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Integration of Factors Controlling Vascular Tone

Overview

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FOR many years, studies of neurohumoral control of the vasculature have been dominated by consideration of the role of catecholamines released from sympathetic perivascular nerves and from the adrenal medulla into the bloodstream. New and improved techniques in immunohistochemistry, electron microscopy, electrophysiology, and pharmacology, introduced in recent years, have led to a wealth of discoveries that have profoundly reshaped our understanding of the autonomic nervous system.[1,2] Although the classic view involved antagonistic cholinergic and adrenergic nerves, many new putative neurotransmitters have been proposed in the last few years. Neuromodulatory mechanisms have been recognized, including prejunctional inhibition or enhancement of transmitter release, postjunctional modulation of transmitter action, and the secondary involvement of locally synthesized hormones and prostaglandins. The existence of more than one transmitter substance in some nerves, or cotransmission, is now also widely recognized.[3,4]

Knowledge of local humoral regulation of blood flow has also rapidly progressed in recent years. This arose from the seminal discovery that endothelial cells, which form the innermost layer of blood vessels, play a crucial role in the vasodilatory response of the vessel to acetylcholine (ACh)[5] and to other substances.[6] The considerable interest in vascular control mechanisms arising from these studies has led to the concept of a dual regulation of blood vessel tone, whereby both nerves and the endothelium are involved.[7,8]

The current review discusses these recent discoveries in neurohumoral control of the vasculature, and suggests a new framework for considering changes in disease.

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Perivascular Nerves

The vascular neuroeffector junction consists of varicose nerve fibers within a plexus at the adventitial-medial border. Transmitter is released *"en passage"* from varicosities to reach vascular smooth muscle cells that are in electrical continuity with each other *via* gap junctions.[9,10] The varicosities do not have a fixed relationship with particular smooth muscle cells, and the junctional cleft varies between about 60 nm and as much as 2 μm in some large arteries; muscle cells rarely (if at all) have postjunctional specializations. This means that the vascular neuromuscular junction differs in a significant way from "synapses" at the motor endplate in striated muscle or within ganglia, where there is a fixed relationship with both pre- and postjunctional specialization. The variable geometry of the vascular neuromuscular junction means that neuromodulation is an important feature. A neuromodulator is defined as a substance that modifies the process of neurotransmission. It may act as a prejunctional modulator by decreasing or increasing the amount of transmitter released by the nerve varicosity, or it may act as a postjunctional modulator by altering the time course or extent of action of the neurotransmitter. Neuromodulators may be circulating neurohormones; local agents, such as prostanoids, bradykinin, or histamine; or neurotransmitter substances released from other nerves nearby or even from the same nerve varicosity.

Multiplicity of Transmitters: Cotransmission and Chemical Coding

For more than 50 yr, the only transmitters considered in perivascular nerves were noradrenaline (NA) and ACh. Since the discovery of nonadrenergic, noncholinergic nerves in the early 1960s, more than 12 new

chemical messengers have been identified, including monoamines, purines, amino acids, polypeptides, and nitric oxide (table 1).

The concept of cotransmission, *i.e.*, that nerves synthesize, store, and release more than one transmitter, was first proposed in 1976.[3] This hypothesis is now generally accepted.[4,11–16]

Although, at first sight, the multiplicity of transmitters released in various computations from different perivascular nerves appears formidable and unnecessary, a pattern is now emerging that clarifies the situation. This is the idea that autonomic nerves have a "chemical coding," *i.e.*, individual neurones contain a particular combination of transmitter substances, have processes that project to identifiable target sites, and have defined central connections. This concept has been developed most fully for the enteric nervous system,[17] but it also applies to perivascular nerves.

Table 1. Established and Putative Transmitters: Perivascular Nerves

Noradrenaline (NA)
Acetylcholine (ACh)
Adenosine 5′-triphosphate (ATP)
5-Hydroxytryptamine (5-HT)
Dopamine (DA)
Enkephalin–dynorphin (ENK–DYN)
Vasoactive intestinal polypeptide (VIP)
Peptide histidine isoleucine (PHI)
Substance P (SP)
Gastrin-releasing peptide (GRP)
Somatostatin (SOM)
Neurotensin (NT)
Vasopressin (VP)
Cholecystokinin–gastrin (CCK–GAS)
Neuropeptide Y–pancreatic polypeptide (NPY–PPP)
Galanin (GAL)
Angiotensin (ANG)
Adrenocorticotrophic hormone (ACH)
Calcitonin gene-related peptide (CGRP)
Nitric oxide (NO)

Sympathetic Perivascular Nerves

There is now a substantial body of evidence showing that NA and ATP act as cotransmitters, being released from sympathetic nerves in variable proportions depending on the tissue and species.[18] Most of the early and more detailed studies were made on the vas deferens,[19] but many studies of sympathetic cotransmission, involving adenosine 5′-triphosphate (ATP) and NA, have now also been carried out on a number of different blood vessels,[20] including rat tail artery,[21,22] rabbit ear artery,[23–25] dog basilar artery,[26] mesenteric artery,[27–32] rabbit pulmonary artery,[33] guinea pig and rabbit saphenous artery,[34–36] and rabbit hepatic artery.[37] Sympathetic cotransmission involving NA and ATP has also been shown in the circulation of skeletal muscle,[38] cat intestine,[39] kidney,[40] dog skin,[41] and the pithed rat.[42,43]

Evidence for purinergic cotransmission includes: block on the prazosin-resistant component of the response to sympathetic nerve stimulation by the ATP antagonist arylazido aminoproprionyl-ATP ($ANAPP_3$), or by the selective desensitizer of the ATP (P_{2X}-purinoceptor) by α,β-methylene ATP; release of ATP during nerve stimulation, which is prevented by tetrodotoxin, guanethidine, or destruction of sympathetic nerves by 6-hydroxydopamine, but is unaffected by selective depletion of NA by reserpine; and mimicry of excitatory junction potentials (EJPs) by ATP, but not by NA.

The purinergic component gives an optimal response during short bursts of sympathetic nerve stimulation (1 s or less), but the traditional period of nerve stimulation in experimental *in vitro* preparations is 30 s or more, under which conditions the NA component dominates the mechanical responses.[44]

The proportions of the cotransmitters NA and ATP vary considerably between different vessels. For example, ATP is the major component of sympathetic cotransmission in the rabbit saphenous artery, intestinal arterioles,[45,46] and mesenteric arteries,[35] but it appears to be a relatively minor component in rabbit ear artery and rat tail artery, in which the relationship between EJPs and mechanical responses is more difficult to demonstrate. A model depicting sympathetic cotransmission is shown in figure 1. The purinergic component of sympathetic cotransmission is selectively affected by the dihydropyridines, nifedipine and Bay K 8644.[48,49]

Recent studies in our laboratory have shown that, in rabbit coronary vessels, in contrast to other vessels in which NA and ATP cause synergistic constriction *via* α_1-adrenoceptors and P_{2X}-purinoceptors, respectively, the predominant effect of ATP is vasodilatation *via* P_{2Y}-purinoceptors.[50] Because, in this vessel, the predominant effect of NA is vasodilatation *via* β-adrenoceptors, this is consistent with the synergism that appears to be characteristic of cotransmission.

Neuropeptide Y (NPY) is also stored in, and released from, sympathetic nerves,[51,52] but, in many vessels, it has little direct postjunctional action.[53,54] However, it

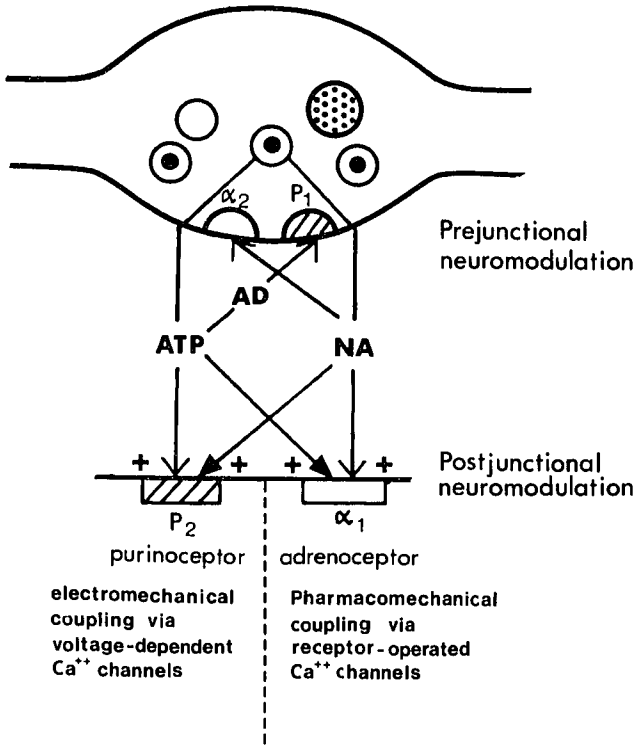

Fig. 1. Diagram showing that adenosine 5'-triphosphate (ATP) and noradrenaline (NA) are released as cotransmitters from the sympathetic nerves supplying the vas deferens and some blood vessels. ATP acts on P_2-purinoceptors on the smooth muscles to initiate excitatory junction potentials, action potentials, and a fast initial contraction involving electromechanical coupling *via* voltage-dependent Ca^{2+} channels. In contrast, NA acts on α_1-adrenoceptors to produce the second slower phase of the contraction by pharmacomechanical (or at least spike-independent) coupling *via* receptor-operated Ca^{2+} channels. Prejunctional α_2-adrenoceptors and P_1-purinoceptors can reduce transmitter release when activated by NA and adenosine (AD), respectively (prejunctional neuromodulation), but NA and ATP enhance each other's actions (postjunctional neuromodulation). (Adapted with permission from Burnstock.[47])

has potent prejunctional actions reducing the release of NA and ATP, and postjunctional actions enhancing the actions of NA and ATP.[19,55,56] The geometry of particular sympathetic neuromuscular junctions appears to influence the type of neuromodulation, *i.e.*, with wide junctions, postjunctional potentiation by NPY dominates; however, narrow clefts favor prejunctional inhibition by NPY.[57] Neuropeptide Y has direct vasoconstrictor actions in some vessels—for example, those in heart, brain, spleen, and skeletal muscle—but its origin may be from intrinsic or local neurons, rather than sympathetic nerves.

In a study of blood vessels in guinea pig skin, a differential chemical coding has been demonstrated, *i.e.*, although sympathetic nerves in the distributing arteries contain NPY and NA, in the smaller arteries, they contain dynorphin (DYN), as well as NPY and NA; and in precapillary arteries, only DYN and NA are present.[58]

5-Hydroxytryptamine (5-HT) immunofluorescent nerves have been localized in a number of vessels.[9,59] However, it seems that, for the most part, 5-HT is not synthesized and stored in separate nerves, but is taken up, stored in, and released as a "false transmitter" from sympathetic nerves.[60]

Parasympathetic Perivascular Nerves

In the salivary gland of the cat, vasoactive intestinal polypeptide (VIP) appears to be stored together with ACh in parasympathetic nerves, probably in separate vesicles. During low-frequency stimulation, ACh is released to increase salivary secretion from acinar cells, and, also, to elicit some minor dilatation of blood vessels in the gland.[61,62] Vasoactive intestinal polypeptide is released from the same nerves, especially at high stimulation frequencies, to produce marked dilatation and, although it has no direct effect on acinar cells, it acts as a neuromodulator to substantially enhance the postjunctional effect of ACh on acinar cell secretion, and to increase the release of ACh from the nerve varicosities *via* prejunctional receptors (fig. 2). Vasoactive intestinal polypeptide is a potent vasodilator of many vessels, notably penile vessels; it appears to play a major role in erection.

Sensory-Motor Perivascular Nerves

Sensory nerves have been claimed to use substance P (SP),[63–65] calcitonin gene-related peptide (CGRP),[66,67] and ATP.[68–71] Calcitonin gene-related peptide and SP have been shown to coexist in sensory nerve terminals in perivascular nerves,[72,73] and, with the use of colloidal gold particles of different sizes, they have been shown to coexist in the same large granular vesicles.[74] By analogy with other systems, it seems likely that ATP coexists in different proportions with these two peptides, perhaps cooperating in axon reflex vasodilatation[75] (fig. 3). Because the role of these nerves during the axon reflex to many organs[76] is motor rather than sensory, they have been termed "sensory-motor nerves," to distinguish them from the other subpopulation of afferent fibers that have an entirely sensory role and whose terminals contain few vesicles and a predominance of mitochondria.[2]

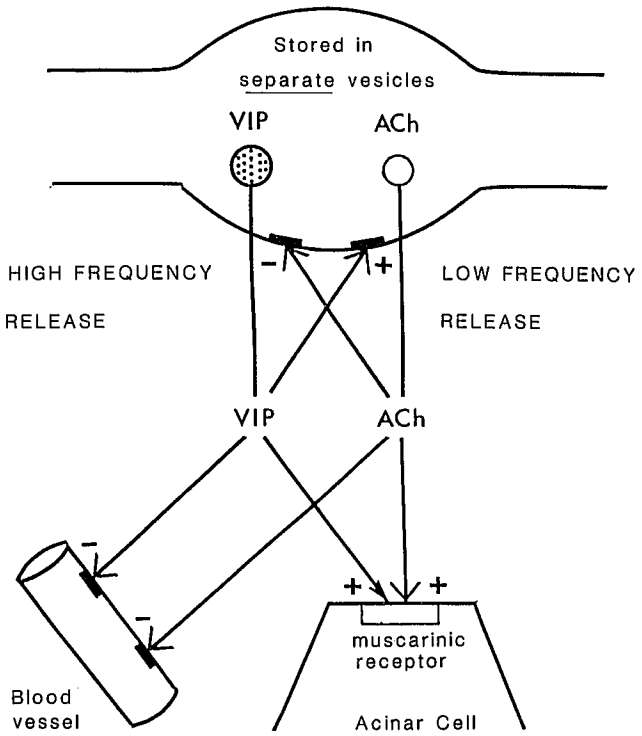

Fig. 2. A classic transmitter acetylcholine (ACh) coexists with vasoactive intestinal polypeptide (VIP) in parasympathetic nerves supplying the cat salivary gland. ACh and VIP are stored in separate vesicles; they can be released differentially at different stimulation frequencies to act on acinar cells and glandular blood vessels. Cooperation is achieved by selective release of ACh at low-impulse frequencies, and of VIP at high frequencies. Pre- and postjunctional modulation is indicated. (Reprinted with permission from Burnstock.[47])

Prejunctional modulation of sensory-motor nerve-mediated vasodilatation of the rat mesenteric arterial bed by adenosine has been demonstrated.[77]

Perivascular Nerves Arising from Intramural Neurones

Little is known about the physiologic roles or the pharmacology of intrinsic neurones of the heart, because it is so difficult to study this *in situ*. However, a novel culture preparation from the atria of newborn guinea pigs has been developed in our laboratory to study the intrinsic innervation of the heart under conditions of unequivocal extrinsic denervation.[78,79] Some of these neurones show immunofluorescence for NPY, some for 5-HT, and some for variable mixtures of both transmitter substances. Projections of these neurones *in situ* form perivascular plexuses in small-resistance coronary vessels.[80] Both NPY and 5-HT are potent vasoconstrictors of coronary vessels and may have synergistic actions. Nitric oxide synthase has also been shown to be localized in a subpopulation of intrinsic cardiac neurones.[81]

Few studies have been carried out on the projections of intrinsic neurones to blood vessels in other organs, but intrinsic enteric neurones are known to supply some vessels in the gut and mesentery, and it is well known that monoamine-containing neurones in the brain contribute to the innervation of some cerebral vessels.[9] Nitric oxide appears to be a transmitter in cerebral and penile vessels, perhaps derived from local neurones.[82]

Endothelium

Since 1980, when Furchgott and Zawadzki[5] first reported that the vasodilatation response to ACh requires the presence of an intact endothelium, the role of the endothelium in the regulation of vascular tone has attracted considerable interest.[6,83] Action on endothelial receptors by a number of vasoactive substances stimulates the production of endothelium-derived relaxing factors (EDRF) or constricting factors (EDCF) and prostaglandins. These subsequently modify vascular tone by causing contraction or relaxation of the vascular smooth muscle. Endothelium-derived relaxing factor has been identified as nitric oxide,[84,85] and the peptide endothelin is considered one of the constricting factors.[86] It should be noted that there is considerable heterogeneity in the endothelium-dependent responses of mammalian blood vessels, with variations between arteries and veins and between different vascular beds. It is likely that such variations would be physiologically useful, particularly with regard to ensuring that the blood supply to the heart and brain are protected under a variety of different conditions.[87]

Endothelium-Mediated Vasodilatation

In addition to ACh, endothelium-dependent vasodilatation has been shown to occur in response to ATP, adenosine 5′-diphosphate (ADP), arachidonic acid, SP, neurokinin A (NKA), 5-HT, bradykinin, histamine, neurotensin, vasopressin (VP), angiotensin II (AgII), and thrombin.[88] Different subtypes of the receptors to such vasoactive substances occur on the endothelium and on the vascular smooth muscle. For example, P_{2x}-

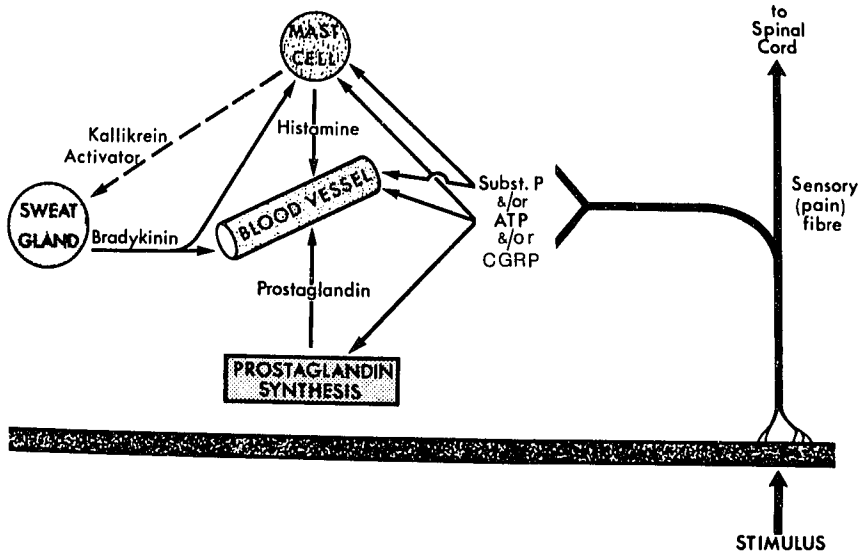

Fig. 3. Diagram showing the basis of the "axon reflex" in the skin leading to vasodilatation and inflammation. It is suggested that calcitonin gene-related peptide (CGRP), substance P (SP), and adenosine 5'-triphosphate (ATP) are released during antidromic activation of sensory collaterals. (Adapted with permission from Burnstock.[75])

purinoceptors are present on the vascular smooth muscle, and are acted on by ATP released from perivascular nerves to produce vasoconstriction; and ATP can cause vasodilatation *via* P_{2Y}-purinoceptors on the endothelial cells.[89,90]

It is clear that neurotransmitters released from perivascular nerves have direct access to vascular smooth muscle cell receptors to produce a response. It is neither likely nor desirable that the same neurotransmitter can diffuse through the media and basal lamina of a large blood vessel (without degradation) to act on endothelial receptors and produce the opposite effect. To establish that endothelium-dependent responses have a role to play in the control of vascular tone in the intact organism, it is necessary to identify the source of the vasoactive substances that act on the endothelial receptors. For some substances, a readily available source is the blood. In the case of ACh and SP, however, circulating levels are low because of their rapid breakdown. The possibility that endothelial cells themselves may be the source of such substances was first proposed in 1985, when Parnavelas *et al.*[91] reported that choline acetyltransferase, the enzyme responsible for the synthesis of ACh, could be localized in endothelial cells lining capillaries and small vessels in the rat cortex. Since this time, using the same technique of immunocytochemical staining combined with electron microscopy, ChAT, SP, 5-HT, VP, and AgII have all been localized in endothelial cells from a variety of blood vessels.[92–95] In addition, SP levels have been measured in endothelium isolated from cerebral arteries and aorta.[96] Others have also demonstrated that endothelial cells have the capability of synthesizing AgII and histamine.[97,98]

Experiments have been carried out to investigate whether certain stimuli can cause the release of vasoactive substances from their endothelial stores, thus providing evidence for a physiologic mechanism for the endothelium-dependent responses to such stimuli to occur. 5-Hydroxytryptamine, ATP, SP, and ACh, all of which are present in coronary endothelial cells, have been shown to be released after hypoxic perfusion of the Langendorf heart preparation from the rat.[93,94,99] Hypoxic vasodilatation has been shown to be endothelium-dependent. In the perfused rat hindlimb, increased flow causes the release of SP, which has been localized in the endothelial cells of the rat femoral artery.[100] After removal of the endothelium by perfusion with air bubbles,[101] increased flow no longer induced the release of SP, although denervation of the hindlimb vasculature of SP-containing nerves by capsaicin had no effect on flow-induced SP release.[102] Substance P has also been shown to be released from columns of endothelial cells grown on microcarrier beads after increased flow.[103] This supports the view that the source of the SP is endothelial cells. Thus, SP and other vasoactive substances within endothelial cells, by their release, contribute to flow-induced vasodilatation, which is known to be an endothelium-dependent response.

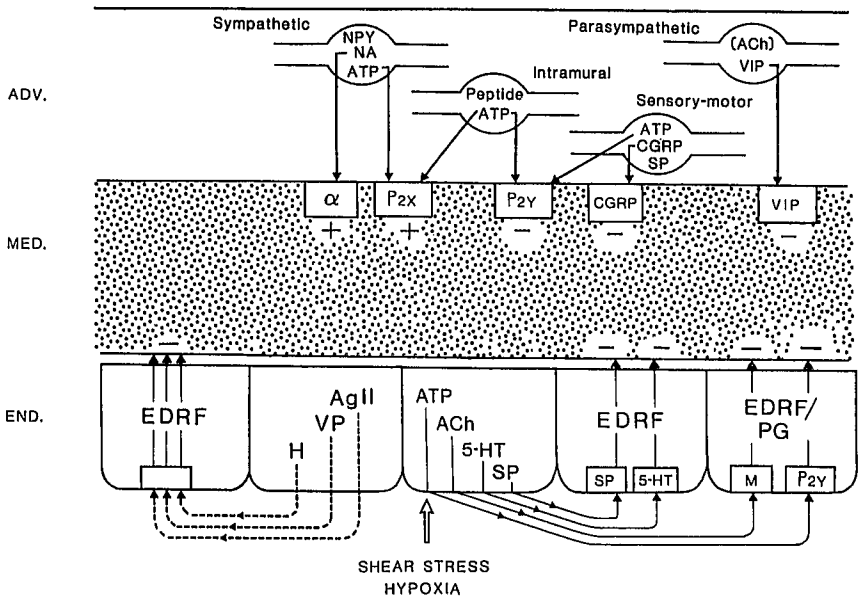

Fig. 4. Regulation of vascular tone by perivascular nerves and endothelial cells. Neuropeptide Y (NPY), noradrenaline (NA), adenosine 5'-triphosphate (ATP), calcitonin gene-related peptide (CGRP), substance P (SP), and vasoactive intestinal polypeptide (VIP) can be released from nerve varicosities in the adventitia (ADV) to act on receptors in the media (MED), causing vasoconstriction or vasodilatation. ATP, ACh, 5-hydroxytryptamine (5-HT), and SP, released from endothelial cells (END) by shear stress or hypoxia, act on their receptors on endothelial cells to cause a release of EDRF or prostaglandins (PG), which, in turn, act on the smooth muscle to cause relaxation. Angiotensin II (AgII), vasopressin (VP), and histamine (H) are also contained in, and may be released from, subpopulations of endothelial cells. In areas denuded of endothelial cells, opposite effects may be produced by receptors on the smooth muscle cells; for example, *via* P_{2X}- and P_{2Y}-purinoceptors and muscarinic receptors (M). (Adapted from Burnstock.[149])

Endothelium-Mediated Vasoconstriction

It has been proposed that the endothelium mediates vasoconstriction *via* production of an EDCF in response to various chemical and physical stimuli, such as NA, thrombin, high extracellular potassium, hypoxia, and stretch.[104–108] In response to stretch, endothelial Ca^{2+} channels have been described and suggested to operate as mechanotransducers.[109] Thus, the role of endothelial cells is twofold, sensory and effector, such that vasoconstriction may occur independently of the action of extraneous vasoactive substances.

Although the nature of EDCF is still uncertain, and appears to be different in blood vessels of different anatomic origin, at least three different classes of endothelial vasoconstrictor substances have been recognized: (1) metabolites of arachidonic acid; (2) a polypeptide-like factor (or factors) produced by cultured endothelial cells; and (3) a still-unidentified diffusible factor released from anoxic/hypoxic endothelial cells.[110] A polypeptide produced by the endothelium in response to various stimuli was demonstrated using cultured bovine aortic endothelial cells, and could represent the EDCF. On release, this was shown to be a potent vasoconstrictor that was unaffected by inhibitors of receptors for known vasoactive substances and by inhibitors of prostaglandin synthesis, but was abolished by several treatments (sodium dodecyl sulphate, trypsin, alkali, or acid hydrolysis) known to affect proteins.[111–113]

An endothelium-derived 21-residue vasoconstrictor peptide, endothelin, has been isolated from porcine aortic endothelial cells, and the complementary DNA of its precursor, preproendothelin, has been cloned and sequenced.[86] It has been shown to be a potent constrictor in, for example, the rabbit skin microvasculature,[114] isolated human resistance vessels,[115] and rat mesenteric resistance vessels.[116] Endothelin and ATP, but not VIP, have been shown to be released from isolated aortic endothelial cells exposed to increased flow.[117] Receptors for endothelin have been localized by autoradiography on cultured rat aortic smooth muscle cells,[118] rat kidney,[119] and human and porcine coronary arteries.[120]

A schematic model of the neural and endothelial factors involved in control of vascular tone is illustrated in figure 4.

Changes in Vascular Control in Aging and Disease

Because perivascular nerves are separated from endothelial cells by vascular smooth muscle, the possibility of trophic interactions between perivascular nerves and endothelial cells has received little direct

investigation. However, studies of the vasculature in disease, and after denervation or mechanical injury, do provide some indication that such interactions may occur.

Perivascular nerve varicosities have been demonstrated in close apposition to endothelial cells in capillaries; this raises the possibility of direct trophic interactions between nerves and endothelial cells in the microvasculature. Adenosine can be formed from the extracellular breakdown of ATP released from nerves. Chronic inhibition of adenosine uptake with dipyridamole has been shown to cause proliferation of capillary endothelium and increased capillary density in skeletal muscle and heart.[121] Furthermore, it has been suggested that neuropeptides may have a role in controlling neurochemical differentiation, cell proliferation, hypertrophy, and regeneration.[122] Such interactions require further investigation in the context of vascular cell biology.

Changes in Perivascular Nerves in Development and Aging

In a study of the changes in density of sympathetic adrenergic nerves in blood vessels of the rabbit, using image analysis quantitation, Cowen *et al.*[123] recognized that the pattern of change with age varied considerably between different vessels. Although the early stages of development of vascular innervation were similar in all the vessels studied, and reached an initial peak density at about 6 weeks after birth, the density of innervation of some vessels (*e.g.*, femoral artery) declined thereafter; other vessels (*e.g.*, renal artery) reached peak density at 6 months and then rapidly declined; however, in the basilar artery, density of innervation continued to increase into old age (3 yr).

Changes in the development of peptide-containing perivascular nerves of guinea pig vessels were studied between 6 weeks *in utero* and old age, and compared with changes in perivascular adrenergic nerves.[124] Again, variation in the pattern of development of perivascular nerves in different vessels was demonstrated. In addition, in mesenteric and carotid arteries, although adrenergic nerve density reached a peak 4 weeks after birth and declined thereafter, the peptide-containing nerves (VIP, CGRP, and SP) reached a peak at birth and declined thereafter to about half of maximum density in old age, raising the possibility that perivascular neuropeptides may play a trophic role in early development.

In another study from our laboratory, it has been shown that, although there is a decrease in expression of vasoconstrictor, cerebrovascular neurotransmitters (NA and 5-HT) in aging rats, there is an increase in vasodilator neurotransmitters (VIP and CGRP).[125]

Changes in Perivascular Nerves After Trauma, Surgery, and Chronic Exposure to Drugs

It has been shown that, 2–8 weeks after sympathetic and sensory denervation of the rabbit ear artery, endothelium-dependent relaxation responses to methacholine are significantly depressed.[126] The reduction in response was not caused by any impairment of the ability of the muscle to relax, because the maximal relaxation to sodium nitroprusside (an endothelium-independent agent) was unaffected by denervation. Long-term sympathetic denervation in rabbits resulted in an increase in the sensitivity of cerebral arteries to hypercapnia, hypoxia, and 5-HT.[127] Although morphologic changes in the endothelial cells were not detected under these conditions,[128] it is possible that alterations in the endothelial control of the cerebral vasculature after sympathetic denervation contributed to this effect. In contrast, when the endothelium of the dog coronary artery was mechanically injured without disruption of the elastic lamina, neuron-specific enolase-positive nerve fibers were increased in number at both 1 and 3 months.[129] An increased density of SP-containing nerve fibers was also observed in the dog coronary artery 3 months after mechanical injury to the endothelium. Surgical sympathectomy or long-term adrenoceptor blockade by propranolol are claimed to prevent or reduce the induction of atherosclerosis by diet.[130] It has been proposed that NPY and NA in cerebral perivascular nerves, which increase during the development of hypertension in rats,[131] are involved in protection against disruption of the blood–brain barrier and cerebral hemorrhage caused by hypertension. Sympathetic denervation before the development of hypertension results in increased incidence of stroke and increased permeability of the blood–brain barrier.

Unilateral removal of the superior cervical ganglion (SCG) results in the reinnervation of denervated cerebral vessels by sprouting nerves from the contralateral SCG.[132] Other marked compensatory changes after superior cervical ganglionectomy include increased SP levels in the ipsilateral iris and ciliary body,[133] increased CGRP content of pial vessels,[134] and increased expression of NPY in nonadrenergic VIP-containing nerves in the cerebral vasculature.[135] Long-term chem-

ical sympathectomy of developing rats, induced by chronic guanethidine treatment, leads to increased brightness and density of CGRP-positive immunofluorescent nerves innervating blood vessels.[136] In late pregnancy, sympathetic innervation of guinea pig uterine blood vessels exhibits a remarkable switch from adrenergic vasoconstrictor to cholinergic vasodilator control,[137] although ultrastructural studies of the guinea pig uterine artery did not show any degeneration of serotonergic or peptidergic (NPY, VIP, SP, and CGRP)-containing nerves in late pregnancy.[138] Four-week treatment with estrogen, but not progesterone, leads to a marked reduction in the density and varicosity diameters of 5-HT-containing nerves supplying the rabbit basilar artery.[139] In view of the possible involvement of 5-HT in the pathogenesis of headache, this finding indicates that contraceptive pills with a high estrogen content may be contraindicated in women prone to migraine attacks. The effect of crush lesions on perivascular noradrenergic nerves has shown differential rates of reinnervation in different blood vessels, indicating the presence of characteristic levels of local neurotrophic activity.[123]

Changes in Perivascular Nerves in Hypertension

The distribution of NA and NPY in nerves was compared during the early development of cerebral vessels in normotensive rats and spontaneously hypertensive rats (SHR) before and after the time when hypertension becomes apparent at about 5 weeks of age.[131] Three interesting findings emerged from this study. First, the levels of both NA and NPY were significantly higher in cerebral perivascular sympathetic nerves in SHR compared with normotensive rats. Second, in both normotensive rats and SHR, there was a discrepancy between the time course of changes in the expression of NA and NPY, *i.e.*, the density of NA-fluorescent fibers increased rapidly between 4 and 6 weeks, but NPY-immunofluorescent nerves showed a rapid increase between 6 and 8 weeks (fig. 4). Because NA and NPY coexist in sympathetic perivascular nerves, this shows that the expression of cotransmitters is not necessarily identical. And, third, the increase in NA and NPY in SHR does not occur in the sympathetic nerve cell bodies in the superior cervical ganglion from which the cerebral perivascular nerves arise.

Diabetes

Perivascular nerves in penile vessels containing VIP and ACh were shown to be damaged or lost in streptozotocin-diabetic rats and in diabetic impotent men.[140–142] A reduction in the expression of VIP and 5-HT, but not NPY and NA, has been demonstrated in perivascular nerves supplying the cerebral blood vessels of streptozotocin-induced diabetic rats.[143]

Attenuation of endothelial-mediated vasodilatation has been claimed in diabetic vessels, perhaps because of reduced release of EDRF.[144]

Atherosclerosis

Although endothelial-mediated vasodilatation has been shown to be seriously attenuated in heavily lesioned vessels, such as aorta and carotid artery, in other vessels in early atherosclerosis a compensatory increase in endothelium-mediated vasodilatation has been reported.[145,146]

Chronic Hypoxia

In a recent study of the effect of chronic hypoxia on the control of endothelial vasoactive substances, it was shown that, in response to sheer stress, endothelial cells isolated from hypoxic rats released less ATP, but more endothelin, compared with cells from normoxic rats.[147] It was suggested that, under conditions of reduced arterial oxygen tension, a dynamic balance between ATP and endothelin could regulate the response of vessels to flow.

Chronic Stimulation of Perivascular Nerves In Vivo

After 10 days of chronic *in vivo* stimulation of perivascular nerves in the rabbit ear artery, the vasoactive peptides NPY and CGRP were shown to appear in subpopulations of endothelial cells, indicating that the level of activity in perivascular nerves has a trophic influence on the expression of peptides in endothelial cells.[148]

Final Comment

The recent developments in our knowledge of both perivascular nerves and endothelial cells described in this article have profound implications on our understanding of the mechanisms controlling blood flow. It can be envisioned that the status of vascular tone will be the result of interactions between the neural and endothelial control mechanisms. It seems likely that spontaneous release of EDRF is responsible for a resting endothelial-mediated vasodilator tone, which is op-

posed by a resting vasoconstrictor tone mediated by sympathetic nerves. Under different physiologic or pathophysiologic circumstances, the balance may be altered so that one or the other may dominate. It seems likely that endothelial release of vasoactive substances may be of greater significance in the response of blood vessels to local changes in their environment, such as hypoxia and increased flow. In contrast, perivascular nerves may be more concerned with the integrative control of blood flow in the organism as a whole.

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