Symposium on the Physiology and the Pharmacology of Vascular Neuroeffector Systems Interlaken 1969

Physiology and Pharmacology of Vascular Neuroeffector Systems

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Neuroeffector Function and Organization

Proc. Symp. Physiol. Pharmacol. Vasc. Neuroeffector Systems, Interlaken 1969, pp. 2–12 (Karger, Basel 1971)

Morphologic Studies of the Vascular Neuroeffector Apparatus

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Our current understanding of the neural control of blood vessels has developed rapidly during the last decade. This has been due to an increased awareness of the clinical significance of such studies, the introduction of electron microscopy alone or coupled with selected techniques for the visualization of the primary neurotransmitter agents, and stricter correlations between morphologic and functional studies.

Methods of Study

A. Light microscopy: (1) Heavy metal impregnation techniques. (2) Supravital and intravital methylene blue staining of nerve fibers. (3) Enzyme substrate histochemistry, e.g. cholinesterase. (4) Fluorescence techniques – monoamine uptake studies – drug depletion effects.

B. Electron microscopy: (1) Qualitative. (2) Quantitative. (3) Histochemical applications.

C. Electron autoradiography: e.g. H^3-norepinephrine.

D. Selective denervation procedures: Such studies as these alone or in combination with functional *in vitro* observations have revealed details of the gross neural organization, nerve-muscle relationship, and density of effective transmitter sites in the neurons.

Organization and Ultrastructure of the Adrenergic Neuroeffector System

Space precludes discussion of smooth muscle architecture or more than passing reference to morphologic features of the supporting elements. We will consider in some detail the anatomy of the neuro-muscular interval, de-

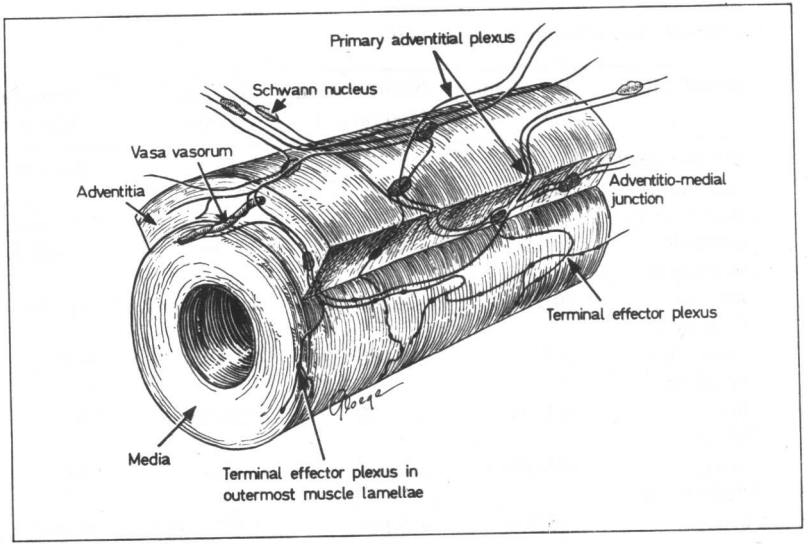

Fig. 1. Schema of neuroeffector organization in a large blood vessel. The terminal effector plexus contains multiple nodal areas of axoplasmic specialization (nerve terminal areas) and is spatially limited to the adventitio-medial junction (reprinted by kind permission of *J. Anat.*, Lond. 1968).

Table I. Differential features of innervation apparatus in blood vessels

	Primary adventitial plexus	Terminal effector plexus
Origin	Autonomic n.	Adventitial plexus
Site	Adventitia	Adventitio-medial junction, Perivasal
Fiber size	0.5–1.5 nm	~0.8 nm
Multiaxonal	3–12	1–3
Schwann n.	+	–
Nerve terminal area	–	+

lineated on the one side by the terminal effector plexus and on the other by the smooth muscle plasmalemma. Firstly, a brief review of the general organization of the neuro-effector system. Post-ganglionic adrenergic fibers ramify in the loose adventitia to form the *primary plexus* (fig. 1). This multiaxonal system contains numerous Schwann cell nuclei which allows for its differentiation from the functional *terminal effector plexus* (table I). This organization is easily recognized in the large arteries [VERITY and BEVAN,

Table II. Nerve terminal ultrastructure

Blood vessel	Ext. diam. (μ)	Neuromuscular interval (Å)	Granular vesicles	Neurotubule-filament (Å)
Pre-capillary arteriole	~18	1,200	2+3	Present
Renal cortex arteriole	25–35	2–4,000	1+2+3	Tubules, ~175 Å
Pancreatic arteriole	20–50	<4,000	1 (10%)+2+3	Present
Coronary arteriole	~50	3–7,000	2+3	NC
Auricular artery	~1,500	~5,000	Present	NC
Mesenteric artery	100–200	~5,000	1+2+3	NC
Pulmonary artery	>5,000	~2.10^4	1 (30%)+2+3	Tubules, ~270 Å Filaments, ~80 Å

[LEVER, GRAHAM, IRVINE and CHICK, 1965; APPENZELLER, 1964; UCHIZONO, 1964; LEVER, AHMED and IRVINE, 1965; SIMPSON and DEVINE, 1966; VERITY and BEVAN, 1968; DEVINE, 1966].

1968]. The axons of the terminal effector plexus contain neurotubules, mitochondria, dense bodies and the characteristic population of granular and agranular vesicles especially prominent in the nerve terminal areas – regional expansions of axoplasm partly surrounded by Schwann cell cytoplasm.

Table II summarizes some characteristic features of nerve terminal ultrastructure in a variety of blood vessel types. Some broad conclusions may be drawn from such data. The mean neuromuscular interval varies from 9.5×10^2 to 2×10^4 Å, and roughly parallels the vessel diameter. Types 2 and 3 granular vesicles are present (in variable amounts) in all nerve terminal areas examined. Such granular vesicles are the presumed storage site for intraneuronal norepinephrine and false neurotransmitters. A small but significant number of larger granular vesicles, Type 1, have been identified. The functional significance of these vesicles is still in some doubt. Finally, neurotubule-filament profiles have been identified in nerve terminal areas but more especially in the adventitial and terminal effector

portions of the plexus and are analogous to such structures found in other portions of the nervous system [ELFVIN, 1961; CRAVIOTO, 1966].

Evidence for Vesicular Localization of Adrenergic Neurotransmitter in Nerve Terminal Areas

Considerable evidence now exists for the presence of norepinephrine in the sympathetic nerve terminations around blood vessels. Amine is preferentially localized in nodal areas of the terminal effector plexus [FALCK, 1962; FUXE and SEDVALL, 1964; NORBERG and HAMBERGER, 1964; KAPELLER and MAYOR, 1969]. Strong evidence can now be presented for the association of granular vesicles of Types 2 and 3 with norepinephrine storage:

(a) Such granular vesicles (~450 Å diameter) have been found in all sympathetically innervated structures [GRILLO, 1966].

(b) Only those nerves containing the specific Type 2 and 3 vesicles degenerate after selective sympathectomy [LEVER, SPRIGGS and GRAHAM, 1968].

(c) The vesicle content in selected tissue preparations known to contain an adrenergic innervation can be correlated with specific monoamine fluorescence reactions [NORBERG and HAMBERGER, 1964; ZELANDER *et al.*, 1962; BLOOM and BARRNETT, 1966].

(d) Electron autoradiography using *in vivo* and *in vitro* preparations has revealed H^3- norepinephrine over granular vesicles in nerve terminal areas [WOLFE *et al.*, 1962; WOLFE and POTTER, 1963; LEVER, SPRIGGS and GRAHAM, 1968].

(e) Electron microscopy with norepinephrine contents of particulate fractions obtained after density gradient centrifugation reveals a high correlation between vesicle content and amine level [ISHII *et al.*, 1965; DE ROBERTIS *et al.*, 1965].

(f) Constricted post-ganglionic sympathetic nerves reveal a close correlation between the increased numbers of granular vesicles seen by electron microscopy, fluorescence intensity and quantitative estimations of norepinephrine content proximal to the constriction [DAHLSTROM, 1965; KAPELLER and MAYOR, 1967; BANKS, MANGNALL, and MAYOR, 1969].

(g) Selective depletion and repletion experiments of vesicle contents by pharmacologic agents has provided functional, histochemical, electron microscopic, and biochemical correlations. In figure 2, data has been assembled to provide a semiquantitative analysis of the percentage change in granule containing vesicles with quantitative measurements of norepineph-

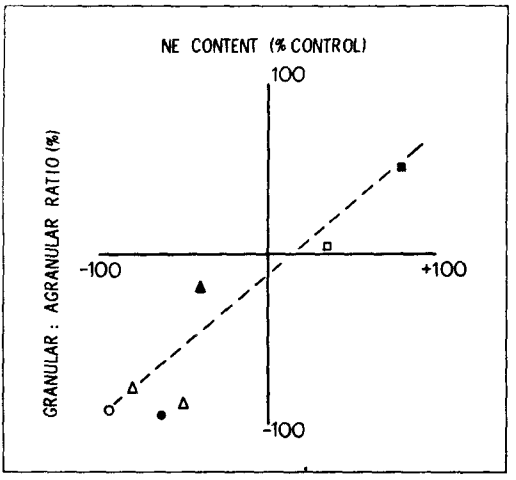

Fig. 2. Correlation of change in ratio of granular: agranular vesicles in nerve terminal areas with norepinephrine content after administration of pharmacologic agents. Data derived from studies of IRALDI and DE ROBERTIS [1961]; VAN ORDEN *et al.* [1966]; DEVINE, ROBERTSON and SIMPSON [1967]; DEVINE and SIMPSON [1968]. ○ Reserpine: Decrease granule binding. ● Guanethidine: Block NE release. □ Bretylium: Block NE release. ■ Pargyline: MAO inhibition. △ α-methyltyrosine: Tyrosine hydroxylase inhibition. ▲ α-methyldopa: DOPA decarboxylase inhibition.

rine content [IRALDI and DE ROBERTIS, 1961; VAN ORDEN *et al.*, 1966; DEVINE, ROBERTSON and SIMPSON, 1967]. These separate studies reveal a linear correlation between norepinephrine content and change in ratio of granular vesicles. Moreover, while guanethidine and bretylium have pharmacologically similar actions, their mode of action at the nerve terminal indicates a significant dissimilarity in that guanethidine has a reserpine-like component inducing vesicle degranulation.

Morpho-pharmacologic Studies of the Significance of the Neuromuscular Interval in Blood Vessels

In blood vessels and other adrenergically innervated tissues, where the presumed neuromuscular interval is less than 1,000 Å in width, there is virtual exclusion of the extracellular space, absence of collagen and microfibrillar material and apparent fusion of the basement membranes of axon

and smooth muscle. The basement membrane component of the smooth muscle sarcolemma varies from 300–700 Å in thickness and a more tenuous basement membrane surrounds the nerve terminal area Schwann cytoplasm extending over the bare area of the axon [VERITY and BEVAN, 1968 (see also fig. 11, 12); SIMPSON and DEVINE, 1966 (fig. 12); MERRILLEES, BURNSTOCK and HOLMAN, 1963]. In such areas there may be increased subplasmal vacuolization. Neuromuscular intervals greater than 1,500 Å are characterized by microfibrillar material, collagen, prolongations of fibroblast cytoplasm and nonopposed basement membranes. This latter organization is the usual in aorta, carotid artery, pulmonary artery of cat and rabbit and coronary arterioles [LEVER, AHMED, and IRVINE, 1965].

In a previous study on the nature of the abnormally long latency in neuromuscular transmission in the pulmonary artery [BEVAN and VERITY, 1966], a preliminary analysis showed that extracellular diffusion would account for only a small part of this latency, the remaining delay presumably could be accounted for by difficulty in access of transmitter to the receptor site or by subsequent rate limiting intracellular events before contraction. More recent morphologic observations strongly suggest that difficulty in access of transmitter to the receptor site may be of greater significance than originally supposed. Such evidence is based upon the finding of single or multiple, long cytoplasmic expansions of adventitial fibroblasts interposed between nerve terminal bare areas and subjacent smooth muscle membrane (fig. 3). Such an architectural feature would greatly modify the minimum diffusion pathway for the liberated transmitter and may increase manyfold the extracellular diffusion time.

A further hindrance to the diffusion of transmitter over distances greater than 1,500 Å is the presence of abundant microfibrillar material in the neuromuscular cleft. Such material, thought analogous to basement membrane, has a glycoprotein composition and may serve for the nonspecific adsorption of neurotransmitter, thus necessitating the release of larger amounts at the nerve terminal area to provide minimum effective concentrations at the receptor site on the smooth muscle membrane. Considerations of the role played by the basement membrane in neurotransmitter-receptor interactions are of necessity speculative. That a well-developed basement membrane exists in vascular smooth muscle is established. Two observations suggest a key role of this material in transmitter-receptor interaction. GILLESPIE and HAMILTON [1966] demonstrated the phenoxybenzamine sensitive binding of norepinephrine to the vascular smooth muscle *surface* in spleen preparations. Moreover, the postulated receptors

were found to be generalized over the entire surface. WOOLLEY and GOMMI [1965] and WESEMANN and ZILLIKEN [1967] have provided evidence on the chemical nature of a neurotransmitter receptor in smooth muscle and postulate a critical role for sialic acid – a significant component of basement membrane material.

Cocaine potentiation of the response of various tissues to sympathetic amine is considered due to its ability to block the reuptake by the nerve terminal of liberated amine [TRENDELENBURG, 1963]. Paradoxically, blood vessels have shown low cocaine induced sensitivity compared with other smooth muscle preparations. A possible explanation may lie in the relatively larger neuromuscular distances to be found in the blood vessels examined. To further investigate this concept a correlation has been made

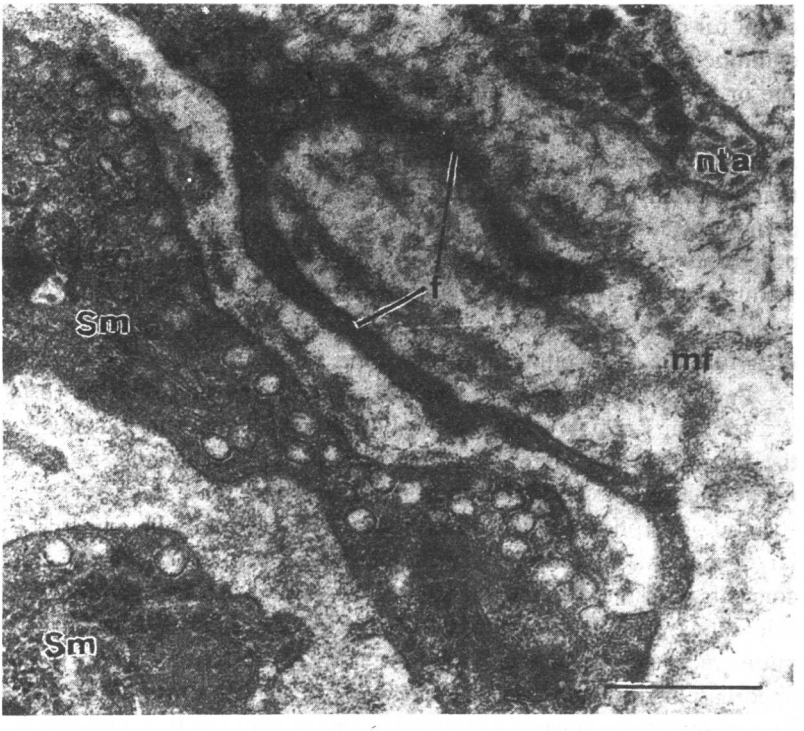

Fig. 3. Extensions of fibroblast cytoplasm (f) are interposed between the nerve terminal area (nta) and subjacent smooth muscle (sm). Microfibrillar material (mf) extends throughout the neuromuscular interval. Bar represents 0.6 μ.

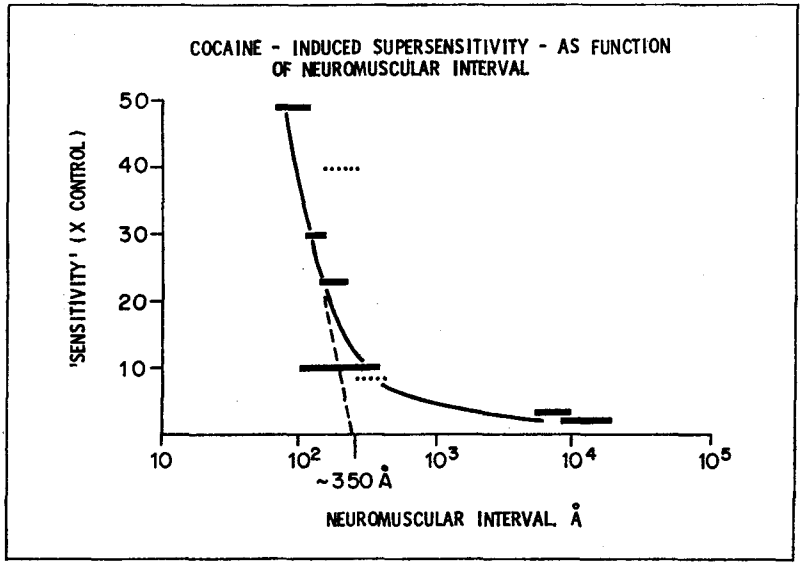

Fig. 4. Correlation between the magnitude of the neuromuscular interval and potentiation of norepinephrine response in the presence of cocaine. Data derived from studies of BENNETT and ROGERS [1967]; BEVAN and VERITY [1967]; FURCHGOTT *et al.* [1963]; GOVIER *et al.*, [1969]; HAEFELY, HÜRLIMANN and THOENEN [1964]; MERRILLEES *et al.* [1963]; PICK [1967]; TRENDELENBURG [1965]; URSILLO and JACOBSON [1965]; VERITY and BEVAN [1968].

between the magnitude of the neuromuscular interval and norepinephrine potentiation in the presence of cocaine in a variety of adrenergically innervated tissues (fig. 4). Two tentative conclusions may be drawn from this preliminary data. Firstly, a nonlinear, inverted hyperbolic relation is seen between the two variables suggesting the existence of two different cocaine induced phenomena. Secondly, the extrapolated point on the × axis at ~350 Å is the minimum neuromuscular distance at which significant cocaine potentiation is seen. The corresponding maximum from the same data lies in the region 800–1,000 Å. In general terms, neuromuscular intervals greater than 1,000 Å in adrenergically innervated tissues will not show cocaine supersensitization. It is perhaps not fortuitous that at this critical interval a break in basement membrane continuum becomes evident and interposition of extracellular components becomes manifest. Although speculative, such data drawn from multiple tissue preparations under dif-

ferent conditions of examination pinpoint a role for basement membrane material in facilitating and controlling transmitter diffusion over relatively large distances.

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