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NONADRENERGIC INNERVATION *of* BLOOD VESSELS

Volume II
Regional Innervation

Geoffrey Burnstock
Susan G. Griffith

CRC PRESS

Nonadrenergic Innervation of Blood Vessels

Volume II: Regional Innervation

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CRC Press, Inc.
Boca Raton, Florida

Library of Congress Cataloging-in-Publication Data

Nonadrenergic innervation of blood vessels.

Includes bibliographies and index.
1. Blood-vessels--Innervation. 2. Adrenergic mechanisms. 3. Regional blood flow--Regulation. 4. Neurotransmitters--Physiological effect. 5. Nervous system, Vasomotor. I. Burnstock, Geoffrey. II. Griffith, Susan G., 1957- . [DNLM: 1. Blood Vessels--innervation. 2. Vasomotor System--physiology. WG 560 N812]
QP109.N66 1988 616.1'3 87-24283
ISBN 0-8493-6680-1 (Set)
ISBN 0-8493-6681-X (V. 1)
ISBN 0-8493-6682-8 (V. 2)

Direct all inquiries to CRC Press, Inc., 2000 Corporate Blvd., N.W., Boca Raton, Florida, 33431.

International Standard Book Number 0-8493-6680-1 (Set)
International Standard Book Number 0-8493-6681-X (Volume I)
International Standard Book Number 0-8493-6682-8 (Volume II)

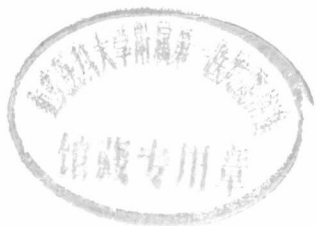
Library of Congress Card Number 87-24283
Printed in the United States

PREFACE

These Volumes are designed to bring together the rapidly increasing number of recent studies of transmitter substances other than noradrenaline involved in the nervous control of blood vessels. Authors have been invited to contribute chapters to provide a multidisciplinary coverage of the field so that structural, functional, pharmacological, and biochemical aspects are all included.

The Chapters in Volume I emphasize the evidence for particular new perivascular neurotransmitters, while those in Volume II are concerned with the interactions of the different transmitters in neural control of particular vascular beds. Inevitably, there is some overlap of material between the two Volumes, but we feel that this is, for the most part, an advantage.

G. Burnstock
S. G. Griffith



DEDICATION

We would like to dedicate these Volumes to Che Su who was an outstanding pioneer in vascular control mechanisms and who had agreed to write the Chapter on ''Purine Nucleotides in Perivascular Nerves'' until his tragic death at the peak of his career prevented him from doing so. He made many most important contributions in the field and he will be sorely missed.

G. Burnstock
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Professor Burnstock has published over 500 papers in scientific and medical journals. He is the Editor-in-Chief of the *Journal of the Autonomic Nervous System* and is currently on the editorial board of 15 other journals. His current research interests include structural and functional analysis of autonomic nervous control of bladder, gut, lung, heart, and blood vessels, and changes in autonomic nerve function in development, old age, and in diseases such as diabetes, hypertension, incontinence, atherosclerosis, vasospasm, and impotence.

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Chapter 1

INNERVATION OF SYSTEMIC BLOOD VESSELS

Ian L. Gibbins, Judith L. Morris, John B. Furness, and Marcello Costa

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I. INTRODUCTION

The systemic blood vessels are defined here as those large vessels supplying and draining regions of the body which include several different organs and tissues. As such, they include the aorta, carotid, subclavian, major splanchnic, and iliac arteries, as well as the caval, brachiocephalic, jugular, subclavian, large abdominal, and iliac veins. Although these vessels serve primarily as major conduits carrying blood to and from the heart, they have a number of other important functions. For example, the elastic nature of the walls of the aorta and the proximal segments of the major distributing arteries have a "Windkessel" effect, stretching to accommodate blood ejected from the left ventricle and recoiling to force blood into the smaller arteries when the aortic valves close. Furthermore, the balance of distribution of the cardiac output to different regions of the body is determined, in part, by the relative diameters of large supplying arteries such as the external carotid or superior mesenteric arteries. Finally, variation in the capacitance of the large systemic veins can actively affect venous return.

There is both anatomical and physiological evidence for the presence of motor nerves, which modify wall tension, and sensory nerves, which may have mechanoreceptive, nociceptive, or chemoreceptive roles, in the walls of systemic blood vessels. Concepts of the ways in which nerves that supply visceral and vascular tissues should be regarded have gradually evolved over the last 200 years,[1-3] and are still changing. During the 19th century, all motor nerves to the vasculature were called sympathetic, involuntary, or vegetative. Gaskell[4] recognized that the nerves were separated anatomically into cranial, thoracolumbar, and sacral groups. Langley, who had introduced the term autonomic for the complete system in 1898, suggested that the thoracolumbar output should be called sympathetic, and the cranial and sacral outputs should be called parasympathetic because the latter groups, in general, had opposite effects to the sympathetic nerves.[5] In particular, sympathetic nerves had pressor effects on the cardiovascular system, whereas parasympathetic nerves were depressor. Moreover, adrenaline had sympathetic-like effects and pilocarpine had parasympathetic-like effects, and so it is understandable that (nor)adrenergic transmission came to be equated with the sympathetic system, and cholinergic transmission with the parasympathetic system.

We have now been overtaken by further discoveries, which have forced us to revise many of our conceptions of both the structure and the function of the autonomic nervous system. First, it is clear that autonomic neurons can release transmitters other than acetylcholine and (nor)adrenaline.[6,7] Most notable of these substances are the neuropeptides, such as neuropeptide-Y (NPY) and vasoactive intestinal polypeptide (VIP), both of which can occur in perivascular axons (see Sections III.B and III.C). Neuropeptides also occur in and can be released from certain classes of sensory neurons, including some of those originating in systemic vessels (see Section III.D and IV.A).

Second, it is equally clear that some, and perhaps all, neurons contain more than one substance which may contribute to neurotransmission.[8,9] Thus, VIP, for example, coexists with acetylcholine in cranial parasympathetic neurons and both substances mediate neurogenic responses in the salivary glands.[7] However, any particular neuropeptide may be associated with neurons having different functions, just as noradrenaline or acetylcholine occur in diverse types of neurons. Somatostatin, for instance, occurs in parasympathetic cholinergic neurons of the toad cardiovascular system,[10,11] in some sympathetic noradrenergic neurons in guinea pigs,[12,13] and in some spinal sensory neurons of rats and cats.[14,15] Within a single species, the same neuropeptide occurs in quite separate populations of neurons; as another example, substance-P (SP) occurs both in spinal sensory neurons (see Section III.D) and in intrinsic enteric neurons whose functions must be quite different from each other.[16] Moreover, several classes of peripheral neurons have been identified in which the individual neurons

contain many different neuropeptides. Particular combinations of the peptides are usually found in neurons associated with specific anatomical pathways.[17-21] As a result of findings like these, it is no longer acceptable, in most cases, to study the distribution of a single neuropeptide under the assumption that it is identifying a single homogeneous population of neurons.

Finally, we cannot distinguish, on structural grounds alone, between the peripheral endings of autonomic motor axons and unmyelinated sensory axons,[22,23] all of which are varicose, and all of which may contain and release vasoactive neuropeptides (see Section II.B).

A re-examination of vascular innervation is thus necessary to determine the patterns of colocalization of substances in perivascular nerve fibers and the origins of these nerve fibers. Many studies have used ultrastructural or histochemical techniques to try to identify putative neurotransmitters present in the nerve endings. Some ultrastructural studies have attempted to correlate the morphology of the storage vesicles in nerve terminals with the transmitter present in the vesicles.[24] However, recent ultrastructural studies in both vascular and non-vascular tissues have indicated that such a correlation often is impossible to make in tissue conventionally fixed for electron microscopy.[22,25] It seems that histochemical reactions or immunohistochemical labeling techniques must be performed before putative neurotransmitters can be localized anatomically.

In this chapter we will describe the distributions of histochemically identified types of axons associated with the systemic vasculature, and the anatomical location of the cell bodies from which the axons originate. Where published histochemical data are scanty, we have examined material from chickens, crocodiles *(Crocodylus porosus)*, guinea pigs, mudpuppies *(Necturus maculosus)*, possums *(Trichosurus vulpecula)*, and rats especially for this chapter. Coexistence of neuropeptides or of neuropeptides and enzymes synthesizing nonpeptide transmitters, has been demonstrated in these tissues with a double-labeling fluorescence immunohistochemical technique. This technique allows the simultaneous visualization of two antigens in whole mounts or sections of tissues. The details and specificity of this procedure have been published previously.[17,20,21,26]

In functional terms, we need to determine whether nerve-mediated responses that are resistant to antagonists of noradrenergic or cholinergic transmission are due to other substances released from noradrenergic or cholinergic axons. Alternatively, the substances mediating these responses may be released from other classes of motor axons utilizing neither noradrenaline nor acetylcholine, or they may even originate from perivascular afferent axons. The evidence for the participation of these mechanisms in motor control of systemic blood vessels will be reviewed. The possible physiological roles of sensory neurons associated with the systemic vessels will also be discussed, with emphasis on the C-fiber afferents.

II. GENERAL ARRANGEMENT AND MORPHOLOGY OF PERIVASCULAR NERVES

A. General Arrangement

Using methylene blue- or silver-staining techniques, anatomists have described an arrangement of nerves supplying the systemic blood vessels which is remarkably similar across the vertebrates, from frog to human.[27-31] In mammals, the ascending aorta and aortic arch receive nerve fibers predominantly from the stellate ganglion, but also from the superior cervical ganglia, upper thoracic ganglia, and the vagi. These nerve fibers reach the aorta in trunks, which divide and anastomose in the adventitia. Small nerve bundles form a dense plexus in the thoracic aorta of most species, which is continuous down the abdominal aorta and along the aortic branches. An additional supply of nerves to the more distal parts of the aorta and its branches arises from the thoracic and lumbar ganglia, and from the coeliac and mesenteric ganglia. Mitchell[29] has also described ganglion cells in the adventitia of the

abdominal aorta of humans, which seem to contribute nerve fibers to the aortic plexus. A dual supply of axons to branches of the aorta, consisting of an extension of the aortic plexus and an additional supply from the sympathetic chains, is also found in nonmammalian vertebrates.

The nerves supplying the caval veins adjacent to the heart seem to extend from the atrial nerve plexuses. Additional nerves reach the caval veins and adjacent veins from the vagi, and from the thoracic sympathetic and sensory ganglia. Nerve fibers arising from ganglion cells in the adventitia of the caval veins have also been observed to contribute to the local nerve plexuses.[29,30]

The nerve trunks supplying the systemic blood vessels consist of a majority of nonmyelinated (nonmedullated) fibers mixed with a few myelinated fibers (Figure 1a). Both nonmyelinated and myelinated nerve fibers contribute to the perivascular plexuses. In larger animals, the branches of the aorta are commonly accompanied by large paravascular nerve trunks of both myelinated and nonmyelinated nerve fibers, which consist of nerves that pass along the artery towards their targets and do not innervate the artery.[27,28]

B. Unmyelinated Nerve Fibers

In the aorta and its branches, small bundles of unmyelinated nerve fibers branch from the adventitial nerve trunks and form a plexus at the junction between the adventitia and the media.[27-31] These nerve bundles are accompanied by Schwann cells, and are orientated mainly along the axis of the vessel. Many nerve fibers in this plexus are varicose.

Early studies described a plexus of fine unmyelinated fibers which penetrate the entire media of the aorta in many vertebrates, and which have a predominantly circular arrangement.[27,28,30,32] These fibers were reported to anastomose and re-anastomose to form a "terminal reticulum", and seemed to contact every smooth muscle cell. The presence or absence of this terminal nerve plexus was the subject of controversy over many years.[31] Ábrahám[31] and Mitchell[29] concluded that, although some arteries did possess some medial innervation (e.g., dog aorta, pig coronary artery; and renal and cerebral arteries), most large arteries did not, and the fibers observed by others were most likely connective tissue elements. Indeed, this view has been supported by many recent histochemical and electron-microscopic studies[33-35] which report the absence of medial innervation in most systemic arteries. In those arteries where nerves do penetrate the media, they are commonly restricted to the outer half of the media. From electron-microscopic studies of the structure of large arteries,[34] it seems most likely that the terminal reticulum corresponds to the fine elastic fibers which branch extensively throughout the media.

The observation that surgical denervation produced by resection or removal of the sympathetic chains caused degeneration of all unmyelinated perivascular axons, led to the conclusion that all unmyelinated perivascular axons originated from the sympathetic ganglia.[27,28,30] However, more recent physiological and immunohistochemical studies have provided evidence that many of the unmyelinated perivascular axons in systemic vessels are sensory, originating from cell bodies in the dorsal root ganglia. These unmyelinated sensory axons run in nerve trunks together with postganglionic sympathetic axons,[36-39] which explains their disappearance after surgical sympathectomy.

In many systemic vessels, the unmyelinated sensory axons that can be detected immunohistochemically also run together with sympathetic axons in the perivascular plexuses. These sensory axons are often varicose, as are the sympathetic terminal axons, and electron-microscopic studies indicate that they have no clearly distinguishing morphological features.[22,40] In large blood vessels, in particular, there is no close association between unmyelinated axons and smooth muscle cells comparable to the neuromuscular junctions in skeletal muscle. Varicose regions of axons, which contain neurotransmitters stored in vesicles, become devoid of Schwann cell cover in the vicinity of vascular smooth muscle cells.

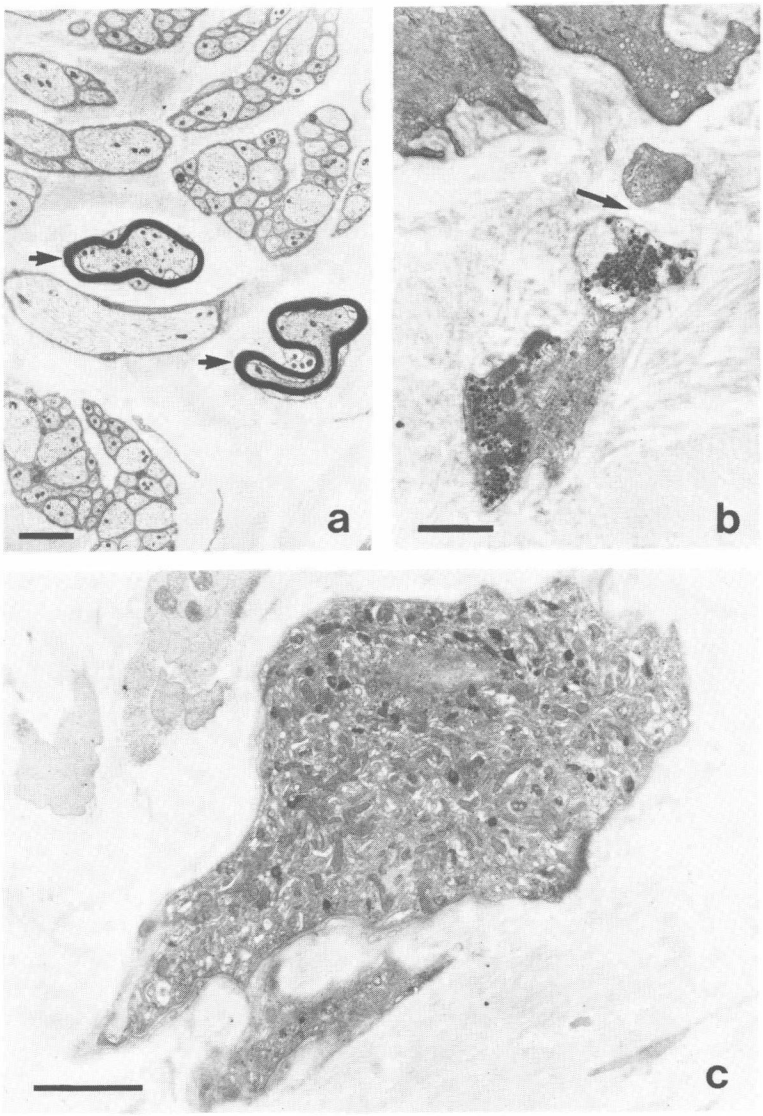

FIGURE 1. Electron micrographs of nerve fibers associated with systemic arteries. (a) Bundles of unmyelinated axons together with two myelinated axons (arrows) in the adventitia of the dorsal aorta, near the origin of a renal artery, in the toad *Bufo marinus*. Scale bar = 2 μm. (b) Adrenergic nerve varicosities in dorsal aorta of *Bufo marinus*. One varicosity is 200 nm away from the closest smooth muscle process (arrow). Scale bar = 1 μm. (Reproduced from Morris, J. L. and Gibbins, I. L. *Cell Tiss. Res.*, 231, 357, 1983. With permission.) (c) Large profile of myelinated axon ending in the truncus arteriosus of the lizard, *Trachydosaurus rugosus*. Scale bar = 2 μm. (Reproduced from Berger, P. J. et al., *Cell Tiss. Res.*, 226, 389, 1982. With permission.)

These regions are thought to be the sites of neuromuscular transmission.[33] The closest neuromuscular distances found in medium to large arteries are in the range 200 to 500 nm (Figure 1b),[41,42] although some varicosities free of Schwann cells may be as far as 1 to 2 μm away from the nearest smooth muscle cells in large elastic arteries.[24,42] In general, neuromuscular distances decrease as vessel diameter decreases.[35]