_1 and α_2 postsynaptic receptors, but the former are the dominant species (Altura and Hershey, 1967; Altura, 1971a, 1978a,b, 1981; Baez, 1977b; Furness and Marshall, 1974). The microcirculatory VSM,

including the precapillary sphincters, responds in graded fashion to catecholamines (Altura, 1981) and, with the exception of the muscular venules, responds to circulating levels of NE and epinephrine (Chin and Evonuk, 1971). Antagonists of α adrenoceptors block the microvascular responses to NE and other α-adrenoceptor stimulants, confirming the thesis that the contractile responses to these agents are mediated by α-receptor stimulation (Altura, 1978a,b, 1981; Miller and Harris, 1975).

A controversy exists concerning the *in vivo* and *in vitro* sensitivity of the venules of the microcirculation to catecholamines, in which the veins appear to be as sensitive or more so than the arteries to catecholamines, whereas with topical administration the venules appear to be less responsive than the arteries (Shepherd and Vanhoutte, 1975; Vanhoutte, 1978; Miller and Weigman, 1977; Greenberg *et al.*, 1973b, 1981; Greenberg and Kadowitz, 1982; Kadowitz *et al.*, 1982a,b; Altura, 1978a,b, 1981). Altura (1981) argues that mesenteric and cremaster arterioles close more and exhibit a greater sensitivity than venules to NE. However, the smallest microscopic venules, endowed with pericytes, are more sensitive to NE than larger venules without pericytes. Therefore, pericytes may contribute to the overall venous response to catecholamines. Although the argument explains the differences in sensitivity of cremaster and mesenteric venules and microscopic venules, it does not answer the question of why differences exist in the *in situ* and *in vitro* studies with topical application. It is possible, but speculative, that differences in rates of diffusion of NE, topography of receptors, or other modulating influences *in vivo* can override the differences in sensitivity of the VSM itself to NE. Moreover, species differences cannot be ruled out. Finally, it is difficult to expect total venous closure in the face of an adequate distending pressure. It is possible that if tension could be measured in these microvessels, or pressure with microtechniques, then the order of sensitivity of the venules might be found to increase more so than the arteries. Further studies must resolve this conflict.

The VSM of the microcirculation appears to be endowed with β adrenoceptors, which mediate vasodilation, suppression of the responses to adrenergic nerve stimulation, and inhibition of the responses to NE and 5-HT (Shepherd and Vanhoutte, 1975; Vanhoutte, 1978). Microvascular β receptors have been found in the mesenteric, hepatic, pancreatic, cerebral, renal, skeletal, and adipose tissue microvascular beds in numerous rodent species (for references see Altura, 1978a,b, 1981). Miller and Harris (1975) failed to find evidence of isoproterenol-induced vasodilation of the bat microcirculation. β-Receptor-mediated vasodilation of arterioles, but not venules, is exquisitely sensitive to inhibition of aging animals (Fleisch and Hooker, 1976; Altura and Altura, 1977b). The absence of responses in

the experiments of Miller and Harris (1975) cannot be due to aging since neither artery nor vein responded to isoproterenol. It is possible that β adrenoceptors may not be present in the microcirculation of all strains and species. Whereas the rat arterioles and venules are endowed with β receptors, as are those of man, the bat may be devoid of this mechanism for vasodilation.

Mechanism of Action of Catecholamines

α-Adrenoceptor-mediated vasoconstriction produces many events within the VSM cell. Some of these events are primary in the process of excitation and contraction, and some merely subserve the contractile process, whereas one mechanism may tend to limit the magnitude of contraction or vasoconstriction.

In VSM in which spontaneous electrical activity is coupled to mechanical activity, NE increases the rate of firing of the action potential and increases mechanical activity (Johansson *et al.*, 1967; Nakajima and Horn, 1967; Keatinge, 1976, 1979). In normally electrically quiescent VSM, NE appears to depolarize the VSM partially and elicit contraction, whereas high concentrations of NE produce an even greater depolarization, equivalent to that of removing calcium from the physiologic salt solution bathing the VSM (Haeusler, 1972, 1973; Mekata and Niu, 1972; Keatinge, 1979; von Loh and Bohr, 1973; Haeusler and Thorens, 1980a,b). Associated with the electrical and mechanical activity of the VSM cell in response to NE is an increase in sodium and calcium influx or, in some VSM, an increase in the release of calcium from sequestered intracellular sites and an increase in potassium efflux from the cell (Guignard and Friedman, 1970; Jones, 1973; Haeusler and Thorens, 1980a,b). The increased influx of calcium ion is believed to occur via both voltage-dependent and voltage-independent calcium channels (Weiss, 1977, 1981a,b) and may also be associated with an increase in magnesium ion into the cell (Greenberg, 1981c). It is now believed that the increased calcium ion interacts with and binds to calmodulin. The calcium–calmodulin complex binds to the dephosphorylated form of myosin light-chain kinase, which is inactive, to form the activated complex calcium–calmodulin–MLCK, which then phosphorylates the higher molecular weight myosin light chain (*p*-myosin). *p*-Myosin is activated by actin and further activated as actomyosin by tropomyosin, with subsequent hydrolysis of ATP, liberation of the energy, and promotion of the sliding-filament mechanism (see Section II,J). Subserving this contraction are the concomitant biochemical changes produced by NE via α-adrenergic receptor activation,

namely, stimulation of glycogenolysis and phosphorylase, and oxygen consumption, which generates ATP from both ADP and breakdown of preformed glycogen and lipid stores (Weston, 1972; Namm, 1971; Diamond, 1978; Paul, 1980). In addition, the aerobic glycolytic pathway, stimulated by NE, may aid in preventing the dissipation of the sodium–potassium gradient by providing energy for the sodium–potassium pump mechanism (Paul, 1980; Hellstrand and Paul, 1980, 1982). The increase in cyclic AMP observed with NE in VSM is not found with phenylephrine and so may be mediated via β adrenoceptors in the VSM (Dunham *et al.*, 1974).

Along with the contractile response to NE is an associated increase in the efflux of prostaglandins from the VSM cell (McGiff *et al.*, 1972; Needleman *et al.*, 1973a,b; Needleman, 1976; Needleman and Isakson, 1980; Greenberg and Kadowitz, 1982). It has been suggested that the prostanoids may limit the magnitude of the contractile response to NE, thus protecting the VSM against excessive contraction. Evidence supporting this thesis is that many inhibitors of prostanoid synthesis enhance the magnitude of NE-induced contraction and enhance the sensitivity of the VSM to this amine (for references see Needleman, 1980; Greenberg, 1982a,b). However, more specific inhibitors such as ETYA do not affect the contractile response of VSM to NE (Goldberg *et al.*, 1975a,b, 1976; Greenberg and Kadowitz, 1982). It is possible that nonspecific perturbations of the membrane produced by vasoconstriction result in the release of prostanoids or that the release of prostanoids from the VSM *in vitro* may reflect the terminal events of a tissue undergoing degeneration and death (Vanhoutte, 1980). Further studies are necessary to evaluate the significance of prostanoid release by NE in VSM.

It has been suggested that the interaction of epinephrine or isoproterenol with β receptors of the VSM may result in an increase in adenylate cyclase activity and cyclic AMP, which both stimulates calcium sequestration by subcellular organelles, such as sarcoplasmic reticulum and mitochondria, and stimulates calcium efflux from the cell. Both of these events decrease the free, ionized calcium concentration, decrease the interaction of calcium with calmodulin, and reverse the contractile events outlined above. In addition, cyclic AMP is synthesized from ATP and may decrease the ATP available for myosin phosphorylation. Finally, cyclic AMP binds to the regulatory subunit of inactive protein kinase to expose the catalytic subunit of protein kinase. This catalytic unit of protein kinase phosphorylates myosin light-chain kinase. Because the phosphorylated form of the myosin light-chain kinase is less capable of binding with calmodulin the myosin light-chain kinase is, in effect, inactivated. A

decreased myosin kinase activity results in a decreased phosphorylation of myosin. Since dephosphorylated or unphosphorylated myosin cannot interact with actin, VSM tension declines.

B. Serotonin

Serotonin released from the vascular wall or the platelets may act as a local modulator of microcirculatory tone (Page, 1968; Jarrott *et al.*, 1975; McGrath, 1977, 1978). Serotonin can directly affect the VSM or act indirectly through adrenergic nerves to increase or decrease neurogenically mediated tone (Haddy *et al.*, 1959; Page, 1968). Serotonin accumulates in adrenergic nerve terminals and dense granules believed to be the intraneuronal storage sites for NE (Nishino *et al.*, 1970; Snipes *et al.*, 1968). Densely innervated blood vessels accumulate both 5-HT and NE in neuronal and extraneuronal storage sites within the VSM (Berkowitz *et al.*, 1975; Curro and Greenberg, 1982a,b; Thoa *et al.*, 1969; Weiss and Rosecrans, 1971a,b).

Serotonin also directly activates the VSM. The contractile responses of VSM to 5-HT are believed to result from an interaction between 5-HT and structurally specific protein moieties (receptors) on the VSM membrane (Freyburger *et al.*, 1952; Innes, 1962; Innes and Kohli, 1970; Page, 1968). McGrath (1978) suggested that the contractile responses of VSM and non-VSM preparations to 5-HT are, in part, mediated by an indirect sympathomimetic effect of 5-HT. 5-Hydroxytryptamine also interacts with the postsynaptic α receptors of VSM. Receptors for 5-HT exist in VSM but cross-reactivity between 5-HT and α receptors exists in some 5-HT and α-receptor agonists and antagonists (Apperley *et al.*, 1976, 1977, 1980; Clement *et al.*, 1969; Curro *et al.*, 1978; Edvinsson and Owman, 1977; Edvinsson *et al.*, 1978; Gyermek, 1966; Offermeier and Ariens, 1966a,b; Wakade *et al.*, 1970). Clement *et al.* (1969) suggested that the parallelism between 5-HT- and NE-induced contractions of VSM indicates that 5-HT and NE may interact with a single population of α receptors or a population of receptors that are very similar. The same conclusions were reached by many investigators utilizing a diverse group of VSM and other smooth muscle preparations (Apperley *et al.*, 1976, 1977, 1980; Clement *et al.*, 1969; Curro *et al.*, 1978; Edvinsson and Owman, 1977; Edvinsson *et al.*, 1978; Gyermek, 1966; Offermeier and Ariens, 1966a,b; Wakade *et al.*, 1970).

Studies indicate that two classes of 5-HT receptors exist in VSM (Curro *et al.*, 1978; Apperley *et al.*, 1980; Black *et al.*, 1981). 5-HT_1 receptors are activated by 5-HT and inhibited by methysergide (MSG), and 5-HT_2 receptors are activated by 5-HT and MSG, inhibited by α-receptor antago-

nists, and refractory to inhibition by MSG. Removal of extracellular magnesium ion (Mg_o) from physiologic salt solutions (PSS) bathing VSM also inhibits contractile responses to 5-HT (Goldstein and Zsoster, 1978), but it is uncertain whether this is an effect on 5-HT_1 or 5-HT_2 receptors.

Curro *et al.* (1978) and Curro and Greenberg (1982a,b) examined the interaction of adrenergic nerves, calcium and magnesium ion, pH, and sulfhydryl group reagents on the receptors for 5-HT in rodent and canine arteries and veins. In the rat mesenteric arteries (RMA), inhibition of neuronal reuptake and extraneuronal reuptake of NE with cocaine and hydrocortisone, respectively, enhanced the responses of the RMA to 5-HT. Depletion of NE with reserpine (1.5 mg/kg daily for 6 days) and inhibition of NE release with guanethidine did not inhibit the responses of RMA to 5-HT. These data support the conclusion that 5-HT is sequestered and/or inactivated by neuronal and extraneuronal mechanisms similar to that of NE but that the contractile responses of the RMA to 5-HT are not mediated by an indirect sympathomimetic effect of 5-HT. The responses of the RMA to 5-HT were inhibited by methysergide, a 5-HT receptor antagonist, and by the α-adrenergic receptor antagonists phentolamine and yohimbine. Tolazoline and prazosin did not affect the responses of RMA to 5-HT. These data support the conclusion that the 5-HT receptor of RMA is a 5-HT_1 receptor but that α_2 antagonists exert cross-reactivity with the 5-HT_1 receptor. An increase in the concentration of extracellular calcium ion (Ca_o) from 0.4 to 3.2 m*M* did not affect the sensitivity of RMA to 5-HT but decreased the antagonistic potency of phentolamine and methysergide. Reductions in the concentration of extracellular magnesium ion (Mg_o) depressed the responses of the RMA to 5-HT and decreased the blocking potency of methysergide and phentolamine. These data support the conclusion that Ca_o and Mg_o are involved in the interaction of 5-HT with 5-HT_1 receptors in RMA and that the 5-HT receptor contains a labile, sulfhydryl, or disulfide bond.

The contractile response of the canine dorsal metatarsal vein (DMV) to 5-HT differed in some aspects from that of the RMA. Agonist–antagonist interactions and ion–agonist and antagonist interactions were evaluated against the contractile responses to 5-HT and phenylephrine and the specific binding of 5-[2-^{14}C]HT to intact DMV. Decreases and increases in pH from pH 7.4 selectively reduce or abolish the responses to 5-HT and MSG. Reduction of disulfide bonds with dithiothreitol also selectively inhibits 5-HT- and MSG-induced contractions of DMV. These changes were associated with concomitant alterations in 5-[2-^{14}C]HT binding and decreases in the affinity for the 5-HT receptor. Reductions in extracellular sodium ion enhanced the contractile responses to 5-HT and MSG, whereas alterations in extracellular potassium ion did not affect the con-

tractile responses of DMW to 5-HT or MSG. Similarly, reductions in extracellular sodium resulted in an increase in 5-[2-^{14}C]HT binding followed by a decrease in this parameter. Alterations in extracellular potassium ion did not affect 5-[2-^{14}C]HT binding at physiologic concentrations of potassium ion. Increases in extracellular calcium ion from 0.8 to 3.2 m*M* did not affect the sensitivity (ED_{50}) of the DMV to 5-HT or MSG but decreased the blocking potency of the antagonists against 5-HT. Reductions in concentration of extracellular magnesium ion from 2.4 to 0 m*M* lowered the ED_{50} for 5-HT but decreased the blocking potency of the antagonists against 5-HT. Increases in extracellular calcium ion concentration did not affect the specific binding of 5-[2-^{14}C]HT to DMV, whereas increases in extracellular magnesium ion concentration from 0 to 2.4 m*M* increased the affinity and maximum binding capacity of 5-[2-^{14}C]HT in DMV. The data support the conclusion that the 5-HT_2 receptor of DMV contains a pH-sensitive, labile, ionizable binding site either containing a reducible disulfide group or maintained in a specific conformation by a disulfide group which is an integral moiety of the 5-HT_2 receptor complex. This receptor complex is not modulated by physiologic concentrations of monovalent cations but is subject to modulation by divalent cations. Since extracellular calcium ion modulates the sensitivity of the antagonists for 5-HT but not 5-HT itself, the data support the conclusion that extracellular calcium ion may allosterically modify the 5-HT_2 receptor complex of DMV. Extracellular magnesium ion alters the sensitivity of the DMV to 5-HT, MSG, and 5-HT receptor antagonists and alters both the affinity and maximum binding capacity of the 5-HT_2 receptors of DMV. These data support the conclusion that magnesium ion is an integral component of the 5-HT_2 receptor complex in DMV.

Kier (1968) and Korolkovas (1970) postulated that a three-point attachment of 5-HT at its receptor site involves the terminal and indole nitrogen atoms and the hydroxyphenyl ring of the indole moiety as absolute requirements for the binding of 5-HT with its receptors in smooth muscle. The minimal steric requirement for α-adrenergic receptor activity appears to be the interatomic distance between the phenyl ring and the terminal amino group on the α-carbon atom (Belleau, 1967). Phentolamine could interact with both postulated receptors because it possesses a hydroxyl group on the 3 position of the phenyl ring, a tertiary amine group, and a second nitrogen atom on the imidazole ring. Similarly, yohimbine possesses a complex ring structure, which also allows it to have three-point attachment to a 5-HT receptor. In contrast, prazosin and tolazoline do not possess a third ring structure allowing for this three-point attachment. Thus, the presence of the third ring or nitrogen atom may enable α-adrenergic receptor blocking agents to interact with 5-HT receptors.

The serotonergic receptor is composed of anionic and cationic binding sites for the complementary attachment of cationic and anionic sites of the serotonergic receptor agonists (Kier, 1968; Korolkovas, 1970). Changes in pH should affect the ionization of the charged amino acid moieties within the peptide chain of the receptor complex. This should result in a decreased number of active binding sites available for interaction with the complementry charged moieties of the 5-HT receptor agonists (Kier, 1968; Korolkovas, 1970). It is clear from the data presented that alkalosis and acidosis resulted in a preferential inhibition of the contractile responses of DMV to 5-HT and MSG, and not to phenylephrine. Moreover, alterations in pH depressed the maximum specific 5-[2-^{14}C]HT binding and increased the K_D (decreased the affinity) of the 5-HT receptor for its substrate. Therefore, the data support the conclusion that the 5-HT_2 receptors of DMV contain pH-sensitive peptide linkages or ionizable groups that either directly, or indirectly at an allosteric site, modulate the binding of 5-HT to its receptor. Moreover, the data add further support for the concept that the 5-HT and α-adrenoceptors differ within both RMA and DMV, as well as other VSM.

It may be argued that alkalosis and acidosis depress VSM contractility by affecting the release of calcium ion from the sarcoplasmic reticulum or by changing the plasticity of the contractile proteins (Alexander, 1968). The decrease in specific 5-HT binding with changes in pH could then be related to degradation of the 5-HT from the pH changes itself. Since the contractile responses of DMV to phenylephrine were not inhibited by the alkalotic or acidotic solution, it is unlikely that pH-induced alterations in the plasticity of DMV or changes in the release of calcium ion from the sarcoplasmic reticulum can explain the preferential inhibition of 5-HT-induced contraction of DMV.

DTT reduces disulfide bonds to form reduced sulfhydryl groups within the VSM (Freidman, 1973; Greenberg *et al.*, 1976). The formation of disulfide bonds between the oxidized form of DTT and the reduced sulfhydryl groups of the DMV should be minimal since the redox potential of this type of reaction would be against an electrochemical potential gradient. Thus, the selective inhibition of MSG- and 5-HT-induced contractions of DMV by DTT and DTNB as well as their inhibitory effects on 5-[2-^{14}C]HT binding supports the conclusion that a disulfide bridge(s) is an integral component of the 5-HT_2 receptor of DMV. The reversal experiments with DTNB also support this conclusion.

DTNB alkylates both sulfhydryl and disulfide groups and inhibits the contractile responses of DMV to each of the agonists tested. However, when added to DMV in which the disulfide groups have been reduced with DTT, DTNB reverses the inhibitory effect of DTT on 5-HT- and

MSG-induced contractions of DMV. This effect is probably related to the formation of a redox potential chain among the VSM sulfhydryl groups, oxidized DTT, and DTNB (Freidman, 1973). With other reactions, such as the formation of a nitrobenzoate–sulfhydryl complex, the effect would be equivalent to alkylation of the reduced sulfhydryl groups with a resultant abolition of the responses to 5-HT and MSG (Freidman, 1973). Therefore, the data with DTT and DTNB support the conclusion that a disulfide bridge is an integral moiety of the 5-HT_2 receptor complex in DMV.

Alterations in $[Na_o]$ from 120 to 160 m*M* and changes in $[K_o]$ from 2 to 10 m*M* did not affect the contractile responses of the DMV to 5-HT or MSG and did not affect the binding of 5-[2-^{14}C]HT in any significant manner. The small changes in the contractile responses of the DMV to 5-HT and MSG could be related to alterations in calcium permeability produced by the changes in $[Na_o]$ since they also affected the contractile responses to phenylephrine in a similar manner (van Breeman *et al.*, 1979). Moreover, the changes in contractile responses of the DMV to 5-HT were not accompanied by changes in either the maximum binding capacity or affinity of the 5-HT receptors within DMV. In addition, alterations in $[Na_o]$ and $[K_o]$ did not affect the antagonist–agonist interaction between 5-HT and phentolamine or cyproheptadine. Thus, it is unlikely that physiologic concentrations of monovalent ions such as Na_o or K_o modulate the 5-HT_2 receptors of DMV.

The roles of Ca_o and Mg_o as modulators of the 5-HT receptors in VSM remain poorly defined. Although a wide variety of calcium-channel-blocking agents inhibit NE- and 5-HT-induced contraction (Triggle, 1976; van Nueten *et al.*, 1978, 1980, 1981; Weiss, 1978, 1981a,b), these experiments do not delineate a direct association between the divalent ions and the 5-HT or NE receptors of VSM. Removal of calcium from PSS inhibits the protective effect of NE against dibenamine blockade of α receptors in rabbit thoracic aorta. This suggests that the NE–receptor interaction is a calcium-dependent process. The pA_2 for the NE–phentolamine interaction in rat thoracic aorta is decreased by calcium-displacing local anesthetics. This suggested that these local anesthetics did not inhibit agonist binding to the receptor but acted allosterically to decrease the affinity of both agonist and antagonist to their receptor (Triggle, 1976; Curro and Greenberg, 1982a,b).

Increases in $[Ca_o]$ from 0.8 to 3.2 m*M* decreased the responses of DMV to phenylephrine but not to 5-HT and MSG. Alterations in $[Ca_o]$ did not affect the maximum binding capacity of affinity of 5-HT for its receptors within DMV. Yet the inhibitory effect of α-adrenoceptor antagonists on 5-Ht and MSG was diminished to a far greater extent than was the inhibitory effect against phenylephrine. Although we did not measure the bind-

ing of the antagonist to DMV, since alterations in $[Ca_o]$ did not affect the agonist–5-HT interaction within DMV yet diminished the antagonist–agonist interaction for these 5-HT_2 receptors, the data support the conclusion that Ca_o appears to modify allosterically the binding of the 5-HT_2 receptor antagonists to sites within the receptor complex. These sites do not appear to be essential for the interaction of 5-HT with its receptor in DMV.

Magnesium ion exerts diverse and complex actions on VSM tone and contractile responses to vasoactive agents. Magnesium ion is essential for the activity of many enzyme systems and exerts both a complementary and opposing action with calcium ion in many biological systems (Altura and Altura, 1974a,b, 1976, 1981). Elevations in $[Mg_o]$ result in competition with Ca_o for binding sites on the sarcolemmal membrane of VSM (Weiner *et al.*, 1980), thereby regulating myogenic tone (Altura and Altura, 1981; Weiner *et al.*, 1980). Removal of Mg_o enhances spontaneous contractions of VSM by increasing passive inward calcium permeability (Weiner *et al.*, 1980), yet relatively selectively decreases the contractile responses of VSM to prostanoids (Altura *et al.*, 1976; Greenberg, 1981c) and 5-HT (Curro and Greenberg, 1982a,b; Greenberg and Curro, 1982a,b; Goldstein and Zsoster, 1978). Moreover, increases in $[Mg_o]$ above 4.8–7.2 m*M* depress the contractile responses of DMV to many agonists in a nonequivalent manner, probably by suppressing membrane excitation and inward calcium permeability and diminishing a pool of activator calcium ion derived from the membrane-bound or extracellular compartment of VSM (Weiner *et al.*, 1980). The relationship of $[Mg_o]$ to the 5-HT receptors of VSM has not been established. Decreases in $[Mg_o]$ depress the contractile responses of rabbit and canine VSM to 5-HT and decrease the sensitivity to this indole-alkylamine (Goldstein and Zsoster, 1978). This suggested that Mg_o may directly modulate the 5-HT receptor of VSM. The data presented herein confirm and extend this observation. Contraction of the DMV by 5-HT and MSG, the antagonistic activity of 5-HT_2 receptor antagonists, and the K_D and maximal binding capacity for 5-[2-^{14}C]HT were directly dependent on $[Mg_o]$ over the range 0–4.8 m*M*. This suggests that Mg_o is directly involved in binding of 5-HT and antagonists to the 5-HT receptor of DMV. The inhibitory effect of decreases in $[Mg_o]$ on 5-HT-induced contraction of DMV appears to be an expression of the decreased affinity of 5-HT for its receptor and a decrease in the total number of receptors available for contraction. This would suggest that Mg_o may form a complex either within the 5-HT receptor, thereby maintaining the conformation of the 5-HT receptor complex, or between the receptor and 5-HT.

A decrease in the contractile responses of DMV to 5-HT occurs with $[Mg_o]$ above 4.8 m*M* despite the increased binding of 5-[2-^{14}C]HT to

DMV. This anomalous action of Mg_o may result from a nonspecific depressant effect of this ion on membrane excitability and subsequent calcium entry into, or mobilization within, DMV. The depressant effect of Mg_o is also observed on the contractile responses to phenylephrine and on the responses of rat VSM to KCl, NA, and other agonists (Altura and Altura, 1981). Since reductions in $[Mg_o]$ do not affect the agonist–antagonist interaction of the α-adrenoceptors nor the contractile responses of DMV to phenylephrine, it is unlikely that Mg_o is an integral moiety within the α-adrenoceptor complex. Thus, the suppression of the maximal contractions to phenylephrine, 5-HT, and MSG by elevated $[Mg_o]$ must reflect a nonspecific depressant action on the processes of excitation or contraction region of the receptor complex to which the indole moiety of the 5-HT molecule was bound, whereas the site of Mg_o would be the region of the terminal amine group. The binding of divalent ions to these sites could decrease the affinity of the antagonists as well as the agonists.

According to the Kier–Korolkovas model of the 5-HT receptor, Ca_o should bind to the portion of the 5-HT receptor to which the indole moiety was bound, since this should also be the anchoring site for antagonists. It is unlikely that Ca_o would bind to the site in the region to which the terminal amino group attaches because this area would affect agonist-mediated contractions and Ca_o is devoid of this action. A similar type of interaction has been postulated for the binding of β-adrenoceptor antagonists with the α adrenoceptor (Janis and Triggle, 1974; Olivares *et al.*, 1967).

Extracellular magnesium ion should bind to the portion of the receptor to which the indole or terminal amino nitrogen is bound since the responses to both agonist (5-HT) and antagonists are altered by changes in $[Mg_o]$ and Mg_o alters the binding of 5-[2-^{14}C]HT to DMV. Since the three-point attachment appears to be obligatory for 5-HT binding to its receptors, a deficiency in Mg_o at these sites could explain the capacity of magnesium to modulate the responses to 5-HT_2 receptor agonists but not α-adrenoceptor agonists in DMV.

When the present study is viewed along with the previous studies of Altura and Altura (1981), Curro and Greenberg (1982a,b), Goldstein and Zsoster (1978), and Weiner *et al.* (1980), it becomes clear that the atypical 5-HT_2 receptors of DMV are diverse and complex but do not differ from 5-HT_1 receptors in their sensitivity to each of these interventions (Curro and Greenberg, 1982a).

In summary, then, four major factors determine the overall VSM response to 5-HT and its antagonists. These include calcium ion, magnesium ion, pH, and disulfide-reducing agents. The concentration of Mg_o is of primary importance in determining the sensitivity of the DMV to 5-HT and its antagonists. This divalent ion can enhance the sensitivity and en-

hance or depress the contractile responses and pA_2 values of the antagonist, depending on its concentration in PSS and the sensitivity of the membrane to its stabilizing actions. The effects of Ca_o are more discreet, in that they alter the antagonist interaction with the 5-HT receptors rather than the agonist. Disulfide groups and ionizable, pH-labile peptide or perhaps disulfide linkages appear to play an important role in maintaining the integrity of the 5-HT_2 receptor in DMV. The relationships between these factors and the functional integrity of the 5-HT_2 receptor deserve careful consideration in the evaluation of responses of the microcirculation to 5-HT in both normal and pathophysiologic conditions. The absence of an effect of 5-HT antagonists may not indicate the absence of 5-HT receptors but rather the predominance of a specific type of 5-HT receptor in the microcirculation.

The following question must be raised. If little difference exists between the 5-HT_1 and 5-HT_2 receptors other than antagonist sensitivity, do these populations of receptors really differ? The contractile responses of VSM from different vascular beds within a multitude of species to 5-HT are inhibited by α-adrenergic receptor antagonists (Apperley *et al.*, 1976, 1980; Clement *et al.*, 1969; Curro and Greenberg, 1982a,b; Curro *et al.*, 1978; Edvinsson *et al.*, 1978; Humphrey, 1978; Innes and Kohli, 1970; Offermeier and Ariens, 1966a,b; van Neuten *et al.*, 1981; Wilton and McCalden, 1977). Based on studies performed in canine DMV (Curro *et al.*, 1978; Humphrey, 1978) and rabbit aortas and ear arteries (Apperley *et al.*, 1976, 1980), the concept emerged that classical, MSG-inhibitable (5-HT_1) and atypical, MSG-resistant, α-adrenergic receptor-sensitive (5-HT_2) 5-HT receptors existed in VSM.

As an alternative to postulating distinct 5-HT_1 and 5-HT_2 receptors in VSM, the capacity of different antagonists to inhibit 5-HT responses in VSM could be explained on the basis of the Monod *et al.* (1965) and Karlin (1967) two-state model for agonist–antagonist binding to their specific receptors. According to this model an agonist and antagonist bind to two distinct conformations or states of the same receptor. These two states are interconvertible and in equilibrium to various degrees. It is possible that in some VSM the antagonist form of the receptor is converted to the agonist form. This would increase the binding of 5-[2-^{14}C]HT to the agonistic form of the receptor, thereby accounting for the increased 5-[2-^{14}C]HT binding. It would also account for the decreased blocking activity of cyproheptadine. Moreover, MSG is a partial agonist. According to the Monod–Karlin transition-state model MSG has affinity for both states of the receptor. A transition of the 5-HT receptor from the antagonist to the agonist state increases the contractile response to MSG and decreases its blocking potency. This transition model can also explain the blocking po-

tency of α-adrenoceptor antagonists against 5-HT, if it is assumed that these antagonists possess affinity for the agonistic form of the receptor but not the antagonist form. Serotonin, under normal conditions, would have a greater affinity than 5-HT for the receptor. When many receptors are present, sufficient antagonist binds and overcomes the contractile responses to 5-HT. Further studies are required to delineate the mechanism of the change in 5-HT receptors in VSM.

The mechanism by which 5-HT elicits contraction of VSM appears to resemble that of NE. However, the responses to 5-HT appear to be more dependent on extracellular calcium ion and cell metabolism than are the responses to NE (Greenberg *et al.*, 1974; Altura and Altura, 1970; van Neuten *et al.*, 1980, 1981). 5-Hydroxytryptamine is a more potent contractor of venules than of arterioles and can cause venostasis (Altura and Hershey, 1967).

C. Angiotensin

Angiotensin II is a potent contractile substance in arteriolar and precapillary arteriolar VSM, as well as larger arteries, but is a poor agonist on most veins (Bohr and Uchida, 1967; Bohr, 1973; Bohr *et al.*, 1978; Peach, 1977; Messina *et al.*, 1975; Altura and Altura, 1977b). The notable exception to the venocontractile action of angiotensin II is the portal vein, in which angiotensin is a potent contractile agent. The order of sensitivity of most arterial smooth muscle to angiotensins is angiotensin II >>> angiotensin III >>> angiotensin I. Contractile responses to angiotensin I may be mediated by its conversion to angiotensin II by the endothelium or by the smooth muscle (Hofbauer, 1973; Itzkowitz and McGiff, 1974) or to an interaction of angiotensin I with angiotensin II receptors on the VSM. The maximal contractile responses of VSM to angiotensin II are usually significantly less than the contractile responses to other contractile substances (Vanhoutte, 1978; Bohr *et al.*, 1978) and may be related to the simultaneous stimulation of prostacyclin and/or PGE_2 from the endothelium and/or VSM (Aiken, 1973, 1974; for other references see Needleman and Isakson, 1980). Indomethacin and other inhibitors of prostanoid synthesis enhance the contractile responses to angiotensin II and prevent the characteristic "fade" response normally observed in VSM (Aiken, 1973; Needleman *et al.*, 1973a,b; McGiff *et al.*, 1975; Needleman and Isakson, 1980).

Angiotensin II exhibits two major characteristics in its contractile response of VSM fade: the diminution of the response despite the maintained concentration of angiotensin I at its receptor site and tachyphyllaxis, the diminution of the response to the repeated administration of the

same concentration of angiotensin II (for references see Bohr *et al.*, 1978; Gross, 1976). Fade may be related to the simultaneous production of vasodilator prostanoids from the VSM which attenuates the pressor response to angiotensin II, which tachyphyllaxis appears to represent the maintained binding of angiotensin II to its receptor site, resulting in autoinhibition to subsequent challenges with angiotensin II. Khairallah *et al.* (1966) showed that washing the VSM with an acidic solution restored the responses of rabbit aorta to angiotensin II at a time when tachyphyllaxis was evident. This procedure resulted in a decline in the binding of angiotensin to the rabbit thoracic aorta. When the blood vessel was reincubated in normal PSS at pH 7.4, the responses to angiotensin II returned. This indicates that tachyphyllaxis is probably a physical phenomenon associated with excessive binding of angiotensin II at its receptor site.

In the microcirculation, angiotensin II seems to exhibit primarily fade (Messina and Kaley, 1982). The latter authors suggested that the *in vivo* system augments prostanoid production so that fade and tachyphyllaxis are readily evident and prostanoid dependent. Under *in vivo* conditions, in which prostanoid production is less than that *in vitro*, tachyphyllaxis and fade are both time and concentration phenomena and are prostanoid independent. Messina *et al.* (1976) evaluated tachyphyllaxis in the whole animal and showed that indomethacin did not interfere with tachyphyllaxis. Moreover, Messina *et al.* (1975) showed that angiotensin II contracted skeletal muscle microvascular smooth muscle only in damaged preparations but did not reduce arteriolar diameter in undamaged preparations. Their results seem to indicate that tachyphyllaxis and the degree of involvement of the prostanoid system in the vascular responses to angiotensin II may be dependent on the degree of vascular damage or unphysiologic nature of the preparation under study.

In addition to its capacity to affect the VSM of the microcirculation directly, angiotensin II may modify microvascular smooth muscle function, including that of the innervated venules, by enhancing the release of NE from adrenergic nerve terminals (Zimmerman *et al.*, 1973; Kadowitz *et al.*, 1975b) and by enhancing the postsynaptic, α-adrenergically mediated vasoconstrictor response to NE (Peach, 1977), as well as by inhibiting the uptake of NE into adrenergic nerves (Peach, 1977; Westfall, 1977). These secondary actions of angiotensin II occur in concentrations below that required to activate the angiotensin II receptors directly in the VSM, suggesting the possibility that these actions of angiotensin may be as important in modulating microcirculatory function as is its direct pressor action.

The receptors for angiotensin II are disulfide dependent, similar to those for 5-HT and neurohypophyseal peptides (see below) and some

prostanoids (Greenberg *et al.*, 1976; M. Johnson and Ramwell, 1973; M. Johnson *et al.*, 1973, 1974a,b; E. Johnson *et al.*, 1974), since dithiothreitol inhibits the responses to angiotensin II (Fleisch *et al.*, 1973). The angiotensin II contraction of VSM is not only magnesium and sodium dependent, but also calcium dependent (Altura and Altura, 1971; Devynck and Meyer, 1976; Devynck *et al.*, 1974). Increases in extracellular sodium enhance the pressor and contractile responses to angiotensin II, whereas decreases in extracellular sodium ion depress responses to this polypeptide (Wright *et al.*, 1982). Moreover, alterations in sodium ion affect the specific binding of 2-^{14}C- or ^{125}I-labeled angiotensin II to membrane fractions of VSM in a related manner. Thus, a disulfide-dependent, sodium-dependent moiety may be involved in the binding of angiotensin with its receptor. Freer (1977) suggested that calcium ion may be an integral component of the angiotensin II receptor in VSM because, in the absence of calcium ion, the contractile response to angiotensin II was maintained but was shifted to the right. Other studies would seem to suggest that the interaction of angiotensin II with its receptor results from a binding of calcium ion to both the histidyl residue of angiotensin II and perhaps a prolyl or related amino acid moiety on the VSM, which then results in angiotensin II displacing calcium ion from binding sites within the cell membrane (Freer, 1977). Angiotensin II has also been shown to stimulate the efflux of calcium from microsomal vesicles of VSM (Baudouin-Legros and Meyer, 1973; Baudouin-Legros *et al.*, 1972), supporting the contention that angiotensin may release calcium ion from sequestered sites within the VSM. The contractile response to angiotensin then results from a biochemical mechanism similar to that of NE.

Wright *et al.* (1982) studied the regulation of angiotensin II receptors in rat mesenteric arteries. The binding of angiotensin II to membrane fractions of this preparation was stimulated by magnesium and calcium ion as well as sodium ion, whereas potassium ion inhibited the binding of angiotensin II to VSM. Guanosine triphosphate (GTP) and other nucleotides inhibited angiotensin II binding by a mechanism that appeared to be related to their capacity to chelate endogenous cations. This conclusion was reached because chelation of endogenous ions with EDTA inhibited the action of GTP. The monovalent and divalent ions affected receptor affinity, whereas GTP and its analogs affected the maximum binding capacity of the VSM for angiotensin II. Wright *et al.* (1982) concluded that cations and guanine nucleotides affected angiotensin II binding by controlling or modulating at least two distinct portions of the receptor complex. They also suggested that the interaction of these sites with cations and guanine nucleotides could be involved in the modulation of VSM responses to angiotensin II.

D. Neurohypophyseal Peptides

Krogh (1929) demonstrated that topical administration of extracts of posterior pituitary gland contracted the microvessels of the webbed feet of frogs and dog ears. The subsequent demonstration that hypophysectomy resulted in an increased microcirculatory blood flow led Krogh (1929) to postulate that the hormones from the posterior pituitary exerted a tonic vasoconstrictor influence on the peripheral vasculature and microcirculation.

1. Heterogeneity of Responses to Neurohypophyseal Peptides

Vascular smooth muscles exhibit a heterogeneity in their responses to oxytocin and arginine vasopressin, the neurohypophyseal peptides. Although it was generally believed that all VSM preparations were relatively insensitive to vasopressin (Douglas, 1975; Saameli, 1968; Sawyer, 1961; Mellander and Johansson, 1968), the experiments of Altura and Altura (1977b) demonstrated that rat terminal arterioles were sensitive to 1 fmol of vasopressin, three orders of magnitude greater than its sensitivity to angiotensin II (Altura, 1973; Altura and Altura, 1977b). Similar results were found in rat thoracic aortas (Altura and Altura, 1977b). In general, although different VSM from different sources within a single mammalian species exhibits a wide divergence of sensitivities to vasopressin, the smaller the VSM the more sensitive it is to vasopressin (for references see Altura, 1978a,b, 1981). It also appears that the muscle venules are the most sensitive of the microcirculatory blood vessels to vasopressin (Altura and Hershey, 1967; Altura, 1973, 1975a) and respond to circulating concentrations (0.1–1 fmol) of this peptide hormone (Lauson, 1974; Forsling, 1976).

Some of the potential mechanisms of the heterogeneity of response of VSM to vasopressin may result from the differential effects of sex hormones on the receptors for vasopressin within the different VSM preparations or on the VSM from discrete vascular beds. Estrogens may increase the concentration of internal sodium ion and subsequently internal calcium ion. This results is an increased or labilized intracellular calcium pool from which vasopressin may release intracellular calcium ion to elicit contraction, thereby potentiating the effects of vasopressin (Altura and Altura, 1971, 1976). Alternatively, the number and/or structure of the receptors for vasopressin and oxytocin (see Section III,C,4) may be highly individual for different VSM (Altura and Altura, 1971, 1976; Vanhoutte, 1978, 1980). Although it had been thought that neurohypophyseal pep-

tides might act on receptors that mediated both relaxation and contraction, the data now suggest that the relaxant action on these polypeptides resulted from the preservative in the commercially available peptides and that these peptides act only on receptors that mediate contraction (Altura, 1978a,b, 1981).

2. *Mechanism of Action of Neurohypophyseal Peptides*

For both vasopressin and oxytocin, two fairly universal and somewhat unique characteristics appear to modulate the peptide–receptor complex interactions. These are (*a*) a requirement for magnesium ion (Somlyo *et al.*, 1967; Altura and Altura, 1970) and (*b*) a requirement for an intact sulfhydryl group on the receptor (Martin and Schild, 1965; Altura and Altura, 1970). In the absence of magnesium ion the contractile responses of VSM to oxytocin and vasopressin are inhibited and/or abolished. Similarly, disulfide bond reduction inhibits the contractile response to these polypeptide vasoconstrictor agonists. Thus, it seems likely that magnesium ion and a disulfide group are integral moieties of the neurohypophyseal peptide–receptor complex.

The contractile responses of vasopressin and oxytocin are inhibited by ethanol, glucose deprivation, hypoxia, alterations in the external concentration of divalent cations, and male sex hormones but are enhanced by female sex hormones. Extracellular sodium concentrations and potassium ion concentrations do not appear to play a major role in the vasoconstrictor or vasopressor action of these hormones (Altura and Altura, 1977a,b; Altura, 1977, 1978a,b, 1981). Although the factors affecting the action of the neurohypophyseal peptides have been elucidated, little progress has been made in defining the mechanism of action of these substances at the cellular or molecular level in VSM. Both oxytocin and vasopressin, as well as ACh, contract uterine smooth muscle *in vitro*. Carsten (1974) showed that oxytocin may impair calcium sequestration by microsomal fractions of uterus, thereby affecting an increase in free, intracellular calcium ion. Similar actions may occur with vasopressin. The cellular action of these peptides differ from that of other contractile agents, such as ACh, in that they do not cause an increase in membrane permeability to potassium ion (Hodgson and Daniels, 1972). Thus, the neurohypophyseal peptides may contract VSM by combining with magnesium- and disulfide-dependent receptors to cause a decrease in intracellular or microsomal sequestration of calcium ion. This would lead to an increase in free calcium ion, which would then interact with calmodulin, activate the myosin light-chain kinase, and promote actomyosin ATPase activity and the sliding-filament

mechanism involved in VSM contraction (see Section II,J). Further studies are necessary to resolve the mechanism of contraction of VSM by neurohypophyseal peptides.

3. *Interaction of Neurohypophyseal Peptides with Vasopressor Substances*

Vasopressin and oxytocin enhance the pressor responses of catecholamines in dogs, cats, and rats (Bartelstone and Nasmyth, 1965; Berde, 1965; Nakano, 1974; Nash *et al.*, 1961) as well as the microcirculatory responses to epinephrine and NE (Altura *et al.*, 1972; Altura, 1977; Altura and Hershey, 1967) in concentrations that do not exert a pressor or constrictor response themselves. Altura and Altura (1979b) suggested that the primary site of these interactions may be at the level of the microvasculature. This finding may be related either to the capacity of these peptide hormones to increase the level of ionized calcium to threshold levels within the VSM cell or to an inhibitory effect on the processes involved in the termination of action of these amines ($uptake_1$ or $uptake_2$). The mechanism remains to be defined. Nevertheless, the findings that elevated levels of vasopressin may play a role in the pathogenesis and/or maintenance of some forms of experimental hypertension (Berecek *et al.*, 1980, 1982; McCaa *et al.*, 1978), that venous smooth muscle reactivity and contractility are enhanced in hypertension (Greenberg *et al.*, 1978, 1981; Greenberg and Bohr, 1975; Bevan *et al.*, 1975; Simon *et al.*, 1975), and that venous smooth muscle undergoes a decrease in diameter in the microcirculation (Bohlen and Gore, 1978) and hypertrophy (Greenberg *et al.*, 1978; Greenberg and Wilborn, 1982; Greenberg, 1981d) in hypertension suggest that neurohypophyseal peptide–catecholamine interactions at the level of the microcirculation may play a role in the hemodynamic alterations in the pathogenesis of hypertension.

4. *Neurohypophyseal Peptides and the Fetal–Maternal Microcirculation*

That the umbilical arteries and veins, as well as some neonatal VSM, are sensitive to the actions of oxytocin and vasotocin is not an unexpected finding (Altura *et al.*, 1972; Dyer and Gough, 1971; Dyer *et al.*, 1972; Turlapaty and Altura, 1978). The actions of oxytocin and vasotocin are inhibited by low oxygen tensions and by the absence or reduction of estrogens (Altura, 1972b; Altura *et al.*, 1972). The latter investigators have suggested that oxytocin may promote closure of the umbilical cord vessel at term by contracting these vessels in the presence of high oxygen tensions and the action of other hormones, such as the prostanoids. Determination

of the exact role of oxytocin and vasotocin in regulating microcirculatory function in the fetal circulation must await the synthesis of specific antagonists of these substances. Moreover, these inhibitors, once developed, should be studied in the microcirculation of pregnant animals at term with established, vital microcirculatory techniques.

E. Kinins

Bradykinin, lysylbradykinin, and methionyllysylbradykinin are derived from kininogen in plasma and exist as both the prohormone and the active moiety. The synthesis and degradation are described in Section II,E. Kinins can also be released from leukocytes (leukokinins) by lysosomal enzyme activity and are believed to play a role in the microvascular response to tissue injury and anaphylaxis (for reference see Greenbaum, 1976; Movat and Habal, 1976). The kinins can initiate vasodilation and increased capillary permeability to fluids and other vasoactive agents, in addition to stimulating pain receptors (for references see Erdos, 1970; Rocha e Silva, 1970, 1974; Pisano and Austen, 1976). They are released in large quantity in response to anaphylaxis, circulatory shock, and tissue injury, and their synthesis and/or release are impaired by the action of nonspecific proteolysis inhibitors, such as aprotinin (for references see Amunsden, 1976).

Bradykinin (and other kinins) are among the most potent dilators of the microcirculation arteriolar smooth muscle, whereas they contract venular and umbilical arterial and venous smooth muscle *in vivo* in concentrations known to be present under physiologic conditions (Melmon *et al.*, 1968; Altura and Altura, 1977a,b; Altura, 1978a,b, 1981). Moreover, bradykinin, in doses that do not affect the level of VSM tone, inhibits the vasoconstrictor responses of the microcirculation to NE, angiotensin II, and epinephrine, but not vasopressin (Altura, 1978a,b, 1981). Thus, kinins may alter microcirculatory function by a direct and indirect effect on the VSM. In addition, more recent studies suggest that bradykinin may inhibit the release of NE from adrenergic nerves, thereby modulating neurogenically mediated vasoconstriction (for references see Vanhoutte, 1978, 1980).

Bradykinin may contract venous smooth muscle and relax arterial smooth muscle by two potential mechanisms. Kinins have been shown to stimulate the release of vasodilator prostanoids from arterial smooth muscle and constrictor prostanoids from venous smooth muscle (Blumberg *et al.*, 1977; Needleman *et al.*, 1973a,b; Palmer *et al.*, 1973; McGiff *et al.*, 1972, 1975, 1976; Aiken, 1973). It has been suggested that kinins may directly activate phospholipase to promote the release of the prostanoids

(McGiff *et al.*, 1976; Needleman *et al.*, 1976, 1978). The prostanoids released from the endothelium or VSM then mediate the vasoconstrictor or vasodilator response to the kinins (for references see Needleman and Isakson, 1980). Evidence in support of this theory is that some inhibitors of prostanoid biosynthesis, such as indomethacin, reverse bradykinin-induced arterial relaxation and inhibit the venoconstriction produced by the kinins (McGiff *et al.*, 1972, 1975, 1976; Messina *et al.*, 1975, 1976; Needleman *et al.*, 1975). Wong *et al.* (1977) and Terragno *et al.* (1975, 1977, 1978) provided direct evidence for a differential effect of kinins on mesenteric artery and vein prostanoid synthesis. Greenberg and Kadowitz (1982) and Goldberg *et al.* (1975a,b, 1976) failed to inhibit bradykinin-induced venoconstriction in canine mesenteric arteries and veins with some inhibitors of prostanoid synthesis, yet found that indomethacin converted bradykinin-induced relaxation to contraction of the mesenteric arteries. Indomethacin-induced inhibition of bradykinin-mediated relaxation was associated with a reduction in prostanoid synthesis by the mesenteric vessels in response to bradykinin. To determine whether the effects of indomethacin were direct or indirect effects of the inhibitor (Northover, 1972, 1973, 1975), Greenberg and Kadowitz (1982) contracted the arteries with NE and obtained the relaxation in response to bradykinin before and after indomethacin, ETYA, or ETYA administered before indomethacin. The data are summarized in Figs. 20 and 21. Indomethacin inhibited bradykinin-induced relaxation in control mesenteric arteries and in blood vessels in which prostanoid synthesis had been already inhibited with ETYA (Table VII). Thus, the data suggest that bradykinin releases prostanoids, but it may not participate in the vasodilator response to the kinins in all species. Needleman *et al.* (1976) proposed another role for prostanoids released by kinins in the microcirculation of the heart. The kinins may mediate the pain and ischemic changes following anoxia and/or ischemia. The prostanoids may mediate the metabolic events subserving the interaction of kinins with their receptors on smooth muscle.

A second mechanism invoked to explain the actions of kinins on VSM presupposes that the endothelium is also obligatory for kinin-mediated relaxation of VSM (Furchgott *et al.*, 1981). This has been described in detail for Ach (see Section II, E). Although Altura and Chand (1981) failed to inhibit bradykinin-induced relaxation with inhibitors of lipoxygenase synthesis, they also found an endothelial obligation for kinin-mediated relaxation. Figure 15 summarizes the action of kinins and other substances on arterial smooth muscle in the presence and absence of the endothelium. It is quite clear, at least for larger arteries, that, in the absence of the endothelium, mesenteric arteries (0.5 mm OD) will not relax in response to kinins, substance P, or, to a limited extent, adenosine and ATP. The sig-

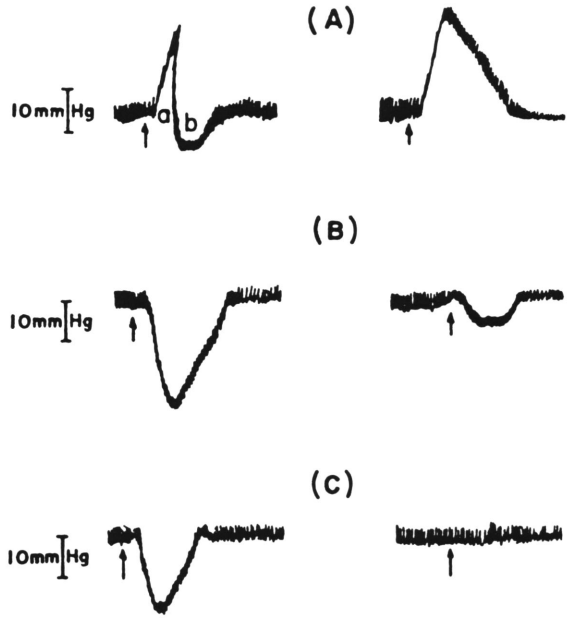

Fig. 20. Typical pressure responses to (A) angiotensin II, (B) bradykinin, and (C) arachidonic acid (controls left) and the effect of indomethacin (right) on these responses. (From Blumberg *et al.*, 1977, with permission.)

	ΔP		
	Control		
Dose	a	b	Indomethacin
Angiotensin II			
50	4 ± 2	−8 ± 1	9 ± 2*
100	17 ± 3	−11 ± 1	33 ± 7*
200	21 ± 4	−15 ± 4	37 ± 11*
	Control		
Bradykinin			
100	−26 ± 3		−8 ± 1*
200	−36 ± 6		−15 ± 4*
400	−37 ± 7		−16 ± 3*
Arachidonic acid			
100	−13 ± 2		0
250	−27 ± 3		−3 ± 3*
500	−42 ± 4		−3 ± 3*

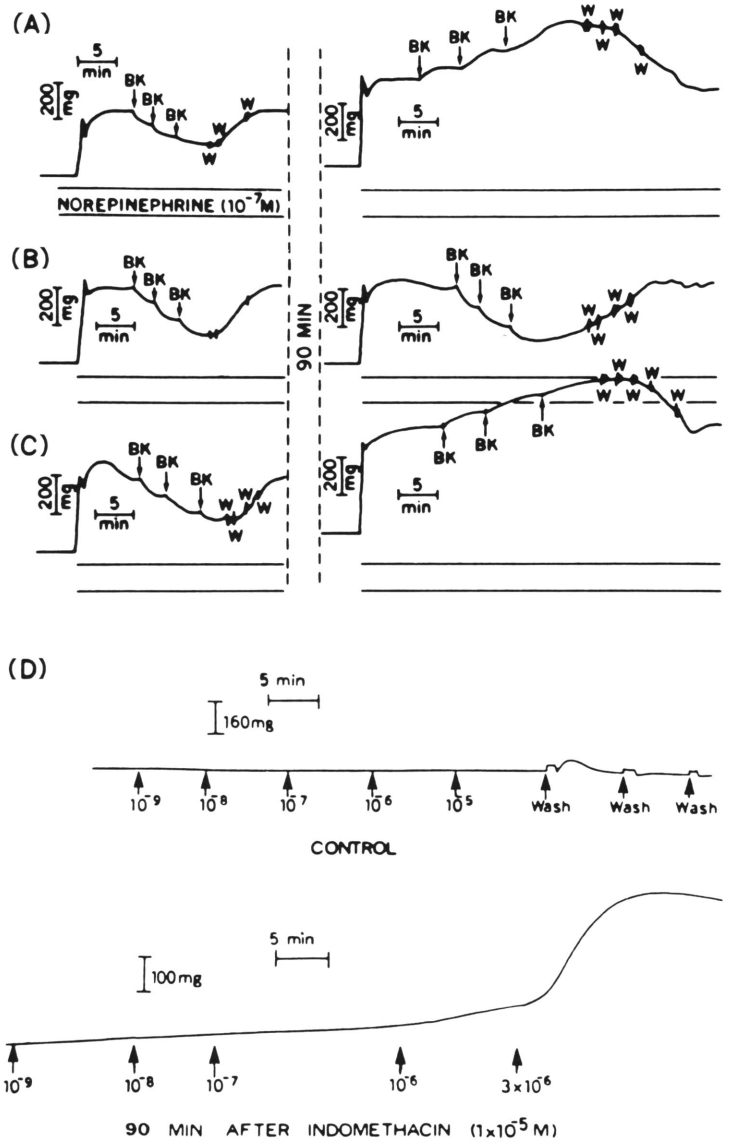

Fig. 21. Effect of indomethacin and eicosatetraynoic acid, alone and in combination, on responses of mesenteric arteries to bradykinin. BK, Bradykinin; W, wash. (From Greenberg and Kadowitz, 1982, with permission.)

TABLE VII

Effect of Indomethacin, Eicosatetraynoic Acid, and Tranylcypromine on Bradykinin- and Norepinephrine-Stimulated Prostaglandin and 6-Keto-$PGF_{1\alpha}$ Synthesis in Canine Arteries and Veins[a,b,c]

Agonist (concentration)	Preparation	INDO[c]	ETYA[c]	TCM[c]	% Inhibition at 10^{-5} M inhibitor			% Inhibition[d] at 10^{-5} M INDO together with 10^{-5} M ETYA
					INDO (PGE_2 + $PGF_{2\alpha}$)	ETYA	TCM (6-Keto-$PGF_{1\alpha}$)	
Bradykinin (10^{-5} M)	Splenic A	2.7 ± 0.6	2.1 ± 0.7	3.3 ± 0.2	99 ± 5	99 ± 3	100 ± 0	99 ± 6
	Mesenteric A	3.6 ± 0.4	3.3 ± 0.5	4.7 ± 0.3	98 ± 2	102 ± 4	100 ± 0	99 ± 7
	Splenic V	2.1 ± 0.5	2.6 ± 0.4	2.6 ± 0.5	98 ± 4	98 ± 4	100 ± 0	99 ± 5
	Mesenteric V	3.4 ± 0.2	2.9 ± 0.5	2.8 ± 0.7	99 ± 5	95 ± 4	100 ± 0	99 ± 4
	Portal V	4.1 ± 0.5	5.2 ± 0.6	2.1 ± 0.9	97 ± 3	99 ± 5	100 ± 0	98 ± 3
Norepinephrine (10^{-5} M)	Splenic A	2.9 ± 0.7	3.6 ± 0.6	2.9 ± 0.4	95 ± 4	101 ± 5	100 ± 0	98 ± 3
	Mesenteric A	3.1 ± 0.5	2.4 ± 0.2	3.7 ± 0.9	98 ± 6	99 ± 3	100 ± 0	99 ± 6
	Splenic V	2.4 ± 0.6	1.9 ± 0.3	2.2 ± 0.4	97 ± 5	98 ± 6	100 ± 0	97 ± 2
	Mesenteric V	4.5 ± 0.9	3.8 ± 0.2	2.6 ± 0.7	98 ± 3	94 ± 5	100 ± 0	99 ± 4
	Portal V	4.8 ± 0.6	5.2 ± 0.4	2.9 ± 0.5	97 ± 5	97 ± 3	100 ± 0	99 ± 3

[a] From Greenberg and Kadowitz (1982) with permission.

[b] Abbreviations: A, artery; ETYA, eicosatetraynoic acid; INDO, indomethacin; PG, prostaglandin; TCM, tranylcypromine; V, vein. Each mean represents the average values obtained from four to seven blood vessels.

[c] IC_{50} = concentration of INDO and ETYA necessary to reduce agonist-induced stimulation of PGE_2 and $PGF_{2\alpha}$ and 6-keto-$PGF_{1\alpha}$ and concentration of TCM necessary to inhibit by 50% the agonist-induced stimulation of 6-keto-$PGF_{1\alpha}$.

[d] Percent inhibition at 10^{-5} M prostaglandin synthesis with both INDO and ETYA present in incubate.

nificance of this finding to microcirculatory function remains to be examined.

F. Histamine

Histamine is a potent mediator of both vasodilation and peripheral edema in experimental animals and man. Although some controversy exists concerning whether histamine can dilate venules as well as arterioles and precapillary sphincters, the concept is generally accepted that the microvascular actions of histamine are mediated both by the classical histamine$_1$ (H_1) and histamine$_2$ (H_2) receptors (Chand and Eyre, 1975; Altura and Halevy, 1978; Owen, 1977; Simionescu *et al.*, 1982). The histamine acting on the microcirculation may be histamine from mast cells, histamine stored in the vascular wall, or histamine released from histaminergic nerves (Beck and Brody, 1961). Alternatively, it has been suggested that adrenergic nerves may stabilize a non-mast-cell pool of histamine. Inhibition of sympathetic nerve activity would then result in a destabilization of this histamine pool, release of histamine, and subsequently histamine-mediated vasodilation and edema formation (Ryan and Brody, 1970; Boerth *et al.*, 1970; Heitz and Brody, 1975) within the microcirculation.

Two distinct receptors mediate the action of histamine on the microcirculatory VSM: H_1 receptors blocked by classical antihistamine blocking agents and histamine receptors blocked by cimetidine and metiamide (for references see Black *et al.*, 1972; Taylor and Richelson, 1982; Altura, 1978a,b, 1981; Vanhoutte, 1978). Altura and Halevy (1978) reported that equivalent topical doses of histamine increased vascular diameter in each of the components of the microcirculation. The order of magnitude of the dilation was arterioles > precapillary sphincters >> venules. The action of histamine is mediated by both H_1 and H_2 receptors since both pyribenzamine and metiamide inhibit the microcirculatory response to histamine (Chand and Eyre, 1975; Altura and Halevy, 1978; Owen, 1977). Antihistamines also contract microvascular VSM, both due to a direct effect on the smooth muscle and by virtue of their capacity to inhibit a dilator component of endogenous, circulating histamine (for references see Altura and Altura, 1974a,b; Altura, 1978, 1981; Vanhoutte, 1978). Thus, histamine released from mast cels, nonneuronal sources, or dietary sources may modify microcirculatory VSM function. In addition, because of the greater arterial versus venous effects, and by virtue of its capacity to increase membrane permeability, histamine can produce peripheral and/or microcirculatory edema and vascular damage.

The mechanism by which histamine relaxes VSM *in vivo* is speculative at present. *In vitro*, many blood vessels contract in response to histamine

(see Vanhoutte, 1978), as do small veins *in vivo* (Shepherd and Vanhoutte, 1975). Microvascular responses to histamine are inhibited by steroids (Kalsner, 1970; Altura, 1966a,b), but this may be related to potentiation of the venoconstrictor action rather than due to a prostanoid-dependent mechanism. It has been suggested that the vasodilator effects of histamine may be mediated indirectly via inhibition of NE release from adrenergic nerves, an event mediated by H_2 receptors (McGrath and Shepherd, 1976). Histamine-mediated relaxation may also be a physical process secondary to the vascular permeability actions of this amine. Vanhoutte (1978) has speculated that histamine may cause endothelial contraction, resulting in fenestration of the endothelial lining and fluid loss from the extracellular space. This would change the osmolarity of the extracellular space, resulting in a hyperosmolar condition that has been shown to promote vasodilation and inhibition of adrenergic neurotransmission (Mellander and Lundvall, 1971; Haddy and Scott, 1968, 1975; McGrath and Shepherd, 1976).

In an attempt to elucidate the site and mechanism of histamine interaction with venular tissue, Simionescu *et al.* (1982) coupled histamine, methylhistamine, and a selective H_1 receptor agonist (4-pyridylethylamine) to ferritin. They measured the distribution of the radioactive forms of the complexes, as well as the electromicrographic localization of these substances in bovine pulmonary and human umbilical venous endothelial cells. They found that approximately 22% of the endothelial receptors for histamine were of the H_1 type, whereas 78% were of the H_2 type. Moreover, the H_2 binding sites were localized to the areas of the contractile proteins of the endothelial cells. It was speculated that contractions of these endothelial cells may allow gaps to form between the cells, increasing permeability. Moreover, since these sites were also associated with the areas of the coated pits and the Pastan (1982) receptosomes, sites of drug transport into the endothelial cell, the localization of histamine receptors at these sites could also play a role in increasing permeability by stimulating the opening of these "pore equivalents."

The biochemical mechanism of histamine action is unclear at present. Endothelial-obligatory relaxing factor does not appear to be important in the relaxation of VSM to histamine (Furchgott *et al.*, 1981). Histamine can stimulate the formation of both cyclic AMP and cyclic GMP. Desensitization to histamine is associated with a loss of the responses to histamine and a decrease in the maximum amount of cyclic GMP that can be formed. However, the affinity of histamine for its receptor is unaltered at the time of desensitization. This suggests that histamine-induced relaxation may be associated with an increase in cyclic GMP and that the number of receptors is important in determining the magnitude of the re-

sponse. Moreover, it appears that H_1 receptor stimulation may be linked to the formation of cyclic GMP, whereas H_2 receptor stimulation may be linked to the formation of cyclic AMP (for references see Taylor and Richelson, 1982). Thus, histamine-mediated relaxation may be dependent on cyclic nucleotide-stimulated protein kinase-dependent dephosphorylation of myosin and phosphorylation of myosin light-chain kinase (see Section II,J).

G. Prostanoids, Thromboxanes, and Leukotrienes and Lipid Peroxides

The prostanoids and the other arachidonate-mediated metabolites represent such a vast family of compounds that entire monographs have been devoted to their action on the microcirculation. Therefore, this section briefly covers new information relating to the action of these substances on the microcirculation and the mechanism of prostanoid action. For a more detailed account the reader is referred to reviews on this subject (Karim, 1976; Messina *et al.*, 1976; Samuelsson and Paoletti, 1982; Salmon, 1982; Heymann, 1980; Greenberg, 1982a,b).

The fatty acid precursors of prostanoids and leukotrienes are also the sources of prostacyclin and thromboxanes. However, the microvascular responses to endogenous prostanoid substances or exogenous fatty acids may not result only from the action of these substances on the microvasculature. The major sites of thromboxane A_2 synthesis are the platelets, whereas the circulating leukocytes metabolize arachidonic acids to hydroperoxides and leukotrienes. Prostacyclin is made primarily by the endothelial cells and VSM, whereas the smooth muscle synthesizes primarily prostanoids and prostacyclin. Thromboxane and prostacyclin have opposing actions on both the VSM and the platelet, whereas the leukotrienes appear to contract VSM and stimulate aggregation (Table VI, p. 74; Fig. 13). Moncada and Vane (1979a,b) suggested that the thromboxane–prostacyclin system acted as an important homeostatic mechanism in the regulation of platelet adherence to the vascular endothelium, platelet aggregation, and thrombi formation (Table VI, Fig. 13).

Prostaglandins of the E series decrease vascular diameter in the microcirculation with a greater effect on arterioles and precapillary arterioles than on venules. Moreover, prostanoids of the E series antagonize the vasoconstrictor action of angiotensin II, NE, and other agonists and enhance the dilator responses to bradykinin (Messina *et al.*, 1976). Thromboxane A_2 and analogs of prostaglandin endoperoxides believed to stimulate thromboxane receptors (U44619) contract arterioles, precapillary

TABLE VIII
Glycogen Phosphorylase Activity, Oxygen Consumption, and Aerobic Glycolysis in Porcine Coronary Arteries[a,b]

Compound added	Change from basal (%)		
	J_{O_2}	J_{lac}	Phosphorylase activity[b]
KCl, 80 m*M*	68 ± 7	67 ± 8	55.8 ± 14.0
Ouabain, 10^{-5} *M*	21 ± 3.5	−47 ± 5	40.7 ± 18.6

[a] From Hellstrand and Paul (1982) with permission.
[b] Total activity in the presence of adenosine monophosphate was 0.14 ± 0.02 μmol/g per minute (n = 20).

arterioles, and venules (Higgs, 1982). Messina and Kaley (1982) incubated PHG_2 with platelet microsomes to generate thromboxane and found *in vivo* coronary dilation and *in vitro* contraction of VSM. Dusting *et al.* (1978) injected PGH_2 with microsomes into the mesenteric circulation of dogs and found only weak constriction. Injection of cold acetone extracts of thromboxane A_2 also resulted in weak responses of the mesenteric circulation. Thus, it is possible that an endogenous circulating inhibitor of thromboxnae action may be present *in vivo* or that *in vitro* concentrations of thromboxane cannot be achieved *in vivo*. Prostacyclin dilates arterioles and precapillary arterioles but does not affect venular tone (Higgs *et al.*, 1978a,b, 1979; Higgs, 1982). Indomethacin enhanced the sensitivity of the vasculature to prostacyclin, possibly by inhibiting endogenous production of prostacyclin. According to Higgs *et al.* (1978, 1979) prostacyclin is the most potent microcirculatory vasodilator agent, whereas Messina and Kaley (1982) suggest that PGE_2 is more potent than prostacyclin. Prostaglandin G_2 contracts microvascular VSM followed by a secondary dilation due to endogenous prostacyclin formation (Lewis *et al.*, 1977; Higgs, 1982; Higgs *et al.*, 1978a,b, 1979). The action of PGH_2 is similar to that of PGG_2. Thus, prostanoid production by VSM or endothelium may modulate microcirculatory VSM function by altering VSM tone or the subsequent effects of other modulators of the level of VSM constriction or dilation.

Prostanoids may also act to modulate leukocyte adherence *in vivo*. Weksler *et al.* (1977) showed that prostacyclin synthesized by endothelial cells modulated leukocyte function. Higgs *et al.* (1978) subsequently showed that the number of flow-moving leukocytes was reduced in hamster cheek pouch microvenules. Boxer *et al.* (1980) demonstrated that prostacyclin reduced the adherence of leukocytes to VSM *in vitro*. Using the hamster cheek pouch preparation, Higgs (1982) showed that prostacy-

clin reduced leukocyte margination by 90% in doses that reduced arterial pressure by 30 mm Hg. Equieffective concentrations of PGE on arterial pressure did not affect leukocyte adherence or margination in the hamster cheek pouch microvessels. Higgs (1982) suggested that prostacyclin inhibited leukocyte adherence to the VSM by increasing cyclic AMP. Moreover, he suggested that local production of prostacyclin by inflammed blood vessels enhances local vasodilation and plays a role in the early stages of inflammation to suppress leukocyte adherence and infiltration into the vascular wall.

The effect of prostanoids on thrombus formation in the microcirculation is dependent on the prostanoid under study. Prostacyclin and PGE_1 inhibit thrombus formation, whereas thromboxane and leukotrienes promote thrombus formation. *In vitro* PGG_2 stimulates platelet aggregation, whereas *in vivo* it is an inhibitor. This would indicate that, *in vivo*, prostacyclin is predominantly formed over that of thromboxane. Figure 13 summarizes the mechanism by which prostacyclin may oppose the action of thromboxane and inhibit thrombus formation (Boxer *et al.*, 1980; Emmons *et al.*, 1967; Gorman *et al.*, 1977; Gorman, 1982; Hamberg *et al.*, 1974; Higgs *et al.*, 1978, 1979; Kloeze, 1967; Moncada *et al.*, 1976a,b; Needleman *et al.*, 1976; Tateson *et al.*, 1977; Westwick, 1977).

The mechanism by which prostanoids may affect VSM tone is described below. However, it appears that the prostanoids may exert their action via discreet receptors located in or on the VSM cell membrane. Tachyphyllaxis to PGA is not associated with tachyphyllaxis to PGE or $PGF_{2\alpha}$, nor is tachphyllaxis to PGD_2 associated with tachyphyllaxis to other prostanoids. Thus, it appears that the receptors for individual prostanoids may differ from each other (for references see Fleisch, 1977; Greenberg *et al.*, 1976). Wakeling and Wyngarden (1974) demonstrated the existence of a specific PGE-binding protein in myometrium. M. Johnson *et al.* (1973a,b, 1974a,b) subsequently showed that ultraviolet light inhibits prostanoid-dependent contraction of uterine smooth muscle, as do ouabain (Kadar and Sunahara, 1969; Greenberg *et al.*, 1974) and inhibitors of disulfide of sulfhydryl groups (E. M. Johnson *et al.*, 1974). Subsequent studies showed that reductions in extracellular magnesium ion concentration from normal (2.4 m*M*) to 0.6 or 0 m*M* inhibited prostanoid-induced contraction and relaxation of VSM (Altura and Altura, 1976; Greenberg and Bohr, 1975; Greenberg, 1981d, 1982a,b). These findings suggest that disulfide groups and magnesium ion are essential components of the prostanoid receptor and that the ouabain-inhibitable sodium transport ATPase of VSM may be an integral component of the contractile and relaxant mechanism activated by prostanoids.

Current data suggest that constrictor prostanoids may act by two dis-

tinct mechanisms. Conventional prostanoids may contract VSM by acting as a calcium ionophore and increase VSM permeability to calcium ion or inhibit (K,Na)-ATPase with a resultant increase in sodium and calcium ion (Greenberg *et al.*, 1974a,b,c; Carsten, 1973, 1974; Carsten and Miller, 1977, 1978). The effects of thromboxanes and endoperoxide analogs, as well as leukotrienes, may be to inhibit intracellular calcium sequestration or promote release of intracellular calcium ion as well as inhibit magnesium uptake into VSM (van Breeman *et al.*, 1979, 1980; McNamara *et al.*, 1980; Greenberg, 1981d). The vasodilator responses to prostaglandins and prostacyclin may be related to an inhibition of calcium influx into the VSM cell and a stimulation of magnesium influx (Carsten and Miller, 1977, 1978; Greenberg *et al.*, 1974; Greenberg, 1981d, 1982a,b). In addition, prostanoids that relax VSM stimulate adenylate cyclase and increase cyclic AMP, thereby potentially affecting the contractile and relaxation process as described above.

H. Acetylcholine

Acetylcholine has been implicated as a mediator and/or regulator of blood flow since the studies of Krogh (1929), which suggested that ACh increases microcirculatory blood flow. Cholinergic nerves innervate some vascular beds such as skeletal muscle (Mellander and Johansson, 1968), cerebral circulation (D'Alacy and Rose, 1977), kidney (Harkness and Brody, 1967), and coronary circulation (D'Alecy and Feigel, 1972). Moreover, ACh acts as a vasodilator to increase blood flow and decrease resistance in many vascular beds (Altura, 1966a,b, 1971a; Mellander and Johansson, 1968; Shepherd and Vanhoutte, 1975; Westfall, 1977). Finally, ACh can modulate the responses to other vasodilator and vasoconstrictor substances as well as nerve stimulation without directly affecting the VSM (Altura, 1978a,b, 1981; Vanhoutte, 1978, 1980). Although a large concentration of ACh is required to affect microcirculatory tone and blood flow *in vivo*, some evidence exists for a role of endogenous ACh in the modulation of microcirculatory VSM function.

Atropine is a selective, cholinergic, muscarinic receptor antagonist. Altura (1966a,b, 1971a) showed that atropine produced concentration-related contractions of precapillary sphincters and arterioles in concentrations that inhibited topically applied ACh-mediated dilation. However, these concentrations of atropine did not affect the vasodilator responses to bradykinin, histamine, or isoproterenol. This effect was not due to an artifact resulting from the topical administration of atropine, because intravenous administration of atropine, in concentrations sufficient to block

topically administered ACh, also produced a decrease in the lumen size of metarterioles, arterioles, precapillary sphincters, and venules (Altura and Hershey, 1967). Since atropine does not contract VSM directly, the contractile responses to atropine may reflect inhibition of endogenous ACh-mediated vasodilation or, as suggested by Altura (1966a,b), the action of some other, unidentified choline–ester moiety.

The remaining evidence for ACh acting as a modulator of VSM tone in the microcirculation is inferred from its actions on larger arteries and the actions of subvasodilator concentrations of ACh on the microcirculation. Acetylcholine inhibits the postsynaptic responses to stimulation of adrenergic nerves by inhibiting the release of the neurotransmitter NE (Vanhoutte, 1974; Vanhoutte *et al.*, 1973). Thus, ACh may modulate the level of VSM tone by altering the concentration of NE released from adrenergic nerves. In addition, subvasodilator concentrations of ACh attenuate the vasoconstrictor responses to NE, angiotension II, 5-HT, and a wide variety of other substances in venules, noninnervated precapillary sphincters, and other microvessels (Altura, 1978a,b, 1981). Thus, ACh may modulate the level of tone of both innervated and noninnervated VSM in the microcirculation.

1. Receptors for Acetylcholine

Previous work with large rabbit, rat, cat, and dog arteries (DeMey and Vanhoutte, 1978, 1981; Furchgott and Zawadski, 1980a,b,c; Furchgott *et al.*, 1981; Altura and Chand, 1981) suggested that ACh acted on a muscarinic receptor on the endothelium to stimulate the release of an endothelial-derived relaxing factor, which in turn acted on the arterial smooth muscle to promote relaxation (see below). Greenberg *et al.* (1982a,b) confirmed the existence of endothelial-mediated relaxation of VSM *in vitro* and demonstrated the existence of distinct cholinergic, muscarinic receptors mediating the endothelial and smooth muscle actions of ACh. Moreover, data obtained with the cholinergic, muscarinic receptor antagonist cetiedil (Boissier *et al.*, 1980; Cho *et al.*, 1979) demonstrated that four distinct cholinergic receptors exist for ACh: two distinct cholinergic, muscarinic receptors on the venous and arterial endothelium and two on the venous and arterial smooth muscle. Cetiedil is avidly bound by VSM (Boissier *et al.*, 1980). Differences in binding between artery and vein may require different concentrations of cetiedil to block cholinergic receptors. Alternatively, cetiedil may have the capacity to differentiate subclasses of cholinergic receptors not amenable to analyses with atropine. Further speculation is beyond the scope of this discussion. However, the data obtained from the atropine and the cetiedil studies clearly demonstrate that

the muscarinic, cholinergic receptors of the endothelial-competent artery and vein cannot be the same receptor subtypes.

Estrada and Krause (1982) also reported that membrane fractions from bovine pia-arachnoid cerebral microvessels could be characterized by two specific binding sites. They speculated that the two binding sites were related to the cholinergic receptors present on the arterial smooth muscle and cerebral capillary endothelium. The data presented herein show that endothelial-dependent responses and responses independent of an intact endothelium can be characterized by two distinct kinetic constants. Moreover, the values obtained by Estrada and Krause (1982) for ACh interacting with the two receptors (2.67×10^{-7} and 1.65×10^{-5} *M*) are relatively the same as the ED_{50} concentrations for ACh-mediated relaxation and contraction of canine arteries in endothelial-intact and endothelial-deficient preparations, respectively. Thus, the existence of distinct cholinergic, muscarinic receptors mediating endothelial-dependent relaxation and smooth muscle contraction may be the rule, rather than the exception, in VSM. Further studies are needed to validate this concept.

2. *Mechanism of Action of Acetylcholine*

In contrast to the *in vivo* vasodilator action of ACh, the *in vitro* action of ACh generally results in a contraction of arterial and smooth muscle. Jeliffe *et al.* (1962), however, reported that rings of aortic smooth muscle relaxed in response to ACh. Furchgott and Zawadski (1979, 1980a,b,c) and DeMey and Vanhoutte (1978, 1981) demonstrated that ACh-mediated relaxation of arterial smooth muscle and some venous smooth muscles (Altura and Chand, 1981; Tanaka *et al.*, 1982a,b) was dependent on the presence of an intact endothelium. Furchgott *et al.* (1981) proposed that ACh-mediated relaxation of arterial smooth muscle is dependent on the release of hydroperoxide metabolites from the endothelium. These substances act on the smooth muscle to stimulate cyclic GMP, which in some manner promotes the relaxation of smooth muscle. This speculation was based on the findings that (*a*) lipoxygenase and cyclooxygenase inhibitors such as ETYA and nordihydroguaiaretic acid (NDGA) and hydroquinone antagonized by ACh-mediated relaxation of endothelial-competent arterial smooth muscle (Furchgott and Zawadski, 1980a,b,c); (*b*) analogs of cyclic GMP relaxed arterial smooth muscle (Schultz *et al.*, 1979); (*c*) ETYA and other lipoxygenase inhibitors inhibited the accumulation of cyclic GMP in smooth muscle (Spies *et al.*, 1980; Goldberg *et al.*, 1978); and (*d*) hydroperoxides and other peroxides derived from phospholipid stimulated the accumulation of cyclic GMP (Hidaka and Asano, 1977). This conclusion has been challenged by Diamond (1982) on the grounds that the changes in cyclic GMP with most vasodilator substances are insuffi-

cient to accommodate or promote relaxation and the temporal changes in cyclic GMP and relaxation do not appear to be temporally related to each other.

The studies of Greenberg *et al.* (1982a,b) with normal and rubbed canine mesenteric arteries and veins confirmed the observations of Furchgott and Zawadski (1978, 1980a,b,c,d) and Furchgott *et al.* (1981) that NDGA and ETYA as well as the other lipoxygenase inhibitors inhibit ACh-mediated relaxation. However, the following findings fail to support the speculation that ACh-mediated endothelial-dependent relaxation is initiated through the formation of endothelial-derived hydroperoxides that act on the smooth muscle to promote relaxation:

1. Each of the lipoxygenase inhibitors inhibited both ACh-mediated relaxation of normal mesenteric arteries and contraction of normal and rubbed mesenteric veins.
2. No significant differences existed in the concentrations of antagonists required to inhibit the concentration and relaxation processes.
3. Arachidonic acid inhibited ACh-mediated relaxation and contraction.
4. Each of the inhibitors inhibited NE-induced contraction in concentrations required to inhibit ACh-mediated relaxation of normal mesenteric arteries and contractions of normal mesenteric veins and rubbed mesenteric arteries and veins.
5. Some lipoxygenase inhibitors inhibited ACh-mediated relaxation, whereas others were devoid of this activity.
6. The concentrations of inhibitors required to block ACh-mediated contraction or relaxation were 10–300 times greater than the concentrations required to inhibit lipoxygenase (Samuelsson and Paoletti, 1982; Tappel *et al.*, 1953; Bray *et al.*, 1980; Volpi *et al.*, 1980).
7. Ouabain, potassium-free saline solution, and high-potassium saline solution, which inhibit the (sodium–potassium)-activated, magnesium-dependent ATPase (electrogenic sodium pump), selectively inhibit ACh-mediated relaxation of endothelial-competent arteries (Greenberg *et al.*, 1982a; Webb *et al.*, 1981; DeMey and Vanhoutte, 1978; Jones, 1980; Haddy, 1978).
8. Concentrations of metabolic inhibitors and hypoxia that do not affect the contractile responses to ACh or other vasoconstrictor agonists abolish ACh-mediated relaxation (DeMey and Vanhoutte, 1978; Furchgott *et al.*, 1981; Greenberg *et al.*, 1982b). The data represented support the conclusion that the effects of some of these inhibitors on ACh-mediated relaxation (and contraction) result from a nonspecific depressant effect of these substances on the processes that mediate both contraction and relaxation.

Some nonsteroidal antiinflammatory agents inhibit superprecipitation of actomyosin from VSM in concentrations that inhibit ACh-mediated contraction and relaxation (Gorog and Kovacs, 1972). This conclusion is also supported by the experiments of Tanaka *et al.* (1982a,b) which demonstrated that 10^{-4} *M* ETYA and NDGA suppress the relaxant effects of cetiedil on both normal and rubbed mesenteric arteries and veins. The relaxant effects of cetiedil are independent of (EDF) or cyclic GMP but depend on a direct stimulation of the electrogenic sodium pump on the smooth muscle of these VSM preparations (Tanaka *et al.*, 1982a,b). In addition, ETYA does not inhibit bradykinin-induced relaxation of canine mesenteric arteries (Greenberg and Kadowitz, 1982) or pulmonary vessels (Altura and Chand, 1981), responses dependent on the presence of an intact endothelium (Furchgott *et al.*, 1981). Furthermore, P. J. Kadowitz (personal communication) failed to block ACh-mediated pulmonary vascular relaxation in the perfused cat lung with 26 mg/kg ETYA. Thus, it is unlikely that ACh-mediated relaxation is dependent on the formation of ETYA- and NDGA-inhibitable hydroperoxides or prostanoids within the endothelium of the mesenteric arteries.

Cyanide and azide are fairly selective inhibitors of oxidative metabolism within VSM at low concentrations (Greenberg *et al.*, 1974a,b,c). The inhibitors decreased the concentration of ATP within the mesenteric arteries and veins and selectively inhibited or completely suppressed ACh-mediated relaxation but not ACh-mediated contraction of mesenteric arteries and veins, respectively. The data support the conclusion that energy derived from oxidative metabolism is obligatory for ACh-mediated relaxation of mesententeric arteries but anaerobic mechanisms or energy derived from the Embden–Myerhoff glycolytic pathway are sufficient to maintain ACh-mediated contraction of mesenteric veins. In support of this conclusion are the experiments of DeMey and Vanhoutte (1978, 1980a) and Furchgott *et al.* (1981), which show that anoxia suppresses ACh-mediated relaxation of canine and rabbit large arteries.

Guanosine triphosphate is essential for the formation of cyclic GMP (Goldberg *et al.*, 1978). A primary source of GTP is derived from the breakdown of glucose via the glycolytic pathway and the tricarboxylic acid cycle (Devlin, 1982). It is tempting to speculate that cyanide and azide inhibit ACh-mediated relaxation of normal mesenteric arteries by decreasing the concentration of GTP and thereby of cyclic GMP. This intriguing hypothesis generated the experiments with pyruvate and phosphoenolpyruvate (PEP), which should have increased the concentration of GTP and therefore of cyclic GMP. However, in the presence of PEP and pyruvate the responses to ACh were not enhanced, but depressed. Moreover, Murad *et al.* (1979) demonstrated that azide, in the presence

of catalase, stimulates cyclic GMP accumulation. Cyanide inhibits cyclic GMP accumulation. However, both compounds inhibit ACh-mediated vasodilation. In addition, cyclic GMP derivatives and cyclic GMP itself relax VSM contracted by high-potassium ion solution. Physiologic salt solution with a high concentration of potassium ion inhibits ACh-mediated relaxation of mesenteric arteries. Therefore, it is unlikely that an inhibitory effect of hypoxia, azide, and cyanide on cyclic GMP formation can explain the inhibition of endothelial-dependent ACh-mediated relaxation of mesenteric arteries.

The findings summarized above clearly demonstrate that ACh-mediated relaxation of canine arteries and contraction of canine veins are two distinct processes mediated by different receptors and by different mechanisms. As summarized in Fig. 15, ACh-mediated relaxation is initiated by ACh interacting with distinct muscarinic receptors located on the endothelial cells. This interaction of ACh with these receptors stimulates the release of an unknown mediator, which appears to act directly on VSM to stimulate relaxation by an ouabain- and high-potassium-inhibitable mechanism. This mechanism appears to be related to the electrogenic sodium pump (Hendrickx and Casteels, 1974). Contraction appears to be related to a direct effect of ACh on the smooth muscle cells of mesenteric veins as well as by an indirect effect mediated through mesenteric vein EDF. These two actions appear to promote an increase in membrane permeability to extracellular calcium ion, as well as a mobilization of intracellular bound calcium. The nature of the EDF mediating VSM relaxation and contraction remains to be elucidated.

I. Adenosine and Nucleotides

Adenosine, ATP, AMP, and ADP relax arterioles, precapillary sphincters, and venules *in vivo*. The magnitude of relaxation depends on the level of VSM tone and follows the order arterioles > precapillary sphincters > venules. Adenosine may arise from the circulation or from tissues surrounding the vasculature during hypoxia, ischemia, or work (for references see Berne *et al.*, 1974; Berne and Rubio, 1974; Rubio and Berne, 1975; Rubio *et al.*, 1975).

The synthesis and degradation of adenosine are described above. Briefly, adenosine is taken up by endothelial cells. This action is inhibited by dipyridamole, a coronary and peripheral vasodilator. The enzyme involved in the degradation of adenine nucleotides, 5′-nucleotidase, is abundant along the luminal surface of endothelial cells primarily in association with caveolae and/or pinocytotic vesicles (Smith and Ryan, 1970, 1971; Ryan and Smith, 1971a,b). Adenosine diphosphate is degraded to

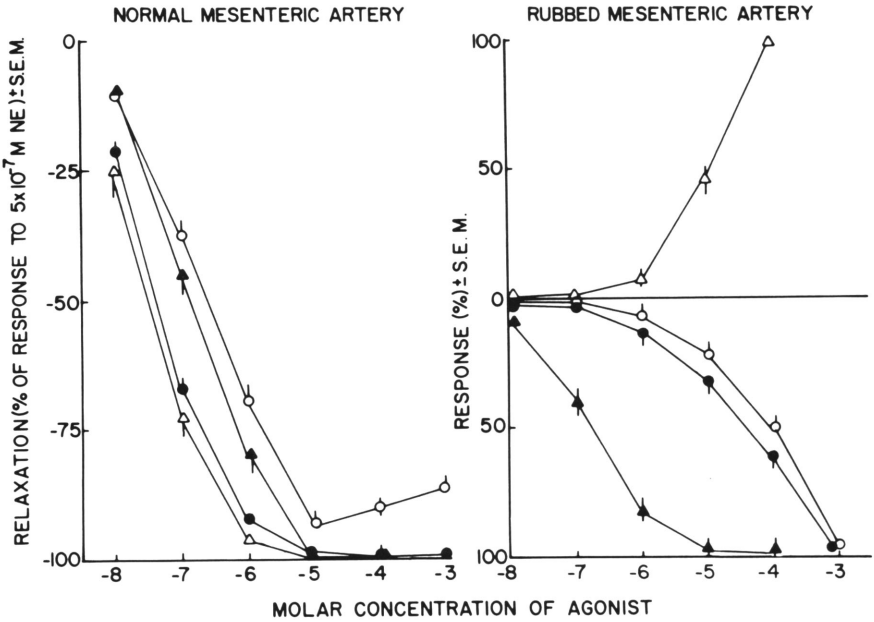

Fig. 22. Effect of endothelial rubbing on vascular relaxation of mesenteric arteries to ACh (△), adenosine (●), ATP (○), and isoproterenol (▲).

AMP, which in turn is metabolized to adenosine and inosine. The adenosine is taken up into the endothelial cell and resynthesized to ADP and ATP. Large arteries relax in response to adenosine and other nucleotides, as do the perfused vascular beds. Frohlich (1962) suggested that the relaxation in response to ATP and other nucleotides was dependent on their capacity to chelate calcium ion. The responses of large arteries to adenosine and other nucleotides appear to be independent of the endothelium, whereas small mesenteric arteries relax in response to adenosine and ATP, and destruction of the endothelium inhibits, but does not abolish, the relaxation (Fig. 22).

Adenine nucleotides produce biphasic effects on many VSM preparations. This is dependent on both the nucleotide under study and the level of VSM tone. Adenosine diphosphate and ATP contract isolated and intact arteries and veins, whereas high concentrations of adenosine enhance the excitatory activity of spontaneously active VSM (Furchgott, 1966; Somlyo and Somlyo, 1968, 1970; Shepherd and Vanhoutte, 1975; Walter and Bassenge, 1968; Sjoberg and Wahlstrom, 1975). The mechanism of the contraction can be explained by an increased permeability to calcium ion (Sjoberg and Wahlstrom, 1975), an increased ATP concentration, and

perhaps an ionophoric action of the nucleotide transporting calcium ion across the cell. The vasodilator and relaxant actions in response to adenosine are greater than those to AMP > ATP > ADP (Walter and Bassenge, 1968; Somlyo and Somlyo, 1968, 1970; Norton *et al.*, 1972; Toda, 1974; Schnaar and Sparks, 1972; Verhaege *et al.*, 1977; Vanhoutte, 1978). The mechanism of the vasodilation produced by these nucleotides seems to be related to an increase in cellular calcium binding, which may result from a direct effect of the adenosine or an indirect effect caused by endothelial-derived relaxing factor, or it may be related to a prostanoid that may be released by adenosine. The vasodilating action of adenosine does not appear to result from its conversion to cyclic AMP. The magnitude and duration of action of adenosine depend on the degree of endothelial competency, the integrity of the transport and metabolizing systems, and the degree of uptake by the VSM (Berne and Rubio, 1974; Rubio and Berne, 1975). Adenosine and other nucleotides may modulate the level of VSM tone both directly and indirectly by altering neurotransmitter release (see Section II,B; for references see Vanhoutte, 1978, 1980).

Adenosine and adenine nucleotides have been implicated in the reactive hyperemia of working skeletal muscle, as well as the cerebral and coronary circulation following occlusion or ischemia (for references see Berne and Rubio, 1974; Rubio and Berne, 1975). Adenosine and other nucleotides are released from ischemic tissue and possibly from purinergic nerves. It has been suggested that adenosine and ATP may either alone, or as comodulators, mediate the cerebral and coronary vascular responses to hypoxia (Berne *et al.*, 1974, Rubio *et al.*, 1975) as well as pulmonary hypoxic vasoconstriction (Mentzer *et al.*, 1975). The question as to which of the mediators are involved is beyond the scope of this discussion and remains to be resolved (for references see Vanhoutte, 1978, 1980). However, if hypoxia damages the vascular endothelium and prevents the uptake and degradation of adenosine, then the amounts of adenosine and other nucleotides released by ischemic insults may modulate microcirculatory function.

J. Anesthetics and Alcohols

The effect of anesthetics and alcohols on the microcirculation has been covered extensively, and the interested reader is referred to Altura 1976, 1978a,b, 1981, 1982), and Longnecker and Harris (1980). Briefly, arterioles, precapillary sphincters, and venules relax in response to ethanol. The sensitivity to the vasodilator action of ethanol is equivalent in arterioles and venules, but the arterioles respond with a greater maximal in-

TABLE IX
Influence of Topical Anesthetic Agents on Arteriolar and Venular Diameters in Rat Mesentery[a]

Anesthetic	Microvessel	
	Arterioles	Muscular venules
Pentobarbital (10^{-4}–10^{-5} *M*)	Vasodilation	Vasodilation
Amobarbital (10^{-4}–10^{-5} *M*)	Vasodilation	Vasodilation
Ethanol (0.1–1%)	Vasodilation	Vasodilation
Procaine (100–1000 μg)	Vasodilation	0[b]
Lidocaine (100–500 μg)	Vasodilation	0[b]

[a] All animals were anesthetized with ketamine hydrochloride. Taken from data presented in Altura (1967), B. T. Altura and Altura (1978), and Altura *et al.* (1976, 1979).
[b] Signifies no effect.

crease in diameter. This is to be expected since the arterioles constrict more than the venules (Altura and Hershey, 1967; Altura, 1971a,b; Altura and Altura, 1974a; Altura *et al.*, 1979; Miller and Weigman, 1977; Longnecker and Harris, 1980; Fig. 23, Table IX). General and local anesthetics as well as barbiturate depressants affect microcirculatory function by depressing the vascular responses to vasoactive agents and by relaxing the VSM. Barbiturates and ethanol affect both arteries and veins but, according to Altura, lidocaine and procaine do not affect muscular venules (Altura, 1981). However, Tanaka *et al.* (1982a,b) showed that lidocaine inhibited adrenergic neurotransmission and relaxed small mesenteric veins *in vitro*. For the inhibitory effect on adrenergic neurotransmission 10^{-5} *M* of lidocaine was required, whereas 10^{-4} *M* lidocaine was required to inhibit or relax VSM contracted with NE or prostanoids. However, at higher concentrations lidocaine contracted the veins and, to a lesser extent, the arteries. Thus, the high concentrations (500 μg topically) of lidocaine used in topical application studies may mask the venodilator effects of lower concentrations. Alternatively, tone may have to be induced in the veins to unmask the relaxant response to local anesthetics.

The mechanism by which local and general anesthetics relax smooth muscle and inhibit microvascular responses to vasoactive agents remains obscure. Anesthetics may inhibit sodium channels to delay or block sodium conductance, and thereby calcium conductance, in VSM. Alternatively, anesthetics may interact with magnesium ion to enhance magnesium binding to the VSM cell, thereby decreasing calcium influx and suppressing reactivity. Finally, anesthetics may interfere with calcium release from sarcoplasmic reticulum and mitochondria or may substitute for

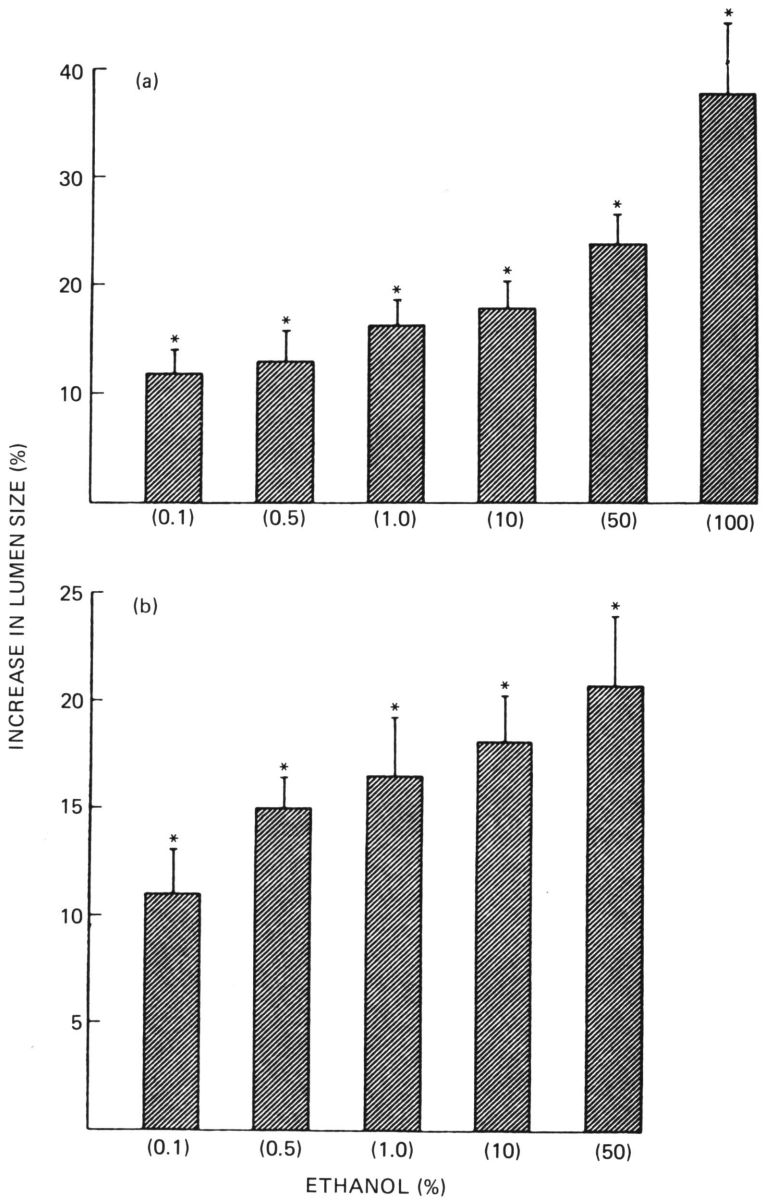

Fig. 23. Effect of ethanol on arteriolar (a) and venular (b) diameter. ▨, Ethanol; *, significantly different from control ($P < 0.02$). (From Altura, 1978, 1981, with permission.)

calcium ion on the VSM membrane, thereby preventing contraction and/or excitation. Further studies are necessary to evaluate the mechanism of anesthetic suppression of microvascular function (for references see Altura, 1981; Longnecker and Harris, 1980).

K. Antihypertensive Vasodilator Compounds

Thiazide diuretics lower blood pressure by a mechanism unrelated to the saliuretic or natriuretic action of thiazide. These diuretics depress both arteriolar and venular reactivity and tone after chronic administration, but acutely do not dilate VSM. Several mechanisms have been invoked to explain the antihypertensive actions of the thiazide diuretics. Thiazide diuretics may decrease the reactivity of the VSM by both inhibiting the release of neurotransmitter and desensitizing the postsynaptic receptors for NE, angiotensin II, and other vasoactive agents to their respective agonists. Evidence for this is derived from studies which show that thiazides inhibit the release of NE from adrenergic nerves and depress VSM responses to NE. Moreover, thiazides suppress the mobilization of calcium ion in VSM obtained from both animals and man. An inability of agonists to release calcium ion from within the cell or an exaggerated accumulation of calcium within the cell, after increased calcium entry from external pools of calcium ion, could diminish the magnitude of the subsequent contraction to vasoactive agents, despite the presence of an adequate stimulus and excitation contraction–coupling mechanism. Finally, the studies of Webster and Dollery (1981) and Watkins *et al.* (1980) suggest that thiazides may stimulate the formation of prostacyclin by the VSM or endothelium which modulates the level of vascular tone produced by the thiazides. The mechanism of action of these compounds remains to be resolved.

Both hydralazine and minoxidil are directly acting vasodilators that preferentially affect arteriolar versus venular tone. The mechanism of action of both compounds is unclear. Both drugs are considered together here since minoxidil appears to be a relative of hydralazine. Hydralazine and minoxidil reduce arterial pressure to equivalent levels (for references see Greenberg, 1980). However, hydralazine depresses vascular reactivity and responses to vasoactive agents, whereas minoxidil is devoid of this action. Thus, it is unlikely that suppression of VSM responsiveness *in vitro* accounts for the arterial smooth muscle effects of these compounds. Greenberg *et al.* (1980) and Pang and Sutter (1981) administered hydralazine and minoxidil chronically to spontaneously hypertensive rats (SHR) and found that despite equivalent reductions in pressure, minoxidil did not

inhibit VSM tone or responses to vasoactive agents. Pang and Sutter (1981) showed that the action of hydralazine was independent of the calcium ion concentration. Thus, these effects of minoxidil and hydralazine on arterial pressure clearly seem unrelated to their incidental effects on vascular reactivity. Haeusler and Gerold (1978) measured an increase in PGE-like material after acute administration of these agents to dogs. Greenwalt *et al.* (1980) and Greenberg *et al.* (1982c) showed that hydralazine inhibited thromboxane A_2 generation in both the platelet and VSM, respectively. However, minoxidil was devoid of this action (Greenberg *et al.*, 1982a,b). Thus, although these drugs may inhibit prostanoid constrictor mechanisms, the relation to their antihypertensive action remains to be elucidated. It must be pointed out that minoxidil does not affect VSM tone unless it passes through the mesenteric–hepatic circulation (Ducharme, personal communication). It is possible that minoxidil forms a labile, hydroperoxide intermediate that affects the synthesis of leukotrienes or other lipid peroxides. Further studies are required to test this hypothesis.

Captopril is an angiotensin-converting enzyme inhibitor that inhibits the conversion of angiotensin I to angiotensin II and prevents the breakdown of bradykinin (Ondetti *et al.*, 1977). This drug decreases arteriolar and venular tone and lowers total peripheral resistance in patients with low, normal, or high renin hypertension (Bengis *et al.*, 1978; Brunner *et al.*, 1980; Gavras *et al.*, 1978; Swartz *et al.*, 1979, 1980; Williams and Hollenberg, 1977). However, the antihypertensive effect is due to factors other than its capacity simply to decrease angiotensin II or stimulate bradykinin accumulation. Bradykinin itself stimulates prostaglandin synthesis (McGiff *et al.*, 1972; Blumberg *et al.*, 1977). Captopril also stimulates prostaglandin and/or prostacyclin formation in VSM and the central nervous system (Dusting *et al.*, 1980; Swartz *et al.*, 1980), which may modulate the decrease in vascular resistance in hypertensive animals and man. Moreover, captopril can directly depress VSM function independent of its prostanoid-stimulating action. Each of these mechanisms may contribute to the antihypertensive and vasodilating effects of captopril.

Nitroprusside is believed to lower arteriolar and venular smooth muscle tone by effecting an increase in cyclic GMP in the VSM. This effect is independent of calcium ion and occurs in broken-cell preparations. Methylene blue inhibits nitroprusside-induced relaxation and the accumulation of cyclic GMP (Kukovetz *et al.*, 1981; Gruetter *et al.*, 1979). Moreover, drugs that inhibit phosphodiesterase potentiate the relaxant effects of nitroprusside (Kukovetz *et al.*, 1979a,b). The reported effects of nitroprusside and other compounds were not related to an increase in cellular calcium ion because (*a*) these compounds increased calcium efflux from VSM without increasing calcium influx (Zsoster *et al.*, 1977); (*b*) in the

absence of extracellular calcium ion, nitrates and nitroprusside decreased calcium efflux from rabbit renal arteries and inhibited KCl-induced contraction, indicating that calcium sequestration was enhanced (Hester *et al.*, 1979); and (*c*) nitrites and nitroprusside do not inhibit directly calcium sequestration by sarcoplasmic reticulum from rabbit aorta (Thorens and Haeusler, 1979). Thus, although the evidence is at best circuitous and circumstantial, it is possible that nitroprusside stimulates cyclic GMP in VSM and that cyclic GMP regulates VSM relaxation by stimulating calcium sequestration by sarcoplasmic reticulum or enhancing calcium extrusion from the VSM cell (Fig. 17).

Diazoxide is a potent antihypertensive agent that relaxes arterial and venous smooth muscle when given by intravenous bolus injection. Because of its high capacity for binding to plasma proteins and the lack of control of the actions of the drug, once administered, its value in the treatment of hypertension is declining as newer drugs, such as minoxidil and the calcium-channel-blocking agents, are developed. The mechanism of this nondiuretic thiazide congener. This compound, like thiazides, depresses VSM reactivity and contractility (Wohl *et al.*, 1967, 1968a,b; McNeil *et al.*, 1969; Rhodes and Sutter, 1971; Janis and Triggle, 1973). Wohl *et al.* (1967) postulated that diazoxide may compete with calcium ion at a membrane site regulating the entry of this divalent ion. This was substantiated by Janis and Triggle (1973), but these investigators found that the competition was noncompetitive rather than competitive. In view of these findings and those of Rhodes and Sutter (1971), who also did not find competitive antagonism of potassium-depolarized portal vein with diazoxide, the postulate arose that diazoxide acted on a membrane-potential-dependent component of the excitation–contraction coupling mechanism. Although diazoxide increases cyclic AMP in VSM, it does not relax uterus by stimulating an increase in cyclic AMP (Polacek *et al.*, 1970; Tabachnik and Gulbekian, 1968). This finding and that of Wohl *et al.* (1968a) and Janis and Triggle (1973) that diazoxide is more effective in VSM from hypertensive than in VSM from normotensive animals suggested that diazoxide acts at a site of membrane derangement in VSM. In view of current knowledge this site may be the voltage-dependent calcium channel of VSM.

The newest class of antihypertensive agents affecting VSM tone consists of the calcium-channel-blocking agents (for references see Weiss, 1981a). These drugs are believed to lower arteriolar and venular vascular resistance, with greater effects on the arterioles than venules, by inhibiting both voltage-dependent and voltage-independent calcium channels in VSM (see Section II,H).

L. Peripheral Vasodilators

The nitrates (amyl nitrate, nitroglycerin, and organic nitrite compounds) are believed to act in a manner analogous to that of nitroprusside. The nitrite compounds affect arterial smooth muscle tone in lower concentrations than that required to affect arteriolar and/or venular smooth muscle tone (Schnaar and Sparks, 1972). The relaxation in response to nitroglycerin and other nitrates is believed to be mediated by an increase in cyclic GMP (for references and mechanism see Section III,K). The nitrites produce tachyphyllaxis with continued administration. Needleman and Johnson (1973) and Needleman *et al.* (1973a,b) showed that tolerance to nitrites could be prevented by dithiothreitol, a sulfide-protecting agent, and enhanced by furosemide, which alkylates sulfhydryl groups. They suggested that relaxation and tolerance depend on the interaction of nitrates and nitrites with sulfhydryl groups on the cell membrane. Fertel (1982) showed that tolerance to nitrites was associated with a capacity to stimulate cyclic GMP formation in VSM. Thus, it is possible that nitrites and nitrates stimulate guanylate cyclase through a sulhydryl-dependent receptor and that tolerance results from desensitization of the receptor and an inability to stimulate guanylate cyclase.

Dipyridamole is an arteriolar vasodilator with little effect on venous smooth muscle. This compound inhibits phosphorylation of adenosine by a direct mechanism and by inhibiting its uptake into endothelium and smooth muscle. It also interferes with phosphodiesterase, thus increasing the accumulation of cyclic AMP, and potentiates the action of prostacyclin on VSM, as well as stimulates prostacyclin production by VSM (McElroy and Philip, 1975; Masotii *et al.*, 1979; Philip and Lemeuix, 1969; Moncada and Korbut, 1978). The mechanism of its action remains to be defined.

Cetiedil is an effective new therapeutic modality for alleviating the pain and constriction associated with vasospastic disorders such as Raynauds' phenomena (Haring *et al.*, 1980; Simaan and Aviado, 1976; Barbe *et al.*, 1980) and sickle cell disease (Asakura *et al.*, 1980; Berkowitz and Orringer, 1981; Glenn *et al.*, 1982). Cetiedil, in concentrations between 50 and 200 μmol/liter, has been found to reverse the sickling of the erythrocyte membrane obtained from patients with sickle cell disease (Asakura *et al.*, 1980), to inhibit the binding of calcium ion to calmodulin obtained from erythrocytes (E. P. Orringer, unpublished), to inhibit methacholine-induced bronchoconstriction in adult man suffering from asthma (Cho *et al.*, 1979), and to act as a nonspecific smooth muscle depressant and vasodilator (Boissier *et al.*, 1980; Simaan and Aviado, 1976). Despite the effi-

cacy of cetiedil in alleviating the vasospasm and pain associated with the aforementioned vasospastic disorders, little is known about the effects of cetiedil on adrenergic neuroeffector mechanisms in vascular smooth muscle.

The actions of cetiedil in producing both relaxation and inhibition of NE- and KCl-mediated contractions differ from that of the calcium-channel-blocking agent verapamil (Tanaka *et al.*, 1982a,b; Weiss, 1981a,b). Verapamil-induced relaxation of mesenteric arteries and veins is antagonized by increased concentrations of extracellular calcium but not by increasing concentrations of extracellular KCl (Weiss, 1981a,b). Verapamil inhibits the responses to KCl in lower concentration than is required to inhibit the contractile responses to NE (Weiss, 1981a,b; Greenberg *et al.*, 1973a,b; Tanaka *et al.*, 1982a,b). In contrast, cetiedil-induced relaxation of mesenteric arteries and veins is not affected by the concentration of extracellular calcium ion, is inhibited by contracting the muscle with 40 m*M* KCl, and is reduced by ouabain. Moreover, no significant differences exist in the concentration of cetiedil required to inhibit the contractile responses to NE and KCl (Tanaka *et al.*, 1982a,b; Boissier *et al.*, 1980). Finally, verapamil-induced inhibition of transmural nerve stimulation to VSM is inhibited by elevations in the concentration of extracellular calcium ion, whereas the inhibitory effect of cetiedil on adrenergic neuroeffector transmission to mesenteric arteries and veins is independent of the extracellular calcium ion concentration (Tanaka *et al.*, 1982a,b). These findings support the conclusion that cetiedil is not a calcium-channel-blocking agent similar to verapamil.

It is also unlikely that cetiedil produces relaxation of mesenteric arteries and veins by stimulating the production of a vasodilator material from the vascular endothelium, in a manner similar to that of ACh and adenosine (Furchgott and Zawadski, 1980a,b,c; Furchgott *et al.*, 1981; Greenberg *et al.*, 1982b). This conclusion is based on the findings that (*a*) endothelial stripping does not affect the responses of the mesenteric arteries and veins to cetiedil but inhibits the relaxant responses to ACh; (*b*) concentrations of ETYA and NDGA that act as specific (relatively) inhibitors of lipoxygenase in other cells (Bray *et al.*, 1980; Tappel *et al.*, 1953; Volpi *et al.*, 1980) do not inhibit cetiedil-induced relaxation of mesenteric arteries or veins; and (*c*) in concentrations that do not relax MA and MV, cetiedil inhibits the relaxant and contractile responses of the mesenteric arteries and veins to ACh (Greenberg *et al.*, 1982b; Cho *et al.*, 1979; Simaan and Aviado, 1976). It can be argued that cetiedil may act directly on the VSM, in a manner similar to that of the mediator released by ACh, to stimulate the production of cyclic GMP within the VSM cell (Furchgott *et*

al., 1981) and thereby promote relaxation of the mesenteric arteries and veins. Evidence in support of this argument is that, in high concentrations, ETYA and NDGA (unpublished) inhibit cetiedil-induced relaxation and the accumulation of cyclic GMP (Spies *et al.*, 1980). Moreover, cetiedil inhibits cyclic nucleotide phosphodiesterase in rat thoracic aortas (Boissier *et al.*, 1980). However, the concentrations of ETYA and NDGA that inhibit the responses to cetiedil also depress the responses of the mesenteric arteries and veins to NE, KCl, verapamil, and ACh, suggesting a nonspecific effect of these inhibitors on the integrity of the muscle. The capacity of cetiedil to inhibit phosphodiesterase occurs with concentrations of cetiedil in excess of 1 m*M*, much greater than that required to produce relaxation (Boissier *et al.*, 1980). Therefore, it is unlikely that cetiedil-induced increases in cyclic GMP can explain the mechanism of cetiedil-induced relaxation of mesenteric arteries and veins.

It is also unlikely that cetiedil relaxes VSM by suppressing the mobilization of calcium ion within the cell. A previous study demonstrated that small canine mesenteric veins were more dependent on the mobilization of intracellular calcium ion to sustain NE-induced tone than were the mesenteric arteries (Greenberg *et al.*, 1973a,b). KCl-induced contraction of mesenteric arteries and veins is dependent on an extracellular source of calcium ion and not on calcium mobilization within the cell (Bohr *et al.*, 1978; Bohr, 1973). If cetiedil acted to suppress calcium mobilization within the cell, then it would be highly unlikely that KCl-induced contracture would be suppressed by cetiedil. Moreover, a differential sensitivity of the mesenteric arteries and veins to cetiedil would exist, and no such evidence has been found.

Both high concentrations of KCl and concentrations of ouabain sufficient to inhibit the (sodium–potassium)-dependent, magnesium-activated ADPase activity of canine VSM (Jones, 1980) produce equivalent ($P > 0.05$) inhibition of cetiedil-induced relaxation of canine mesenteric VSM. Both of these interventions inhibit the sodium–potassium pumping mechanism. This would indirectly suggest that cetiedil may act to promote relaxation of mesenteric arteries and veins by stimulating a (sodium–potassium)-dependent electrogenic sodium transport mechanism and promoting potassium entry into the cell. Moreover, cetiedil did not relax canine mesenteric arteries and veins in PSS deficient in KCl. Potassium-deficient solutions inhibit the electrogenic sodium pump mechanism in VSM and thereby inhibit relaxation dependent on this mechanism (Webb *et al.*, 1981; Haddy, 1978; Jones, 1980). The greater inhibitory of KCl-deficient solution on cetiedil-induced relaxation of mesenteric arteries and veins may occur because cetiedil can overcome the block of the pump

mechanism caused by high KCl and ouabain concentrations. However, in potassium-free PSS, there is an absolute deficiency in KCl and thereby an irreversible inhibition of the pump. Further studies are necessary to verify or refute this speculation.

It is also possible that cetiedil may relax canine mesenteric arteries and veins by inhibiting calcium binding to their calcium–calmodulin system. Our studies do not rule out this possibility. A positive correlation exists between the capacity of smooth muscle relaxants such as prenylamine and chlorpromazine to inhibit calcium binding to calmodulin and to promote relaxation of skinned smooth muscle (Cassidy *et al.*, 1980) as well as between the capacity of these drugs to bind to calmodulin, inhibit phosphorylation of myosin light-chain kinase, and promote relaxation of intact VSM *in vitro* (Hidaka *et al.*, 1979). Cetiedil (in concentrations greater than 5×10^{-5} *M*) inhibits the binding of calcium to calmodulin (E. P. Orringer, unpublished) and KCl-mediated calcium accumulation in erythrocytes (Berkowitz and Orringer, 1981). Further studies measuring the calcium-binding capacity of the VSM calmodulin obtained from cetiedil-treated animals are necessary to refute or verify the possibility that calmodulin is involved in the VSM relaxant action of cetiedil.

IV. Directions for Future Research

The mechanism of physiologic and pharmacologic control of microvascular VSM function has been ascertained by inference from the events that occur in larger vessels. Whether this is valid remains to be elucidated. Studies are required which will assess the capacity of microvessels to synthesize vasoactive compounds, relax in response to known stimulants of discreet metabolic pathways, and respond to specific calcium-channel-blocking agents. Moreover, microperfusion studies are required in which the effect of vasoactive agents on ion fluxes, metabolism, and prostanoid synthesis and release *in vivo* can be defined. In addition, studies in which physiologic humoral agents and pharmacologic moieties are administered *in vivo*, rather than topically, should be performed to ascertain the site of action of drugs under conditions of actual hemodynamic efficacy. Finally, studies of microvascular biochemistry are needed to elucidate the role of endothelium and smooth muscle mechanisms in microvascular function. With the current incipient stages of microvessel research, it is hoped that some of the questions raised in this review will find answers and provide new directions for the treatment of aberrant vascular function in pathologic disease states.

Acknowledgments

The authors' research was supported in part by grants HL 22216 and RCDAHL7K04-00428 from the National Heart, Lung, and Blood Institute. S. Greenberg was a recipient of Research Career Development Award 7-K04 HL00428 from the Hypertension Branch of the National Institutes of Health.

References

Adelstein, R. S. (1978). *Trends Biochem. Sci. (Pers. Ed.)* **3,** 27–30.
Adelstein, R. S., and Eisenberg, E. (1980). *Annu. Rev. Biochem.* **49,** 921–956.
Adelstein, R. S., and Klee, C. B. (1980). *In* "Calcium and Cell Function" (W. Y. Cheung, ed.), Vol. 1, pp. 167–182. Academic Press, New York.
Aiken, J. W. (1973). *J. Pharmacol. Exp. Ther.* **184,** 678–687.
Aiken, J. W. (1974). *Pol. J. Pharmacol. Pharm.* **26,** 217–227.
Alexander, R. S. (1968). *Microvasc. Res.* **3,** 3–17.
Alquist, R. P. (1948). *Am. J. Physiol.* **153,** 586–599.
Altura, B. M. (1966a). *Am. J. Physiol.* **211,** 1393–1397.
Altura, B. M. (1966b). *Am. J. Physiol.* **212,** 1447–1454.
Altura, B. M. (1971a). *Microvasc. Res.* **3,** 361–384.
Altura, B. M. (1971b). *Proc. Soc. Exp. Biol. Med.* **138,** 273–276.
Altura, B. M. (1972a). *Microvasc. Res.* **4,** 319
Altura, B. M. (1972b). *Eur. J. Pharmacol.* **19,** 171–179.
Altura, B. M. (1973). *Eur. J. Pharmacol.* **24,** 49–60.
Altura, B. M. (1975a). *J. Pharmacol. Exp. Ther.* **193,** 403–412.
Altura, B. M. (1975b). *Circ. Res.* **36–37** (Suppl. I), 233–240.
Altura, B. M. (1975c). *Am. J. Physiol.* **228,** 1615–1620.
Altura, B. M. (1976). *Artery (Fulton, Mich.)* **2,** 18–25.
Altura, B. M. (1977). *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **36,** 1840–1847.
Altura, B. M. (1978a). *Microvasc. Res.* **16,** 91–117.
Altura, B. M. (1978b). *In* "Microcirculation" (G. Kaley and B. M. Altura, eds.), Vol. 2, pp. 431–502. Univ. Park Press, Baltimore, Maryland.
Altura, B. M. (1979). *In* "Current Concepts in Kinin Research" (G. L. Haberland, and U. Hamberg, eds.), pp. 47–55. Pergamon, Oxford.
Altura, B. M. (1981). *In* "Microcirculation, Current Physiologic, Medical and Surgical Concepts" (R. M. Effros, H. Schmid-Shonbein, and J. Ditzel, eds.), pp. 52–105. Academic Press, New York.
Altura, B. M., and Altura, B. T. (1970). *Am. J. Physiol.* **219,** 1698–1705.
Altura, B. M., and Altura, B. T. (1971). *Am. J. Physiol.* **220,** 938–944.
Altura, B. M., and Altura, B. T. (1974a). *Anesthesiology* **41,** 197–214.
Altura, B. M., and Altura, B. T. (1974b). *Microvasc. Res.* **7,** 145–155.
Altura, B. M., and Altura, B. T. (1976). *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **35,** 2360–2366.
Altura, B. M., and Altura, B. T. (1977a). *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **36,** 1853–1860.
Altura, B. M., and Altura, B. T. (1977b). *In* "Excitation–Contraction Coupling in Smooth Muscle" (R. Casteels, T. Godfraind, and J. C. Ruegg, eds.), pp. 137–144. Elsevier, Amsterdam.

Altura, B. M., and Altura, B. T. (1981). *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **39,** 234–241.
Altura, B. M., and Chand, N. (1981). *Br. J. Pharmacol.* **68,** 245–247.
Altura, B. M., and Halevy, S. (1978). *Proc. Natl. Acad. Sci. USA* **75,** 2941–2944.
Altura, B. M., and Hershey, S. G. (1967). *Angiology* **18,** 428–439.
Altura, B. M., Malaviya, D., Reich, C. F., and Orkin, L. R. (1972). *Am. J. Physiol.* **222,** 345–355.
Altura, B. M., Altura, B. T., and Waldemar, Y. (1976). *Artery (Fulton, Mich.)* **2,** 326–336.
Altura, B. M., Ogunkoya, A., and Gebrewold, A., and Altura, B. T. (1979). *J. Cardiovasc. Pharmacol.* **1,** 97–113.
Amer, M. S. (1973). *Science (Washington, D.C.)* **179,** 807–809.
Amer, M. S. (1977). *In* "Cyclic 3′5′-Nucleotides: Mechanism of Action" (H. Kramer and J. Schultz, eds.), pp. 381–396. Wiley, New York.
Amer, M. S., Doba, M., and Reis, D. J. (1974). *Proc. Natl. Acad. Sci. USA,* **72,** 2135–2139.
Amunsden, E. (1976). *In* "Chemistry and Biology of the Kallekrein-Kinin System in Health and Disease" (J. J. Pisano, and K. F. Austen, eds.), pp. 517–524. US Govt. Printing Office, Washington, D.C.
Anderson, D. K. (1976). *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **35,** 1294–1297.
Andersson, R., Lundholm, L., Mohme-Lundholm, E., and Nilsson, K. (1972). *Adv. Cyclic Nucleotide Res.* **1,** 213–230.
Angles-d'Auriac, G., Badouin, M., and Meyer, P. (1972). *Circ. Res.* **31,** (Suppl. II), 151–157.
Apperley, E., Humphrey, P. P. A., and Levey, G. P. (1976). *Br. J. Pharmacol.* **58,** 211–221.
Apperley, E., Humphrey, P. P. A., and Levey, G. P. (1977). *Br. J. Pharmacol.* **61,** 465P
Apperley, E., Humphrey, P. P. A., and Levey, G. P. (1980). *Br. J. Pharmacol.* **58,** 211–221.
Armstrong, J. M. (1982). *In* "Cardiovascular Pharmacology of the Prostaglandins" (A. G. Herman, P. M. Vanhoutte, H. Denolin, and A. Goosens, eds.), pp. 51–64. Raven, New York.
Asakura, T., Ohnishi, S. T., Adachi, K., Oxuc, M., Hashimoto, K., Singer, M., Russell, M. O., and Schwartz, E. (1980). *Proc. Natl. Acad. Sci. USA* **77,** 2955–2959.
Ashton, F. T., Somlyo, A. V., and Somlyo, A. P. (1975). *J. Mol. Biol.* **98,** 17–29.
Axelsson, J., Johansson, B., Jonsson, O., and Wahstrom, B. (1967). *Bibl. Anat.* **8,** 16–20.
Bar, H. P. (1974). *Adv. Cyclic Nucleotide Res.* **54,** 195–238.
Barbe, R., Amiel, M., Pouzeratte, B., Veyre, B., Villard, J., and Grivet, G. (1980). *Clin. Trials J.* **17,** 1–25.
Bartelstone, H. J., and Nasmyth, P. A. (1965). *Am. J. Physiol.* **208,** 754–762.
Baudouin-Legros, M., and Meyer, P. (1973). *Br. J. Pharmacol.* **47,** 377–385.
Baudouin-Legros, M., and Meyer, P., Fermandijan, S., and Morgat, J. (1972). *Nature (London)* **235,** 336–338.
Beck, L., and Brody, M. J. (1961). *Angiology* **12,** 202–221.
Bell, R. L., and Majerius, P. W. (1980). *J. Biol. Chem.* **255,** 1790–1792.
Bell, R. L., and Baenziger, N. L., and Majerius, P. W. (1980). *Prostaglandins* **20,** 269–274.
Belleau, B. (1967). *Ann. N.Y. Acad. Sci.* **139,** 580–605.
Bengis, R. G., Coleman, T. G., Young, D. B., and McCaa, R. E. (1978). *Circ. Res.* **43,** (Suppl. I), 45–53.
Berde, B. (1965). *Oxytocin Res. Proc. Symp.* pp. 11–35.
Berecek, K. H., Stocker, M., and Gross, F. (1980). *Circ. Res.* **46,** 619–624.
Berecek, K. H., Stocker, M., and Gross, G. (1982). *Hypertension (Dallas)* **4,**
Berkowitz, L. R., and Orringer, E. P. (1981). *J. Clin. Invest.* **68,** 1215–1220.

Berkowitz, B. A., Lee, C. H., and Spector, S. (1975). *Clin. Exp. Pharmacol. Physiol.* **1,** 397–400.
Berne, R. M., and Rubio, R. (1974). *Adv. Cardiol.* **12,** 303–317.
Berne, R. M., Rubio, R., and Curnish, R. R. (1974). *Circ. Res.* **35,** 262–271.
Berridge, M. J. (1975). *Adv. Cyclic Nucleotide Res.* **6,** 1–98.
Bertelsen, S., and Pettinger, W. A. (1977). *Life Sci.* **21,** 595–606.
Bevan, J. A. (1982). *In* "Prostaglandins Organ and Tissue Specific Actions" (S. Greenberg, P. J. Kadowitz, and T. F. Burks, eds.), pp. 1–11. Dekker, New York.
Bevan, J. A., and Su, C. (1973). *Annu. Rev. Pharmacol.* **13,** 269–285.
Bevan, J. A., Gartska, W., Su, C., and Su, M. O. (1973). *Eur. J. Pharmacol.* **22,** 47–53.
Bevan, J. A., Bevan, R. D., Chang, P. C., Pegram, B. L., Purdy, R. E., and Su, C. (1975). *Circ. Res.* **37,** 183–198.
Bito, L. Z., and Barody, R. A. (1975). *Prostaglandins* **10,** 633–638.
Black, J. L., French, R. J., and Mylecharane, E. J. (1981). *Br. J. Pharmacol.* **74,** 619–626.
Black, J. W., Duncan, W. A. M., Durant, C. J., Ganellin, C. R., and Parsons, E. M. (1972). *Nature (London)* **236,** 385–390.
Blackwell, G. J., Flower, R. J., Nijkamp, F. P., and Vane, J. R. (1978). *Br. J. Pharmacol.* **62,** 79–89.
Blackwell, G. J., Carnucio, R., DiRosa, M., Flower, R. J., Parente, L., and Perisco, P. (1980). *Nature (London)* **287,** 147–149.
Bloom, D. S., Cole, A. W. G., and Palmer, T. N. (1979). *Br. J. Pharmacol.* **65,** 587–592.
Blumberg, A. L., Denny, S. F., Marshall, G. R., and Needleman, P. (1977). *Am. J. Physiol.* **232,** H305–H310.
Boerth, R. C., Ryan, M. J., and Brody, M. J. (1970). *J. Pharmacol. Exp. Ther.* **172,** 52–61.
Bohlen, P., and Gore, M. B. (1978). *Am. J. Physiol.* **235,** 886–892.
Bohr, D. F. (1973). *Circ. Res.* **32,** 665–672.
Bohr, D. F., and Uchida, E. (1967). *Circ. Res.* **21,** (Suppl. II), 135–143.
Bohr, D. F., Greenberg, S., and Bonacorrsi, A. (1978). *In* "Microcirculation" (G. Kaley and B. Altura, eds.), Vol. 2, pp. 311–348. Univ. Park Press, Baltimore, Maryland.
Boissier, J. R., Aurousseau, M., Guidicelli, J. F., and Duval, D. (1980). *Arzneim. Forsch.* **28,** 2222–2228.
Bonaccorsi, A., Hermsmeyer, K., Aprigliano, O., Smith, C. B., and Bohr, D. F. (1977). *Blood Vessels* **14,** 261–276.
Boxer, L. A., Allen, J. M., Schmidt, M., Yoder, M., and Baehner, R. L. (1980). *J. Lab. Clin. Med.* **95,** 672–678.
Bray, M. A., Ford-Hutchinson, A. W., Shipley, M. E., and Smith, M. J. H. (1980). *Br. J. Pharmacol.* **71,** 507–512.
Brown, M. S., and Goldstein, J. L. (1979). *Proc. Natl. Acad. Sci. USA* **76,** 3330–3337.
Brunner, H. R., Gavras, H., Waeber, B., Turini, G. A., and Wauters, J. P. (1980). *Arch. Int. Pharmacodyn. Ther.* **247,** (Suppl. 2), 188–212.
Burnstock, G. (1976). *Neuroscience* **1,** 239–248.
Carsten, M. E. (1973). *Gynecol. Invest.* **4,** 95–105.
Carsten, M. E. (1974). *Prostaglandins* **5,** 33–40.
Carsten, M. E., and Miller, J. D. (1977). *J. Biol. Chem.* **252,** 2576–2582.
Carsten, M. E., and Miller, J. D. (1978). *Arch. Biochem. Biophys.* **185,** 282–295.
Cassidy, P., Hoar, P. E., and Kerrick, W. G. L. (1980). *Pfleugers Arch.* **387,** 115–120.
Chand, N., and Eyre, P. (1975). *Agents Actions* **5,** 277–295.
Chernukh, A. M., and Timkina, M. I. (1976). *In* "Physiology of Smooth Muscle" (E. Bulbring, and M. F. Shuba, eds.), pp. 403–410. Raven, New York.
Chin, A. K., and Evonuk, E. (1971). *J. Appl. Physiol.* **30,** 205–207.

Cho, Y. W., Han, H. C., Oh, S. Y., and Kuemmerle, H. P. (1979). *Int. J. Tissue React.* **1,** 155–158.

Clement, D., Vanhoutte, P. M., and Leusen, I. (1969). *Arch. Int. Physiol. Biochim.* **77,** 73–87.

Conti, M. A., and Adelstein, R. S. (1980). *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **39,** 1569–1573.

Crain, K. R., and Appleman, M. M. (1978). *Adv. Cyclic Nucleotide Res.* **9,** 221–230.

Curro, F. A., and Greenberg, S. (1982a). *J. Pharmacol. Exp. Ther.*

Curro, F. A., and Greenberg, S. (1982b). *Methods Findings Clin. Exp. Pharmacol. Physiol.* (in press).

Curro, F. A., Greenberg, S., Verbeuren, T., and Vanhoutte, P. M. (1978). *J. Pharmacol. Exp. Ther.* **207,** 936–949.

Dabrowska, R., Aromatorio, D., Sherry, J. M. F., and Hartshorne, D. J. (1978). *Biochemistry* **17,** 253–258.

D'Alecy, L. G., and Feigel, E. (1972). *Circ. Res.* **30,** 214–224.

D'Alecy, L. G., and Rose, C. J. (1977). *Circ. Res.* **41,** 324–331.

DeMey, J. G., and Vanhoutte, P. M. (1978). *Arch. Int. Pharmacodyn. Ther.* **234,** 339 (Abs.)

DeMey, J. G., and Vanhoutte, P. M. (1980a). *Blood Vessels* **17,** 27–40.

DeMey, J. G., and Vanhoutte, P. M. (1980b). *Circ. Res.* **46,** 826–835.

DeMey, J. G., and Vanhoutte, P. M. (1980c). *Eur. J. Pharmacol.* **67,** 159–164.

DeMey, J. G., and Vanhoutte, P. M. (1980d). *Pharmacologist* **22,** 282. (Abs.).

DeMey, J. G., and Vanhoutte, P. M. (1981). *Br. J. Pharmacol.* **72,** 501P. (Abs.).

DeMey, J. G., Burnstock, G., and Vanhoutte, P. M. (1979). *Eur. J. Pharmacol.* **55,** 401–405.

Devlin, T. M. (ed.) (1982). "Textbook of Biochemistry with Clinical Correlations" Wiley, New York.

Devynck, M. A., and Meyer, P. (1976). *Am. J. Med.* **61,** 758–767.

Devynck, M. A., Pernollet, M. G., Meyer, P., Fermandijan, S., Fromageot, P., and Bumpus, M. (1974). *Nature (London)* **249,** 67–69.

Diamond, J. (1978). *Adv. Cyclic Nucleotide Res.* **9,** 327–340.

Diamond, J. (1982). *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **41,**

Douglas, W. W. (1975). *In* "The Pharmacological Basis of Therapeutics" (L. S. Goodman and A. Gilman, eds.), pp. 630–652. MacMillan, New York.

Dunham, E. W., Haddox, M. K., and Goldberg, N. D. (1974). *Proc. Natl. Acad. Sci. USA* **71,** 815–819.

Dusting, J. G., Moncada, S., and Vane, J. R. (1978). *Eur. J. Pharmacol.* **49,** 65–72.

Dusting, J. G., Mullins, E. M., and Doyle, A. E. (1980). *Adv. Prostaglandin Thromboxane Res.* **7,** 815–819.

Dyer, D. C., and Gough, E. D. (1971). *Am. J. Obstet. Gynecol.* **111,** 820–825.

Dyer, D. C., Ueland, K., and Eng, M. (1972). *Arch. Int. Pharmacodyn. Ther.* **200,** 213–221.

Ebashi, S., Mikawa, T., Hirata, M., and Nonomura, Y. (1978). *Ann. N.Y. Acad. Sci.* **307,** 451–461.

Edvinsson, L., Hardebo, J. E., and Owman, C. (1978). *Circ. Res.* **42,** 143–151.

Eling, T. E., and Anderson, M. W. (1976). *Agents Actions* **6,** 543–547.

Emmons, P. R., Hampton, J. R., Harrison, M. J. C., Honour, A. J., and Mitchell, R. J. A. (1967). *Br. Med. J.* **2,** 468–472.

Erdos, E. G. (1970). *Handb. Exp. Pharmacol.* **25,**

Estrada, C., and Krause, D. N. (1982). *J. Pharmacol. Exp. Ther.* **221,** 85–90.

Evensen, S. A. (1979). *Haemostasis* **8,** 203–210.
Fertel, R. (1982). *J. Pharmacol. Exp. Ther.*
Fleisch, J. R. (1977). *In* "Factors Influencing Vascular Reactivity" (O. Carrier, and S. Shibata, eds.), pp. 78–95. Igaku-Shoin, Tokyo.
Fleisch, J. H., and Hooker, C. S. (1976). *Circ. Res.* **38,** 243–249.
Fleisch, J. H., Krzan, M. C., and Titus, E. (1973). *Circ. Res.* **33,** 284–290.
Forsling, M. L. (1976). "Anti-Diuretic Hormone." Edan, Montreal.
Freer, R. J. (1977). *Am. J. Physiol.* **232,** 231–239.
Freidman, M. (1973). "Chemistry and Biochemistry of the Sulfhydryl Group in Amino Acids, Peptides and Proteins." Pergamon, New York.
Freyburger, W. A., Graham, B. E., Rapport, M. M., Seay, P. H., Govier, W. N., Swoap, O. F., and Vanderbrook, M. J. (1952). *J. Pharmacol. Exp. Ther.* **101,** 80–86.
Frohlich, E. D. (1962). *Am. J. Physiol.* **203,** 162–166.
Funaki, S. (1961). *Nature (London)* **191,** 1102–1103.
Funaki, S., and Bohr, D. F. (1964). *Nature (London)* **203,** 192–194.
Furchgott, R. F. (1955). *Pharmacol. Rev.* **7,** 183–265.
Furchgott, R. F. (1966). *Bull. N.Y. Acad. Med.* **42,** 996–1006.
Furchgott, R. F., and Zawadski, J. V. (1979). *Pharmacologist* **21,** 271 (Abs.).
Furchgott, R. F., and Zawadski, J. V. (1980a). *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **39,** 581 (Abs.).
Furchgott, R. F., and Zawadski, J. V. (1980b). *Nature (London)* **288,** 373–376.
Furchgott, R. F., and Zawadski, J. V. (1980c). *Pharmacologist* **22,** 271 (Abs.).
Furchgott, R. F., Zawadski, J. V., and Cherry, P. D. (1981). *In* "Vasodilation" (P. M. Vanhoutte, and I. Leusen, eds.), pp. 49–66. Raven, New York.
Furness, J. B., and Marshall, J. M. (1974). *J. Physiol. (London)* **239,** 75–88.
Gabella, G. (1981). *In* "Smooth Muscle: An Assessment of Current Knowledge" (E. Bulbring, A. F. Brading, A. W. Jones, and T. Tomita, eds.), pp. 1–46. Univ. of Texas Press, Austin.
Gavras, H., Brunner, H. R., Turini, G. A., Kershaw, G. R., Tifft, C. P., Cuttlelod, S., Gavras, I., Vukovich, R. A., and McKinstry, D. N. (1978). *N. Engl. J. Med.* **298,** 991–995.
Gellai, M., Norton, J. M., and Detar, R. (1973). *Circ. Res.* **32,** 279–289.
Gerritsen, M. (1982). *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **41,** 879 (Abs.).
Glenn, T. M., Sakane, Y., and Cho, Y. W. (1982). *J. Clin. Pharmacol.*
Gluck, E. V., and Paul, R. J. (1977). *Pfleugers Arch.* **370,** 9–18.
Goldberg, M. R., Joiner, P. D., Greenberg, S., Hyman, A. L., and Kadowitz, P. J. (1975a). *Prostaglandins* **9,** 385–390.
Goldberg, M. R., Kadowitz, P. J., and Greenberg, S. (1975b). *Pharmacologist* **17,** 221 (Abs.).
Goldberg, M. R., Chapnick, B. M., Joiner, P. D., Hyman, A. L., and Kadowitz, P. J. (1976). *J. Pharmacol. Exp. Ther.* **198,** 357–365.
Goldberg, N. D., Haddox, M. K., Hartle, D. K., and Hadden, J. W. (1973). *In* "Pharmacology and the Future of Man: Proceedings of the 5th International Conference on Pharmacology" (R. A. Maxwell, and G. H. Acheson, eds.), pp. 149–169. Karger, Basle.
Goldberg, N. D., Graff, G., Haddox, M. K., Stephenson, J. H., Glass, D. B., and Moser, M. E. (1978). *Adv. Cyclic Nucleotide Res.* **9,** 101–130.
Goldstein, S., and Zsoster, T. T. (1978). *Br. J. Pharmacol.* **62,** 507–514.
Golenhofen, K., and Lammel, E. (1972). *Pflugers Arch.* **331,** 233–243.
Golenhofen, K., Hermstein, N., and Lammel, E. (1973). *Microvasc. Res.* **5,** 73–80.

Gorman, R. R. (1982). *In* "Prostaglandins: Cardiovascular and Cardiopulmonary Actions" (S. Greenberg, and T. M. Glenn, eds.). Academic Press, New York.
Gorman, R. R., Bunting, S., and Miller, O. V. (1977). *Prostaglandins* **13,** 377–388.
Gorog, P., and Kovacs, I. B. (1972). *Biochem. Pharmacol.* **21,** 1713–1723.
Greenbaum, L. M. (1976). *In* "Chemistry and Biology of the Kallekrein-Kinin System in Health and Disease" (J. J. Pisano, and K. F. Austen, eds.), pp. 455–462. U.S. Govt. Printing Office, Washington, D.C.
Greenberg, S. (1980). *J. Pharmacol. Exp. Ther.* **215,** 279–286.
Greenberg, S. (1981a). *Am. J. Physiol.* **242,** H525–H538.
Greenberg, S. (1981b). *Circ. Res.* **48,** 895–906.
Greenberg, S. (1981c). *J. Pharmacol. Exp. Ther.* **219,** 326–337.
Greenberg, S. (1981d). *J. Pharmacol. Exp. Ther.* **219,** 279–292.
Greenberg, S. (1982a). *In* "Prostaglandins: Tissue and Organ Specific Actions" (S. Greenberg, P. J. Kadowitz, and T. F. Burks, eds.). Dekker, New York.
Greenberg, S. (1982b). *In* "Prostaglandins: Cardiovascular and Cardiopulmonary Actions" (S. Greenberg, and T. M. Glenn, eds.). Academic Press, New York.
Greenberg, S., and Bohr, D. F. (1975). *Circ. Res. Suppl.* **36,** 213–218.
Greenberg, S., and Curro, F. A. (1982a). *Hypertension (Dallas)* (in press).
Greenberg, S., and Curro, F. A. (1982b). *Circ. Res.* (in press).
Greenberg, S., and Kadowitz, P. J. (1982). *Methods Find. Exp. Clin. Pharmacol.* **4,** 7–24.
Greenberg, S., and Wilborn, W. M. (1982). *Arch. Int. Pharmacodyn. Ther.* (in press).
Greenberg, S., Wilson, W. R., and Long, J. P. (1973a). *Arch. Int. Pharmacodyn. Ther.* **206,** 213–228.
Greenberg, S., Diecke, F. P. J., and Long, J. P. (1973b). *J. Pharmacol. Exp. Ther.* **185,** 493–503.
Greenberg, S., Heitz, D. C., Brody, M. J., Diecke, F. P. J., Wilson, W. R., and Long, J. P. (1974a). *J. Pharmacol. Exp. Ther.* **191,** 458–467.
Greenberg, S., Heitz, D., and Long, J. P. (1974b). *Can. J. Physiol. Pharmacol.* **52,** 649–650.
Greenberg, S., Englebrecht, J., Howard, L., and Long, J. P. (1974c). *Prostaglandins* **5,** 49–61.
Greenberg, S., Kadowitz, P. J., Diecke, F. P. J., and Long, J. P. (1974d). *Proc. Soc. Exp. Biol. Med.* **143,** 1008–1013.
Greenberg, S., Kadowitz, P. J., Long, J. P., and Wilson, W. R. (1976). *Circ. Res.* **39,** 66–76.
Greenberg, S., Palmer, E. C., Palmer, S. J., and Wilborn, W. (1978). *Clin. Sci. Molec. Med.* **51, 31**–36.
Greenberg, S., Glenn, T. M., Eddy, L. J., and Rebert, R. R. (1980). *Adv. Shock Res.* **3,** 238–263.
Greenberg, S., Glenn, T. M., and McGowan, C. (1981a). *Am. J. Physiol.* H528–H535.
Greenberg, S., Gaines, K., and Sweatt, D. (1981b). *Am. J. Physiol.* H343–H352.
Greenberg, S., Kadowitz, P. J., Hyman, A. L., and Curro, F. A. (1981a). *Am. J. Physiol.* **9,** 274–285.
Greenberg, S., McGowan, C., and Gaida, M. M. (1982a). *Clin. Exp. Hypertens.* (in press).
Greenberg, S., McGowan, C., and Gaida, M. M. (1982b). *Can. J. Physiol. Pharmacol.* (in press).
Greenberg, S., Tanaka, T. P., and Peevey, K. (1982c). *Proc. Soc. Exp. Biol. Med.* (in press).

Greenberg, S., Tanaka, T. P., Peevey, K., and Blackburn, W. (1982b). *Circ. Res.* (in press).

Greenberg, S., Gaines, K., and Glenn, T. M. (1982e). *Proc. Symp. Hydroxamic Acids Biol.* (in press).

Greenwalt, J. E., Wong, L. K., Alexander, M., and Bianchine, J. R. (1980). *Adv. Prostaglandin Thromboxane Res.* **6,** 293–296.

Gross, F. (1976). *Handb. Exp. Pharmacol.* **35,** 378–456.

Gruetter, C. A., Barry, B. K., McNamara, D. B., Gruetter, D. Y., Kadowitz, P. J., and Ignarro, L. J. (1979). *J. Cyclic Nucleotide Res.* **5,** 211–224.

Guignard, J. P., and Friedman, S. (1970). *Circ. Res.* **27,** 505–512.

Gyermek, L. (1966). *Handb. Exp. Pharmacol.* **19,** 471–528.

Haddy, F. J. (1978). *In* "Mechanisms of Vasodilation" (P. M. Vanhoutte, and I. Leusen, eds.), pp. 200–205. Karger, Basel.

Haddy, F. J., and Scott, J. B. (1968). *Physiol. Rev.* **48,** 688–707.

Haddy, F. J., and Scott, J. B. (1975). *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **34,** 2006–2011.

Haddy, F. J., Gordon, P., and Emmanuel, D. (1959). *Circ. Res.* **7,** 123–130.

Haeusler, G. (1972). *J. Pharmacol. Exp. Ther.* **180,** 672–682.

Haeusler, G. (1973). *Experientia* **29,** 762–763.

Haeusler, G., and Gerold, M. (1978). *Proc. Int. Congr. Pharmacol. 7th,* p. 806. (Abs).

Haeusler, G., and Thorens, S. (1980a). *J. Physiol. (London)* **303,** 203–224.

Haeusler, G., and Thorens, S. (1980b). *J. Physiol. (London)* **303,** 225–241.

Hagen, I. H., and White, R. L. (1978). *Stroke* **4,** 68–71.

Hamberg, M., Svensson, J., Wakabayashi, T., and Samuelsson, B. (1974). *Proc. Natl. Acad. Sci. USA* **71,** 345–349.

Haring, J., Mesangeau, D., Huet, Y., and Aurousseau, M. (1980). *Int. J. Clin. Pharmacol. Ther. Toxicol.* **18,** 467–481.

Harkness, H., and Brody, M. J. (1967). *Am. J. Physiol.* **213,** 424–428.

Hedquist, P. (1977). *Annu. Rev. Pharmacol. Toxicol.* **17,** 259–279.

Heistad, D. D., and Marcus, M. L. (1978). *Circ. Res.* **42,** 295–302.

Heitz, D. C., and Brody, M. J. (1975). *Am. J. Physiol.* **228,** 1351–1357.

Hellstrand, P., and Paul, R. J. (1980). *Physiologist* **23,** 95 (Abs.).

Hellstrand, P., and Paul, R. J. (1982). *In* "Vascular Smooth Muscle: Metabolic, Ionic and Contractile Mechanisms" (M. F. Crass, and C. D. Barnes, eds.), pp. 1–35. Academic Press, New York.

Henderson, R. M. (1975). *In* "Methods in Pharmacology" (E. E. Daniel, and D. M. Paton, eds.), Vol. 3, pp. 47–77. Plenum, New York.

Hendrickx, H., and Casteels, R. (1974). *Pfluegers Arch.* **346,** 299–306.

Hester, R. K., Weiss, G. B., and Fry, W. Y. (1979). *J. Pharmacol. Exp. Ther.* **208,** 155–160.

Heymann, M. A. (1980). "Prostaglandins in the Perinatal Period." Grune & Stratton, New York.

Hidaka, H., and Asano, T. (1977). *Proc. Natl. Acad. Sci. USA* **74,** 3657–3661.

Hidaka, H., Yamaki, T., Tatsuka, T., and Asano, M. (1979). *Mol. Pharmacol.* **15,** 49–59.

Higgs, G. A. (1982). *In* "Cardiovascular Pharmacology of the Prostaglandins" (A. G. Herman, P. M. Vanhoutte, H. Denolin, and A. Goosens, eds.), pp. 315–325. Raven, New York.

Higgs, E. A., Higgs, G. A., Moncada, S., and Vane, J. R. (1978a). *Br. J. Pharmacol.* **63,** 535–539.

Higgs, G. A., Moncada, S., and Vane, J. R. (1978b). *J. Physiol. (London)* **280,** 30–31P.

Higgs, G. A., Moncada, S., and Vane, J. R. (1978c). *J. Physiol. (London)* **280,** 55–56P.
Higgs, G. A., Cardinale, D. C., Moncada, S., and Vane, J. R. (1979). *Microvasc. Res.* **18,** 245–254.
Hinke, J. A. M. (1965). *In* "Muscle" (W. M. Paul, E. E. Daniel, C. M. McKay, and G. Monckton, eds.), pp. 269–285. Pergamon, Oxford.
Hirst, G. D. S. (1977). *J. Physiol (London)* **273,** 263–275.
Hodgson, B. J., and Daniels, E. E. (1972). *Can. J. Physiol. Pharmacol.* **50,** 725–730.
Hofbauer, J. H. (1973). *Clin. Sci. Mol. Med.* **39,** 263–278.
Horrobin, D. F., Mtajabi, J. P., and Manku, M. S. (1976). *Med. Hypotheses* **2,** 219–226.
Hoult, J. R. S., and Moore, P. K. (1982). *In* "Cardiovascular Pharmacology of the Prostaglandins" (A. G. Herman, P. M. Vanhoutte, H. Denolin, and A. Goosens, eds.), pp. 35–49. Raven, New York.
Hudgins, P. M., and Weiss, G. B. (1968). *J. Pharmacol. Exp. Ther.* **159,** 91–97.
Hughes, J., Gillis, C. N., and Bloom, F. E. (1969). *J. Pharmacol. Exp. Ther.* **169,** 237–248.
Humphrey, P. P. A. (1978). *Br. J. Pharmacol.* **63,** 671–675.
Huxley, J., and Hanson, J. (1954). *Nature (London)* **154.**
Innes, I. R. (1962). *Br. J. Pharmacol. Chemother.* **19,** 427–441.
Innes, I. R., and Kohli, J. D. (1970). *Arch. Int. Pharmacodyn. Ther.* **188,** 287–297.
Itzkowitz, H. D., and McGiff, J. C. (1974). *Circ. Res.* **34–35** (Suppl.), 65–73.
Janis, R. A., and Triggle, D. J. (1973). *Can. J. Physiol. Pharmacol.* **51,** 621–626.
Janis, R. A., and Triggle, D. J. (1974). *Pharmacol. Res. Commun.* **6,** 55–60.
Jarrot, B., McQueen, A., Graf, L., and Louis, W. J. (1975). *Clin. Exp. Pharmacol. Physiol.* **3** (Suppl. 2), 65–73.
Jeliffe, R. W. (1962). *J. Pharmacol. Exp. Ther.* **135,** 349–353.
Johansson, B., Jonsson, O., Axelsson, J., and Wahlstrom, B. (1967). *Circ. Res.* **21,** 619–633.
Johnson, E. M., Jr., Marshall, G. R., and Needleman, P. (1974). *Br. J. Pharmacol.* **51,** 541–547.
Johnson, M., and Ramwell, P. W. (1973). *Prostaglandins* **3,** 703–723.
Johnson, M., Jessup, R., and Ramwell, P. (1973a). *Prostaglandins* **4,** 593–605.
Johnson, M., Jessup, R., and Ramwell, P. (1974a). *Prostaglandins* **5,** 125–136.
Johnson, M., Jessup, R., Jessup, S., and Ramwell, P. (1974b). *Prostaglandins* **6,** 433–449.
Joiner, P. D., Kadowitz, P. J., Hughes, J. P., and Hyman, A. L. (1975). *Proc. Soc. Exp. Biol. Med.* **150,** 414–421.
Jones, A. W. (1973). *Circ. Res.* **33,** 563–572.
Jones, A. W. (1980). *Handb. Physiol. Sect. 2 Cardiovasc. Sys.* **2,** 253–299.
Junod, A. F. (1972a). *J. Pharmacol. Exp. Ther.* **183,** 182–187.
Junod, A. F. (1972b). *J. Pharmacol. Exp. Ther.* **183,** 341–344.
Kadar, D., and Sunahara, F. A. (1969). *Can. J. Physiol. Pharmacol.* **47,** 871–879.
Kadowitz, P. J., and Hyman, A. L. (1973). *Circ. Res.* **32,** 221–227.
Kadowitz, P. J., Sweet, C. S., and Brody, M. J. (1973). *Adv. Biosci.* **9,** 243–252.
Kadowitz, P. J., Joiner, P. D., and Hyman, A. L. (1975a). *J. Pharmacol. Exp. Ther.* **191,** 432–505.
Kadowitz, P. J., Joiner, P. D., and Hyman, A. L. (1975b). *Annu. Rev. Pharmacol.* **15,** 285–306.
Kadowitz, P. J., Knight, D. S., Hibbs, R. G., Elbson, J. P., Joiner, P. D., Brody, M. J., and Hyman, A. L. (1976). *Circ. Res.* **39,** 191–198.
Kadowitz, P. J., Greenberg S., Knight, D. S., Gruetter, C. A., Greenberg, S., and Hyman, A. L. (1981). *In* "Microcirculation: Current Physiologic, Medical and Surgical Con-

cepts" (R. M. Effros, H. Schmid-Shonbein, and J. Ditzel, eds.), pp. 107–124. Academic Press, New York.
Kadowitz, P. J., Greenberg, S., and Hyman, A. L. (1982a). *In* "Prostanoids, Cardiopulmonary and Cardiovascular Actions" (S. Greenberg, and T. M. Glenn, eds.). Academic Press, New York.
Kadowitz, P. J., Knight, J. P., Greenberg, S., and Hyman, A. L. (1982b). *In* "Prostaglandins: Organ and Tissue Specific Actions" (S. Greenberg, P. J. Kadowitz, and T. F. Burks, eds.). Dekker, New York.
Kaley, G., and Altura, B. M. (1978). "Microcirculation" Vol. 2 Univ. Park Press, Baltimore, Maryland.
Kalsner, S. (1970). *Can. J. Physiol. Pharmacol.* **48,** 443–449.
Karim, S. M. M. (1976). "Prostaglandins: Physiological, Pharmacological and Pathological Aspects." Lancester MTP Press, Lancester, England.
Karlin, A. (1967). *J. Theor. Biol.* **16,** 306–320.
Katsuki, S., Arnold, W., Mittal, C., and Murad, F. (1977). *J. Cyclic Nucleotide Res.* **3,** 23–35.
Keatinge, W. R. (1964). *J. Physiol. (London)* **174,** 184–205.
Keatinge, W. R. (1968a). *J. Physiol. (London)* **194,** 169–182.
Keatinge, W. R. (1968b). *J. Physiol. (London)* **194,** 183–200.
Keatinge, W. R. (1976). *J. Physiol. (London)* **258,** 73P–74P.
Keatinge, W. R. (1977). *In* "Excitation Contraction Coupling in Smooth Muscle" (R. Casteels, T. Godfraind, and J. C. Ruegg, eds.), pp. 47–52. Elsevier–North Holland Biomedical Press, Amsterdam.
Keatinge, W. R. (1978). *J. Physiol. (London)* **279,** 275–289.
Keatinge, W. R. (1979). *Br. Med. Bull.* **35,** 249–254.
Khairallah, P. A., Page, I. H., Bumpus, F. M., and Turker, R. K. (1966). *Circ. Res.* **14,** 247–254.
Kier, L. B. (1968). *J. Pharm. Sci.* **57,** 1188–1191.
Kimura, H., Mittal, C., and Murad, F. (1975). *J. Biol. Chem.* **250,** 8016–8022.
Kloeze, J. (1967). *In* "Prostaglandins" (S. Bergstrom, and B. Samuelsson, eds.), Vol. 2, pp. 241–252. Armquist & Wiksell, Stockholm.
Korolkovas, A. (1970). "Essentials of Molecular Pharmacology: Background for Drug Design." Wiley, New York.
Krogh, A. (1929). "The Anatomy and Physiology of Capillaries." Yale Univ. Press, New Haven, Connecticut.
Kukovetz, W. R., Holtzmann, S., Wurm, A., and Poch, G. (1979a). *J. Cyclic Nucleotide Res.* **5,** 469–476.
Kukovetz, W. R., Holtzmann, S., Wurm, A., and Poch, G. (1979b). *Naunyn Schmiedebergs Arch. Pharmacol.* **310,** 129–138.
Kukovetz, W. R., Poch, G., and Holtzmann, S. (1981). *In* "Vasodilation" (P. M. Vanhoutte, and I. Leusen, eds.), pp. 339–351. Raven, New York.
Kumamoto, M. (1977). *In* "Factors Influencing Vascular Reactivity" (O. Carrier and S. Shibata, eds.), pp. 106–131. Igaku-Shoin, Tokyo.
Kuriyama, H., and Suzuki, H. (1978). *Br. J. Pharmacol.* **64,** 493–501.
Langer, S. Z., Shepperson, N. B., and Massingham, R. (1981). *Hypertension (Dallas)* **3** (Suppl.), 112–118.
Lauson, H. D. (1974). *Handb. Physiol. Sect. 7 Endocrinol.* **4,** 287–393.
Lee, T. J. F., Hume, W. R., Su, C., and Bevan, J. A. (1978). *Circ. Res.* **42,** 535–542.
Levitt, B., and Westfall, D. P. (1982). *Blood Vessels* **19,** 30–40.
Lewis, G. P., Westwicke, J., and Williams, T. J. (1977). *Br. J. Pharmacol.* **59,** 442P (Abs.).

Limas, C. J., and Cohn, J. N. (1974). *Circ. Res.* **35,** 601–607.
Longnecker, D., and Harris, P. D. (1980). *In* "Microcirculation" (G. Kaley, and B. M. Altura, eds.), Vol. 3, pp. 384–405. Univ. Park Press, Baltimore, Maryland.
Majno, G. (1965). *Handb. Physiol. Sect. 2 Cardiovasc. Sys.* **3,** 2293–2375.
Marcus, A. J., Broekman, M. J., Weksler, B. B. Jaffe, E. A., Safier, L. B., Ullman, H. L., and Tack-Goldman, K. (1982). *In* "Cardiovascular Pharmacology of the Prostaglandins" (A. G. Herman, P. M. Vanhoutte, H. Denolin, and A. Goosens, eds.), pp. 125–136. Raven, New York.
Martin, P. J., and Schild, H. O. (1965). *Br. J. Pharmacol.* **25,** 418–431.
Masotti, G., Poggessi, L., Galanti, G., and Neri Serneri, G. G. (1979). *Thromb. Haemostasis* **42,** 197 (Abs.).
McCaa, R. E., Hall, J. E., and McCaa, C. S. (1978). *Circ. Res.* **43** (Suppl. 1), 32–39.
McElroy, F. A., and Philip, R. B. (1975). *Life Sci.* **17,** 1479–1493.
McGiff, J. C., Terragno, N. A., Malik, K. A., and Lonigro, A. J. (1972). *Circ. Res.* **31,** 36–43.
McGiff, J. C., Itskowitz, H. D., and Terragno, N. A. (1975). *Clin. Sci. Mol. Med.* **49,** 125–128.
McGiff, J. C., Itskowitz, H. D., Terragno, A., and Wong, P. K. Y. (1976). *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **35,** 175–180.
McGrath, M. M. (1977). *Circ. Res.* **41,** 428–435.
McGrath, M. M. (1978). *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **37,** 187–193.
McGrath, M. M., and Shepherd, J. T. (1976). *Circ. Res.* **39,** 566–573.
McNamara, D. B., Roulet, M. J., Gruetter, C. A., Hyman, A. L., and Kadowitz, P. J. (1980). *Prostaglandins* **20,** 311–320.
McNeil, J. H., Barnes, R. V., Davis, R. S., and Hook, J. B. (1969). *Can. J. Physiol. Pharmacol.* **47,** 663–669.
Mekata, F. (1971). *J. Gen. Physiol.* **57,** 738–751.
Mekata, F., and Niu, H. (1972). *J. Gen. Physiol.* **59,** 92–102.
Mellander, S., and Johansson, B. (1968). *Pharmacol. Rev.* **20,** 117–196.
Mellander, S., and Lundvall, J. (1971). *Circ. Res.* **28–29** (Suppl. I), 39–45.
Melmon, K. L., Kline, M. J., Hughes, T., and Nies, A. S. (1968). *J. Clin. Invest* **47,** 1295–1302.
Mentzer, R. M., Jr., Rubio, R., and Berne, R. M. (1975). *Am. J. Physiol.* **229,** 1625–1631.
Messina, E. J., and Kaley, G. (1982). *In* "Prostaglandins: Cardiovascular and Cardiopulmonary Actions" (S. Greenberg, and T. M. Glenn, eds.). Academic Press, New York.
Messina, E. J., Weiner, R., and Kaley, G. (1975). *Circ. Res.* **37,** 430–437.
Messina, E. J., Weiner, R., and Kaley, G. (1976). *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **35,** 2367–2375.
Miller, F. N., and Harris, P. D. (1975). *Microvasc. Res.* **10,** 340–351.
Miller, F. N., and Weigman, D. L. (1977). *Eur. J. Pharmacol.* **44,** 331–337.
Moncada, S., and Korbut, R. (1978). *Lancet* **1,** 1286–1289.
Moncada, S., and Vane, J. R. (1979a). *In* "Prostacyclin" (J. R. Vane, and S. Bergstrom, eds.), pp. 5–16. Raven, New York.
Moncada, S., and Vane, J. R. (1979b). *Pharmacol. Rev.* **30,** 293–331.
Moncada, S., Gryglewski, R. J., Bunting, S., and Vane, J. R. (1976a). *Prostaglandins* **12,** 715–737.
Moncada, S., Gryglewski, R. J., Bunting, S., and Vane, J. R. (1976b). *Nature (London)* **263,** 663–665.
Monod, J., Wyman, J., and Changeux, J. P. (1965). *J. Mol. Biol.* **12,** 88–118.

Moretti, R. L., and Abraham, S. (1978a). *Circ. Res.* **42,** 317–323.
Moretti, R. L., and Abraham, S. (1978b). *Prostaglandins* **15,** 603–622.
Moretti, R. L., and Lin, C. Y. (1980). *Prostaglandins* **19,** 99–108.
Moretti, R. L., Abraham, S., and Ecker, R. R. (1976). *Circ. Res.* **39,** 231–238.
Movat, H. Z., and Habal, F. M. (1976). *In* "Chemistry and Biology of the Kallekrein-Kinin System in Health and Disease" (J. J. Pisano and F. K. Austen, eds.), pp. 463–469. US Govt. Printing Office, Washington, D.C.
Moylan, R. D., and Westfall, T. C. (1979). *Blood Vessels* **16,** 302–310.
Murad, F., Arnold, W. P., Mittal, C. K., and Braughler, J. M. (1979). *Adv. Cyclic Nucleotide Res.* **2,** 175–204.
Nakajima, A., and Horn, L. (1967). *Am. J. Physiol.* **213,** 25–30.
Nakano, J. (1974). *Handb. Physiol. Sect. 7 Endocrinol.* **4,** 395–442.
Namm, D. H. (1971). *J. Pharmacol. Exp. Ther.* **178,** 299–310.
Nash, C. B., Boyaji, L. D., and Manley, E. S. (1961). *Arch. Int. Pharmacodyn. Ther.* **133,** 433–443.
Needleman, P. (1976). *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **35,** 2376–2383.
Needleman, P., and Isakson, P. C. (1980). *Handb. Physiol. Sect. 2 Cardiovasc. Sys.* **2,** 613–634.
Needleman, P., and Johnson, E. M. (1973). *J. Pharmacol. Exp. Ther.* **184,** 709–715.
Needleman, P., Jakschik, B., and Johnson, E. M., Jr. (1973a). *J. Pharmacol. Exp. Ther.* **187,** 324–331.
Needleman, P., Kauffman, A. H., Douglas, J. R., Jr., Johnson, E. M., Jr., and Marshall, G. R. (1973b). *Am. J. Physiol.* **224,** 1415–1419.
Needleman, P., Douglas, J. R., Jakschik, B., Stoechlein, P. B., and Johnson, E. M. (1974). *J. Pharmacol. Exp. Ther.* **188,** 453–465.
Needleman, P., Keys, S. L., Denny, S. E., Isaksson, P. C., and Marshall, G. R. (1975). *Proc. Natl. Acad. Sci. USA* **72,** 2060–2063.
Needleman, P., Moncada, S., Bunting, S., Vane, J. R., Hamberg, M., and Samuelsson, B. (1976). *Nature (London)* **261,** 558–560.
Needleman, P., Bronson, S. D., Wyche, A., Sivakoff, M., and Nichalou, K. C. (1978). *J. Clin. Invest.* **61,** 839–846.
Nishiki, K., Erecinska, M., and Wilson, D. F. (1978). *Am. J. Physiol.* **234,** C73–C81.
Nishino, K., Irikura, T., and Takayanagi, I. (1970). *Nature (London)* **228,** 564–565.
Northover, B. J. (1972). *Br. J. Pharmacol.* **45,** 651–659.
Northover, B. J. (1973). *Br. J. Pharmacol.* **48,** 496–504.
Northover, B. J. (1975). *Br. J. Pharmacol.* **53,** 113–120.
Norton, J. M., Gellai, M., and Detar, R. (1972). *Pfleugers Arch.* **335,** 279–286.
Offermeier, J., and Ariens, E. J. (1966a). *Arch. Int. Pharmacodyn. Ther.* **164,** 192–215.
Offermeier, J., and Ariens, E. J. (1966b). *Arch. Int. Pharmacodyn. Ther.* **164,** 216–245.
Olivares, G. J., Smith, N. T., and Aranow, L. (1967). *Br. J. Pharmacol. Chemother.* **30,** 240–250.
Ondetti, M. A., Rubin, B., and Cushman, D. W. (1977). *Science (Washington, D.C.)* **196,** 441–444.
Orton, T. C., Anderson, M. W., Pickett, R. D., Eling, T., and Fouts, J. R. (1975). *J. Pharmacol. Exp. Ther.* **186,** 482–497.
Owen, D. A. A. (1977). *Gen. Pharmacol.* **8,** 141–156.
Page, I. H. (1968). "Serotonin." Yearbook Publ. Chicago, Illinois.
Palmer, M. A., Piper, P. J., and Vane, J. R. (1973). *Br. J. Pharmacol.* **49,** 226–242.
Pang, C. C. Y., and Sutter, M. C. (1981). *Blood Vessels* **17,** 293–301.
Paul, R. J. (1980). *Handb. Physiol. Sect. 2 Cardiovasc. Sys.* **2,** 174–239.

Peach, M. J. (1977). *Physiol. Rev.* **57,** 313–370.
Pearson, J. D. (1982). *In* "Cardiovascular Pharmacology of the Prostaglandins" (A. G. Herman, P. M. Vanhoutte, H. Denolin, and A. Goosens, eds.), pp. 23–34. Raven, New York.
Pearson, J. D., Carleton, J. S., Hutchins, A., and Gordon, J. L. (1978). *Biochem. J.* **170,** 265–271.
Perry, S. V., and Grand, R. J. A. (1979). *Br. Med. Bull.* **35,** 219–226.
Peterson, J. W., and Paul, R. J. (1974). *Biochim. Biphys. Acta* **357,** 167–176.
Philip, R. B., and Lemieux, J. P. V. (1969). *Nature (London)* **221,** 1162
Pisano, J. J., and Austen, F. K. (eds.) (1976). "Chemistry and Biology of the Kallekrein-Kinin System in Health and Disease." US Govt. Printing Office, Washington, D.C.
Polacek, I. J., Bolan, J., and Daniel, E. E. (1970). *Can. J. Physiol. Pharmacol.* **49,** 999–1004.
Popescu, L. M. (1974). *Stud. Biophys.* **44,** S141–S153.
Rhodes, H. J., and Sutter, M. C. (1971). *Can. J. Physiol. Pharmacol.* **49,** 276–287.
Rhodin, J. A. G. (1968). *J. Ultrastruct. Res.* **25,** 452–500.
Rhodin, J. A. G. (1974). *In* "Histology, a Text and Atlas" (J. A. G. Rhodin, ed.). Oxford Univ. Press, London.
Rhodin, J. A. G. (1980). *Handb. Physiol. Sect. 2 Cardiovasc. Sys.* **2,** 1–31.
Rhodin, J. A. G. (1981). *In* "Microcirculation: Current Physiologic, Medical and Surgical Concepts" (R. M. Effros, H. Schmid-Shonbein, and J. Ditzel, eds.), pp. 11–17. Academic Press, New York.
Rocha e Silva, M. (1970). "Kinin Hormones." Springfield, Illinois.
Rocha e Silva, M. (1974). *Life Sci.* **15,** 7–22.
Rubio, R., and Berne, R. M. (1975). *Prog. Cardiovasc. Dis.* **18,** 105–122.
Rubio, R., Berne, R. M., Bockman, E. L., and Curnish, R. R. (1975). *Am. J. Physiol.* **228,** 1896–1901.
Ryan, M. J., and Brody, M. J. (1970). *J. Pharmacol. Exp. Ther.* **174,** 123–132.
Ryan, J. W., and Ryan, U. S. (1977a). *Am. J. Med.* **63,** 595–603.
Ryan, J. W., and Ryan, U. S. (1977b). *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **36,** 2683–2691.
Ryan, J. W., and Ryan, U. S. (1980). *In* "Enzymatic Release of Vasoactive Peptides" (F. Gross, and H. F. Vogel, eds.), pp. 259–274. Raven, New York.
Ryan, J. W., and Ryan, U. S. (1981). *In* "Microcirculation: Current Physiologic, Medical and Surgical Concepts" (R. M. Effros, H. Schmid-Shonbein, and J. Ditzel, eds.), pp. 147–169. Academic Press, New York.
Ryan, J. W., and Smith, U. (1971a). *Biochim. Biophys. Acta* **249,** 177–180.
Ryan, J. W., and Smith, U. (1971b). *Trans. Assoc. Am. Physicians* **84,** 297–306.
Saameli, K. (1968). *Handb. Exp. Pharmacol.* **23,** 545–612.
Salmon, J. A. (1982). *In* "Cardiovascular Pharmacology of the Prostaglandins" (A. G. Herman, P. M. Vanhoutte, H. Denolin, and A. Goosens, eds.), pp. 7–22. Raven, New York.
Salmon, J. A., Smith, D. R., Flower, R. J., Moncada, S., and Vane, J. R. (1978). *Biochim. Biophys. Acta* **523,** 250–262.
Samuelsson, E., and Paoletti, R. (1982). *Adv. Prostaglandin Thromboxane Leukotriene Res.* **9,** 1–343.
Sawyer, W. H. (1961). *Pharmacol. Rev.* **13,** 225–277.
Schnaar, R. L., and Sparks, H. V. (1972). *Am. J. Physiol.* **223,** 223–228.
Schultz, K. D., Bohme, E., Kreye, V. W., and Schultz, G. (1979). *Naunyn Schmiedebergs Arch. Pharmacol.* **301,** 1–9.
Shepherd, J. T., and Vanhoutte, P. M. (1975). "Veins and Their Control." Sauders, Philadelphia, Pennsylvania.

Shepro, D., Batbouta, J. C., Carson, M. P., Robblee, L., and Belamarich, F. A. (1975). *Circ. Res.* **36,** 799–806.

Sigurdsson, S. B., Uvelius, B., and Johansson, B. (1975). *Acta Physiol. Scand.* **95,** 263–269.

Simaan, J. A., and Aviado, D. M. (1976). *J. Pharmacol. Exp. Ther.* **198,** 176–186.

Simionescu, N., Heltianu, C., Antohe, F., and Simionescu, M. (1982). *N.Y. Acad. Sci.*

Simon, G. M., Pamnami, G., Dunkel, J. F., and Overbeck, H. W. (1975). *Circ. Res.* **36,** 791–798.

Sjoberg, B., and Wahlstrom, B. A. (1975). *Acta Physiol. Scand.* **94,** 46–53.

Small, J. V., and Sobieszek, A. (1977). *Eur. J. Biochem.* **76,** 521–530.

Small, J. V., and Sobieszek, A. (1980). *Int. Rev. Cytol.* **64,** 241–306.

Smith, A. D., and Winkler, H. (1972). *Handb. Exp. Pharmacol.* **33,** 538–617.

Smith, U., and Ryan, J. W. (1970). *Adv. Exp. Med. Biol.* **8,** 249–262.

Smith, U., and Ryan, J. W. (1971). *Chest* **59,** 13.

Snipes, R. L., Thoenen, H., and Tranzer, J. P. (1968). *Experientia* **20,** 1026–1027.

Somlyo, A. P., and Somlyo, A. V. (1968). *Pharmacol. Rev.* **20,** 197–272.

Somlyo, A. V., and Somlyo, A. P. (1970). *Pharmacol. Rev.* **22,** 249–253.

Somlyo, A. P., Somlyo, A. V., and Woo, C. Y. (1967). *J. Physiol. (London)* **192,** 657–668.

Speden, R. N. (1964). *Nature (London)* **202,** 193–194.

Spies, C., Schultz, K. D., and Schultz, G. (1980). *Naunyn Schmiedebergs Arch. Pharmacol.* **311,** 71–77.

Stalcup, S. A. (1982). *Ann. N.Y. Acad. Sci.*

Stalcup, S. A., Lipset, J. S., Legant, P. M., Leuenberger, P. J., and Mellins, R. B. (1979a). *J. Appl. Physiol.* **46,** 227–234.

Stalcup, S. A., Lipset, J. S., Woam, J. M., Leuenberger, P. J., and Mellins, R. B. (1979b). *J. Clin. Invest.* **63,** 966–976.

Strum, J. M., and Junod, A. F. (1972). *J. Cell Biol.* **54,** 456–467.

Stull, J. T., and Sanford, C. F. (1981). *In* "New Perspectives on Calcium Antagonists" (G. B. Weiss, ed.), pp. 35–46. Amer. Physiol. Soc., Bethesda, Maryland.

Su, C. (1975). *J. Pharmacol. Exp. Ther.* **195,** 159–166.

Su, C. (1977). *J. Pharmacol. Exp. Ther.* **204,** 351–361.

Su, C., and Bevan, J. A. (1976). *Pharmacol. Ther. Part B.* **2,** 275–288.

Su, C., Bevan, R. D., Duckles, S. D., and Bevan, J. A. (1978). *Microvasc. Res.* **15,** 37–44.

Swartz, S. L., Williams, G. H., Hollenberg, N. K., Moore, T. J., and Dluhy, R. G. (1979). *Hypertension (Dallas)* **1,** 106–111.

Swartz, S. L., Williams, G. H., Hollenberg, N. K., Levin, L., Dluhy, R. G., and Moore, T. J. (1980). *J. Clin. Invest.* **65,** 1257–1264.

Tabachnik, U. I. A., and Gulbekian, A. (1968). *Ann. N.Y. Acad. Sci.* **150,** 204–218.

Tanaka, P., Greenberg, S., Glenn, T. M., Peevy, K. J., and Cho, Y. W. (1982a). *Eur. J. Pharmacol.* (in press).

Tanaka, P., Greenberg, S., Glenn, T. M., and Cho, Y. W. (1982b). *J. Pharmacol. Exp. Ther.*

Tani, E., Yamagama, S., and Ito, Y. (1977). *Cell Tissue Res.* **179,** 131–142.

Tappel, A. L., Lundberg, W. O., and Boyer, P. D. (1953). *Arch. Biochem. Biophys.* **33,** 293–303.

Tateson, J. E., Moncada, S., and Vane, J. R. (1977). *Prostaglandins* **13,** 389–399.

Taylor, J. E., and Richelson, E. (1982). *In* "Receptors and Recognition" (H. I. Yamamura, and S. J. Enna, eds.), pp. 71–100. Chapman & Hall, Cambridge.

Terragno, D. A., Crowshaw, K., Terragno, N. A., and McGiff, J. C. (1975). *Circ. Res.* **36–37** (Suppl. I), 76–80.

Terragno, N. A., Terragno, D. A., and McGiff, J. C. (1977). *Circ. Res.* **40,** 590–598.

Terragno, N. A., Terragno, D. A., Early, J. A., Roberts, M. A., and McGiff, J. C. (1978). *Clin. Sci. Mol. Med.* **55,** s199–s202.
Thoa, N. A., Eccleston, D., and Axelrod, J. (1969). *J. Pharmacol. Exp. Ther.* **169,** 68–73.
Thorens, S., and Haeusler, G. (1979). *Eur. J. Pharmacol.* **54,** 79–91.
Toda, N. (1974). *J. Pharmacol. Exp. Ther.* **191,** 139–146.
Todd, A. S. (1959). *J. Pathol. Bacteriol.* **78,** 281–283.
Todd, A. S. (1964). *J. Clin. Pathol.* **17,** 324–327.
Triggle, D. J. (1976). *In* "Chemical Pharmacology of the Synapse" (D. J. Triggle, and C. R. Triggle, eds.), pp. 431–594. Academic Press, New York.
Triggle, D. J. (1981). *Chest* **84,** 278–283.
Turlapaty, P., and Altura, B. M. (1978). *Eur. J. Pharmacol.* **52,** 421–423.
van Breeman, C., Aaronson, P., and Loutzenhiser, R. (1979). *Pharmacol. Rev.* **30,** 167–208.
Vanhoutte, P. M. (1974). *Circ. Res.* **34,** 317–326.
Vanhoutte, P. M. (1978). *In* "Microcirculation" (G. Kaley, and B. M. Altura, eds.), Vol. 3, pp. 181–309. Univ. Park Press, Baltimore, Maryland.
Vanhoutte, P. M. (1980). *Handb. Physiol. Sect. 2 Cardiovasc. Sys.* **2,** 443–474.
Vanhoutte, P. M. (1981a). *In* "New Perspectives on Calcium Antagonists" (G. B. Weiss, ed.), pp. 109–121. Amer. Physiol. Soc., Bethesda, Maryland.
Vanhoutte, P. M. (1981b). *In* "Vasodilation" (P. M. Vanhoutte, and I. Leusen, eds.), pp. 67–72. Raven, New York.
Vanhoutte, P. M. (1982). *J. Cardiovasc. Pharmacol.* **4** (Suppl. 1), S91–S96.
Vanhoutte, P. M., and Leusen, I. (eds.) (1981). "Vasodilation" Raven, New York.
Vanhoutte, P. M., Lorenz, R. R., and Tyce, G. M. (1973). *J. Pharmacol. Exp. Ther.* **185,** 386–394.
Van Nueten, J. M., Van Beek, J., and Janssen, P. A. J. (1978). *Arch. Int. Pharmacodyn. Ther.* **232,** 42–52.
Van Nueten, J. M., Van Beek, J., and Vanhoutte, P. M. (1980). *J. Pharmacol. Exp. Ther.* **213,** 179–187.
Van Nueten, J. M., Janssen, P. A. J., Van Beek, J., Xhonneux, R., Verbeuren, T. J., and Vanhoutte, P. M. (1981). *J. Pharmacol. Exp. Ther.* **218,** 217–230.
Verhaege, R. H., Vanhoutte, P. M., and Shepherd, J. T. (1977). *Circ. Res.* **40,** 208–215.
Volpi, M., Naccache, P. H., and Sha'afi, R. I. (1980). *Biochem. Biophys. Res. Commun.* **92,** 1231–1237.
Von Loh, D., and Bohr, D. F. (1973). *Proc. Soc. Exp. Biol. Med.* **144,** 513–516.
Wakade, A. R., Kanwar, R. S., and Gulati, O. D. (1970). *J. Pharmacol. Exp. Ther.* **175,** 189–196.
Wakeling, A. E., and Wyngarden, L. J. (1974). *Prostaglandins* **5,** 291–301.
Walter, P., and Bassenge, E. (1968). *Pfleugers Arch.* **299,** 52–65.
Watkins, J., Abbot, E. C., Hensby, C. N., Webster, J., and Dollery, C. T. (1980). *Br. Med. J.* **281,** 702–705.
Webb, R. C., Lockette, W. E., Vanhoutte, P. M., and Bohr, D. F. (1981). *In* "Vasodilation" (P. M. Vanhoutte, and I. Leusen, eds.), pp. 319–330. Raven, New York.
Webster, J., and Dollery, C. T. (1981). *Br. J. Clin. Pharmacol.* **4,** 201–203.
Weibel, E. R. (1974). *Microvasc. Res.* **8,** 218–235.
Weiner, R., Turlapaty, P., and Altura, B. M. (1980). *Eur. J. Pharmacol.* **63,** 241–249.
Weiss, G. B. (1977). *Adv. Gen. Cell. Pharmacol.* **2,** 71–154.
Weiss, G. B. (1978). *In* "Calcium in Drug Action" (G. B. Weiss, ed.), pp. 57–74. Plenum, New York.
Weiss, G. B. (1981a). *In* "Vasodilation" (P. M. Vanhoutte, and I. Leusen, eds.), pp. 307–310. Raven, New York.

Weiss, G. B. (1981b). *In* "New Perspectives on Calcium Antagonists" (G. B. Weiss, ed.), pp. 83–94. Amer. Physiol. Soc., Bethesda, Maryland.
Weiss, G. B., and Rosecrans, J. A. (1971a). *Eur. J. Pharmacol.* **13,** 197–207.
Weiss, G. B., and Rosecrans, J. A. (1971b). *Eur. J. Pharmacol.* **14,** 130–139.
Weksler, B. B., Knapp, J. M., and Jaffe, E. A. (1977). *Blood* **50** (Suppl. 1), 287S (Abs.).
Westfall, T. C. (1977). *Physiol. Rev.* **57,** 659–728.
Weston, A. H. (1972). *Br. J. Pharmacol.* **45,** 95–103.
Westwick, J. (1977). *Br. J. Pharmacol.* **61,** 138P–139P (Abs.).
White, R. (1982). *In* "Prostanoids: Cariopulmonary and Cardiovascular Actions" (S. Greenberg, and T. M. Glenn, eds.). Academic Press, New York.
Wikberg, J. E. S. (1979). *Acta Physiol. Scand.* (Suppl. 468), 1–89.
Williams, G. H., and Hollenberg, N. K. (1977). *N. Engl. J. Med.* **297,** 184–188.
Williamson, J. R., Vogler, N. J., and Kilo, C. (1969). *Diabetes* **18,** 567–568.
Wilton, P. B., and McCalden, T. A. (1977). *Eur. J. Pharmacol.* **46,** 213–219.
Wohl, A. J., Haeusler, L. M., and Roth, F. E. (1967). *J. Pharmacol. Exp. Ther.* **158,** 531–539.
Wohl, A. J., Hausler, L. M., and Roth, F. E. (1968a). *J. Pharmacol. Exp. Ther.* **162,** 109–114.
Wohl, A. J., Hausler, L. M., and Roth, F. E. (1968b). *Life Sci.* **7,** 381–387.
Wong, P. K. Y., Terragno, D. A., Terragno, N. A., and McGiff, J. C. (1977). *Prostaglandins* **13,** 1113–1125.
Wright, G. B., Alexander, R. W., Ekstein, L. S., and Gimbrone, M. A., Jr. (1982). *Circ. Res.* **50,** 462–469.
Zimmerman, B. G. (1973). *J. Pharmacol. Exp. Ther.* **168,** 303–309.
Zimmerman, B. G., Ryan, M. J., Gomer, S., and Kraft, E. (1973). *J. Pharmacol. Exp. Ther.* **187,** 315–323.
Zimmerman, B. G., Ryan, M. J., Gomer, S., and Kraft, E. (1974). *Life Sci.* **11,** 1104–1112.
Zins, G. R. (1975). *Am. J. Med.* **58,** 14–24.
Zsoster, T. T., Heneim, N. F., and Wolchinsky, C. (1977). *Eur. J. Pharmacol.* **45,** 7–12.

ISBN 0-12-508301-7