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Non-synaptic transmission at autonomic neuroeffector junctions

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Abstract

Non-synaptic transmission is characteristic of autonomic neuroeffector junctions. The structure of the autonomic neuromuscular junction is described. The essential features are that: the terminal portions of autonomic nerve fibers are varicose and mobile, transmitters being released '*en passage*' from varying distances from the effector cells; while there is no structural post-junctional specialization on effector cells, receptors for neurotransmitters accumulate on cell membranes at close junctions; muscle effectors are bundles rather than single smooth muscle cells, that are connected by gap junctions which allow electrotonic spread of activity between cells. A multiplicity of transmitters are utilized by autonomic nerves, and cotransmission occurs often involving synergistic actions of the cotransmitters, although pre- and post-junctional neuromodulation of neurotransmitter release also take place. It is suggested that autonomic neural control of immune, epithelial and endothelial cells also involves non-synaptic transmission.

Keywords: Cotransmission; Enteric; Gap junctions; Heart; Junctional cleft; Lung; Neurotransmitters; Nerve varicosities; Parasympathetic; Sensory-motor; Smooth muscle; Sympathetic; Ureter; Vas deferens

1. Introduction

Non-synaptic transmission at autonomic neuromuscular junctions has been recognised for some time (see Burnstock and Iwayama, 1971; Burnstock, 1986, 2004b; Gabella, 1995). It also occurs in the central nervous system (see Vizi, this issue). Evidence in support of non-synaptic neuromuscular transmission will be presented in this article and also for neuroeffector transmission to immune, epithelial and endothelial cells.

2. Structure of the autonomic neuromuscular junction

The autonomic neuromuscular junction differs in several important respects from the better known skeletal neuromuscular junction; it is not a synapse with the well defined prejunctional and postjunctional specializations established for the skeletal neuromuscular synapse or ganglionic synapses. A model of the autonomic neuroeffector junction has been proposed on the basis of combined electrophysiologic, histochemical and electron-microscopical studies. The essential features of this model are that the terminal portions of autonomic nerve fibers are varicose, transmitter being released *en passage* from varicosities during conduction of an impulse, although excitatory and inhibitory junction potentials are probably elicited only at close junctions. Furthermore, the effectors are muscle bundles rather than single smooth muscle cells, which are connected by low-resistance pathways (gap junctions) that allow electrotonic spread of activity within the effector bundle. In blood vessels, the nerves are confined to the adventitial side of the media muscle coat, and this geometry appears to facilitate dual control of vascular smooth muscle by perivascular nerves and by endothelial relaxing and contracting factors. Neuroeffector junctions do not have a permanent geometry with postjunctional specializations, but rather the varicosities are continuously moving and their special relation with muscle cell membranes changes with time, including dispersal and reformation of receptor clusters. For example, varicosity movement is likely to occur in cerebral blood arteries, where there is a continuously increasing density of sympathetic innervation during development and aging and in hypertensive vessels or those that have been stimulated chronically *in vivo*, where there can be an increase in innervation density of up to threefold.

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2.1. Varicose terminal axons

In the vicinity of the effector tissue, axons become varicose, varicosities occurring at 2–10 μm intervals (Figs. 1a,b, 2a,d and 3a,c,d) and branches intermingle with other axons to form the autonomic ground plexus, first described by Hillarp (1946). The extent of the branching, and the area of effector tissue affected by individual neurons, varies with the tissue. Autonomic axons combined in bundles are enveloped by Schwann cells. Within the effector tissue they partially lose their Schwann cell envelope (Figs. 2a,e and 3b), usually leaving the last few varicosities naked (Figs. 2b,c,f,g and 4c). Varicosities are up to 2 μm in diameter and about 3 μm in length and are packed with vesicles and mitochondria, while intervaricosities are often as little as 0.2 μm in diameter and contain neurofilaments (Figs. 2a,d and 4a).

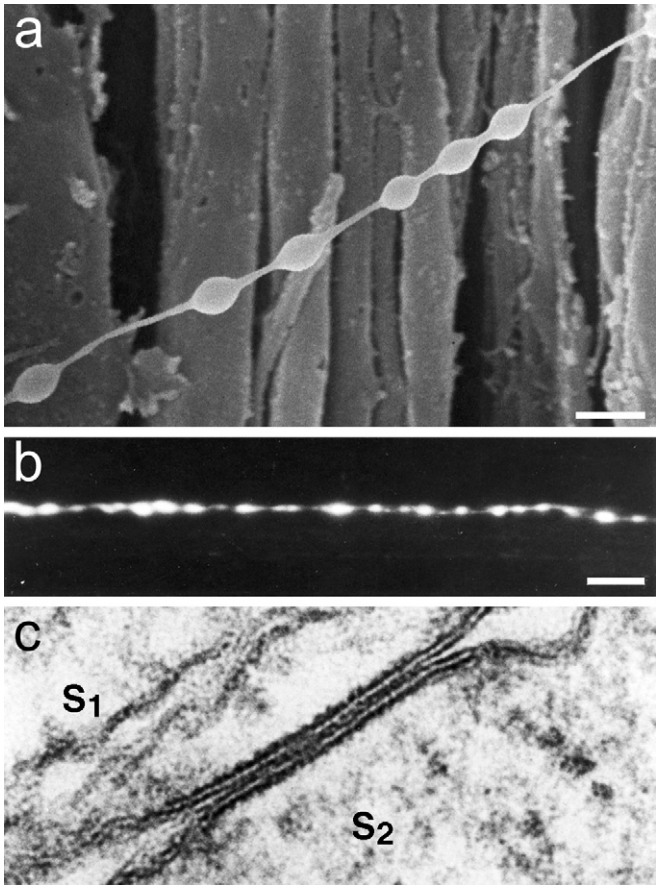

Fig. 1. (a) A scanning electron micrograph of a single terminal varicose nerve fiber lying over smooth muscle of the small intestine of the rat. The intestine was pre-treated to remove connective tissue components by digestion with trypsin and hydrolysis with HCl. Scale bar = 3 μm (reproduced from Burnstock (1988), with permission from Marcel Dekker). (b) A single adrenergic axon in the guinea pig mesentery. Fluorescence histochemical method for catecholamines on a whole mount preparation. Scale bar = 10 μm (reproduced from Burnstock and Costa (1975), with permission from Chapman and Hall). (c) Gap junction between two cultured smooth muscle cells (S_1, S_2). A gap of up to about 3 nm can be seen between the outer leaflets of the unit membrane; there are a few short areas of fusion. The inner leaflets of the membranes are lined by an accumulation of electron-opaque material. Magnification, ×280,000 (reproduced from Campbell et al. (1971), with permission from The Rockerfeller University Press).

The density of innervation, in terms of the number of axon profiles per 100 muscle cells in cross-section, also varies considerably in different organs. For example, it is very high in the vas deferens, iris, nictitating membrane, and sphincteric parts of the gastrointestinal tract, but low in the ureter, uterus, and longitudinal muscle coat of the gastrointestinal tract. In most blood vessels, the varicose nerve plexus is placed at the adventitial border and fibers rarely penetrate into the medial muscle coat (Fig. 3a and c).

2.2. Junctional cleft

The width of the junctional cleft varies considerably in different organs. In the vas deferens, nictitating membrane, sphincter pupillae, rat parotid gland, and atrioventricular and sinoatrial nodes in the heart, the smallest neuromuscular distances range from 10 to 30 nm (Fig. 2d). The minimum neuromuscular distance varies considerably in different blood vessels. Generally, the greater the vessel diameter, the greater the separation of nerve and muscle. Thus, minimal neuromuscular distances in arterioles and in small arteries and veins are about 50–100 nm, in medium to large arteries the separation is 200–500 nm (Fig. 3b), whereas in large elastic arteries where the innervation is sparser, the minimum neuromuscular distances are as wide as 1000–2000 nm. Serial sectioning has shown that at close junctions in both visceral and vascular organs there is fusion of prejunctional and postjunctional basal lamina. In the longitudinal muscle coat of the gastrointestinal tract, autonomic nerves and smooth muscle are rarely separated by less than 100 nm (Fig. 4a). However, in the circular muscle coat, close (20 nm) junctions are common, sometimes several axon profiles being closely apposed with single muscle cells. The wide and variable cleft characteristic of non-synaptic autonomic neuroeffector junctions makes them particularly amenable to the different ways in which cotransmitters and neuromodulators can interact to affect neurotransmission (Burnstock, 1987a), illustrated in Fig. 6d.

2.3. Prejunctional and postjunctional specialization

Although there are many examples of prejunctional thickenings of nerve membranes in varicosities associated with accumulations of small synaptic vesicles, representing sites of transmitter release (see Fig. 2c), there are no convincing demonstrations of postjunctional specializations, such as membrane thickening or folding or indeed absence of micropinocytic vesicles; this is in keeping with the view that even close junctions might be temporary liaisons. Thus pre-and postjunctional sites are particularly accessible for neuromodulatory influences, where local agents may enhance or exacerbate release of neurotransmitter or alter the extent or time course of neurotransmitter action.

2.4. Muscle effector bundles and gap junctions

The smooth muscle effector is a muscle bundle rather than a single muscle cell – that is, individual muscle cells being

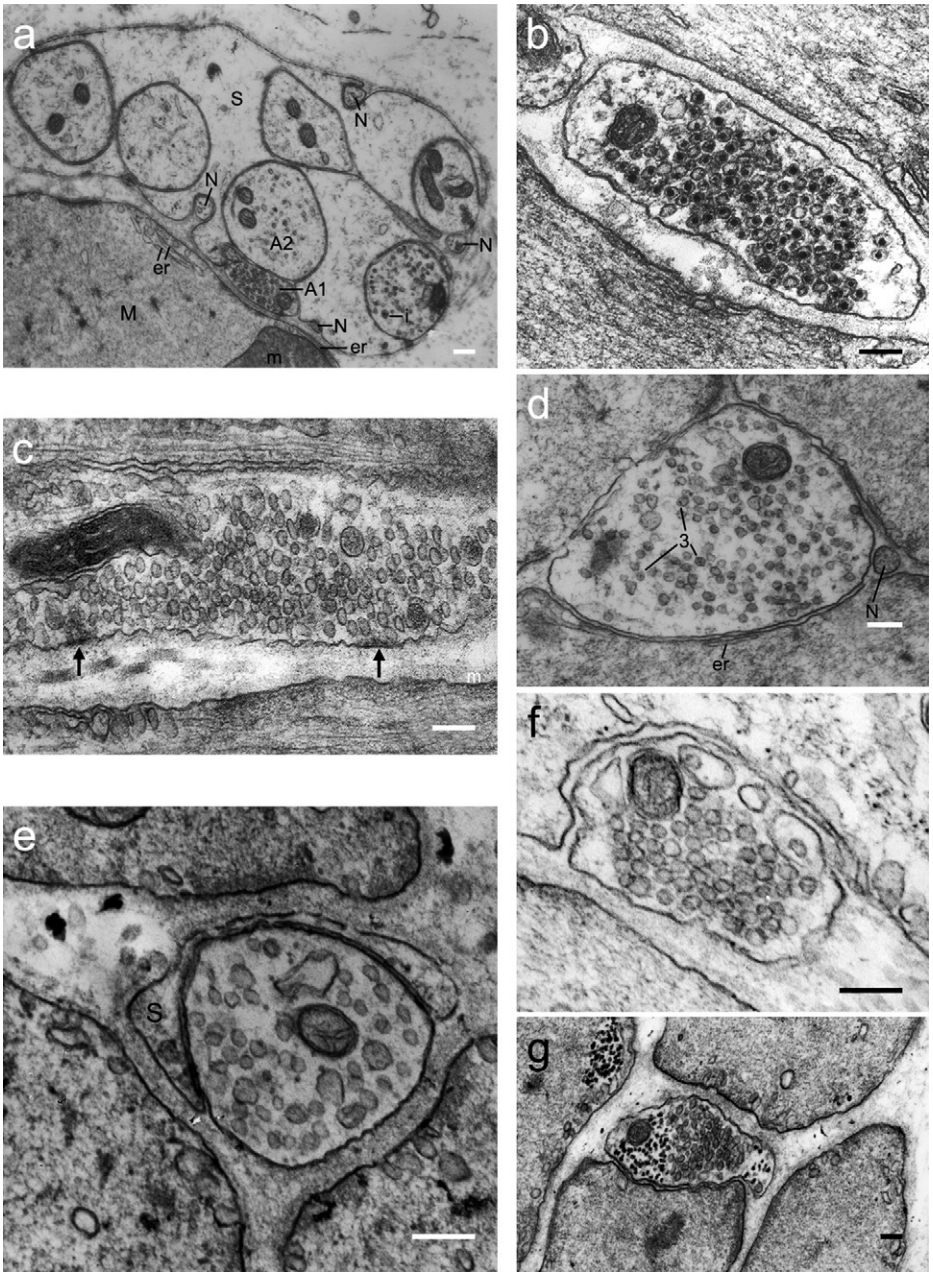

Fig. 2. (a) A medium-sized intramuscular bundle of axons within a single Schwann cell (S) in the guinea pig vas deferens. Varicosity A1 containing many vesicles, perhaps related to the proximity (80 nm) to the muscle cell (M). The small profiles (N), less than 0.25 μm in diameter, are probably sections through intervaricosities and contain neurofilaments. er = endoplasmic reticulum (reproduced from Merrillees et al. (1963), with permission from The Rockerfeller University Press). (b) A single axon in the vas deferens of a mouse sacrificed one hour after treatment with 250 mg/kg of 6-OHDA. Most small vesicles contain large, dense, granular cores indicating catecholamines (reproduced from Furness et al. (1970), with permission from the American Society for Pharmacology and Experimental Therapeutics). (c) An autonomic varicosity in guinea pig vas deferens showing dense prejunctional thickenings and bunching of vesicles, probably representing transmitter release sites (*arrows*), but there is no postjunctional specialization (reproduced from Burnstock (2004b), with permission from Elsevier). (d) A large naked axon varicosity in guinea pig vas deferens is in close apposition (~20 nm) with three muscle fibres at once. Another nerve profile (N) is a section through an intervaricose nerve fiber. er = endoplasmic reticulum (reproduced from Merrillees et al. (1963), with permission from The Rockerfeller University Press). (e) A nerve varicosity in possum bladder showing a surface free of Schwann cells (S) in apposition to a muscle cell. (f) A nerve varicosity free of Schwann cells in sheep ureter. (g) A nerve varicosity free of Schwann cells in close apposition to smooth muscle in lizard lung (electron micrographs in e, f and g are courtesy of Y. Uehara). Scale bar in a–g = 0.2 μm.

connected by low-resistance pathways that allow electrotonic spread of activity within the effector bundle. Sites of electrotonic coupling are represented morphologically by areas of close apposition between the plasma membranes of adjacent muscle cells. High-resolution electron micrographs have shown that the membranes at these sites consist of ''gap junctions'' (see Fig. 1c). Gap junctions (or nexuses) vary in size between punctate junctions, which are not easily recognized except in freeze-fracture preparations, and junctional areas more than 1 μm in diameter. The number and arrangement of gap junctions in muscle effector bundles of different sizes in different organs and their relation to density of autonomic

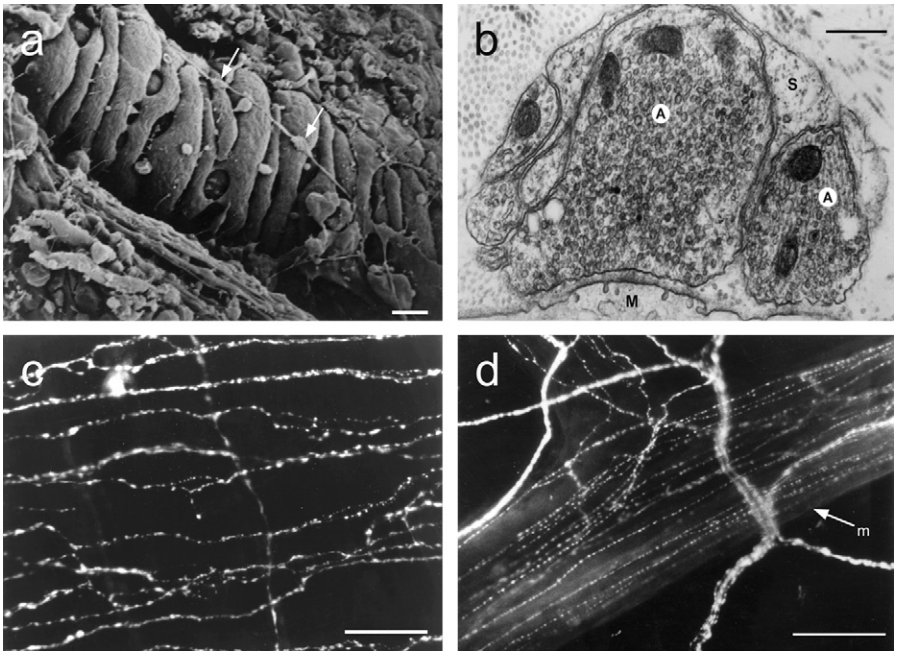

Fig. 3. (a) Scanning electron micrograph of the adventitial surface of the smooth muscle cells of the central arteriole of the rat retina digested by collagenase and trypsin before fixation. Arrows point to varicosities in a single nerve fiber. Scale bar = 3.2 μm (reproduced from Uehara and Suyama (1978), with permission of Oxford University Press). (b) Electron micrograph of relation of axons (A) and smooth muscle (M) at the adventitial-medial border of the anterior cerebral artery of the rat. Axons profiles are devoid of Schwann cytoplasm (S) on the side facing the muscle and approach the muscle surface as close as 80 nm. Basement membrane material is interposed between axonal and smooth muscle membranes. There are many vesicles and mitochondria in terminal varicosities. Scale bar = 0.6 μm (reproduced from Burnstock et al. (1970), with permission from the American Heart Association). (c) Whole mount preparation of the sheep mesenteric vein at the level of the inner surface of the adventitia, showing innervation of the medial muscle coat by an autonomic ground plexus consisting of bundles of fine varicose nerves containing noradrenaline. Incubated in formaldehyde vapour for 1 h. Scale bar = 50 μm (reproduced from Burnstock (1970), with permission from Edward Arnold). (d) A muscle band (m) in the lizard lung innervated by a number of fine fluorescent varicose fibres running along their length. Whole mounts incubated in formaldehyde vapour for 1 h. Scale bar = 200 μm (reproduced from McLean and Burnstock (1967), with permission from Elsevier).

innervation have not been fully analyzed. It is interesting that partial denervation has been shown to result in an increase in gap junctions.

2.5. Receptor localization on smooth muscle cells

The distribution of P2X purinoceptors on smooth muscle cells in relation to autonomic nerve varicosities in urinary bladder, vas deferens, and blood vessels has been examined by using immunofluorescence and confocal microscopy. Antibodies against the $P2X_1$ receptor, the dominant receptor subtype found in smooth muscle, and an antibody against the synaptic vesicle proteoglycan SV2 showed clusters of receptors (about 0.9 μm × 0.2 μm in size) located beneath varicosities (Hansen et al., 1999; Vial and Evans, 2005). Many more small clusters (about 0.4 μm × 0.04 μm) were present on the whole surface of smooth muscle cells unrelated to varicosities; they may represent pools of receptors that can migrate toward varicosities to form large clusters. In blood vessels, small clusters of P2X receptors are present on cells throughout the medial muscle coat, whereas large clusters are restricted to the muscle cells at the adventitial surface. α-Adrenoceptors appear to be located only in extrajunctional regions, so that the possibility that noradrenaline (NA) is released from more distant varicosities has been raised. There are hints from studies of receptor-coupled green fluorescent protein chimeras that the receptor clusters are labile, dispersing when a varicosity moves to a new site where clusters reform, perhaps within a 20- to 30-min time scale.

3. Autonomic neurotransmission, cotransmission and neuromodulation

A neurotransmitter is a chemical substance released from nerves upon electrical stimulation and which acts on specific receptors on adjacent effector cells to bring about a response, thus acting as a chemical messenger of neural activation. Neurotransmitter is released *en passage* from varicosities during conduction of an impulse along an autonomic axon. It is likely that a given impulse will evoke release from only some of the varicosities that it encounters (Blakeley and Cunnane, 1979; Blakeley et al., 1982; Brock and Cunnane, 1995).

Release of neurotransmitter causes a transient change in membrane potential of the postjunctional cell (Burnstock, 1981, 1986). If the result of a single pulse is a depolarization the response is called an excitatory junction potential (EJP) (Fig. 5a). EJPs summate and facilitate with repetitive stimulation and upon reaching sufficient amplitude the threshold for generation of an action potential is reached and results in a mechanical contraction. If the result of a single pulse of neurotransmitter release is a hyperpolarization, the response is called an inhibitory junction potential (IJP) (Fig. 5b). IJPs

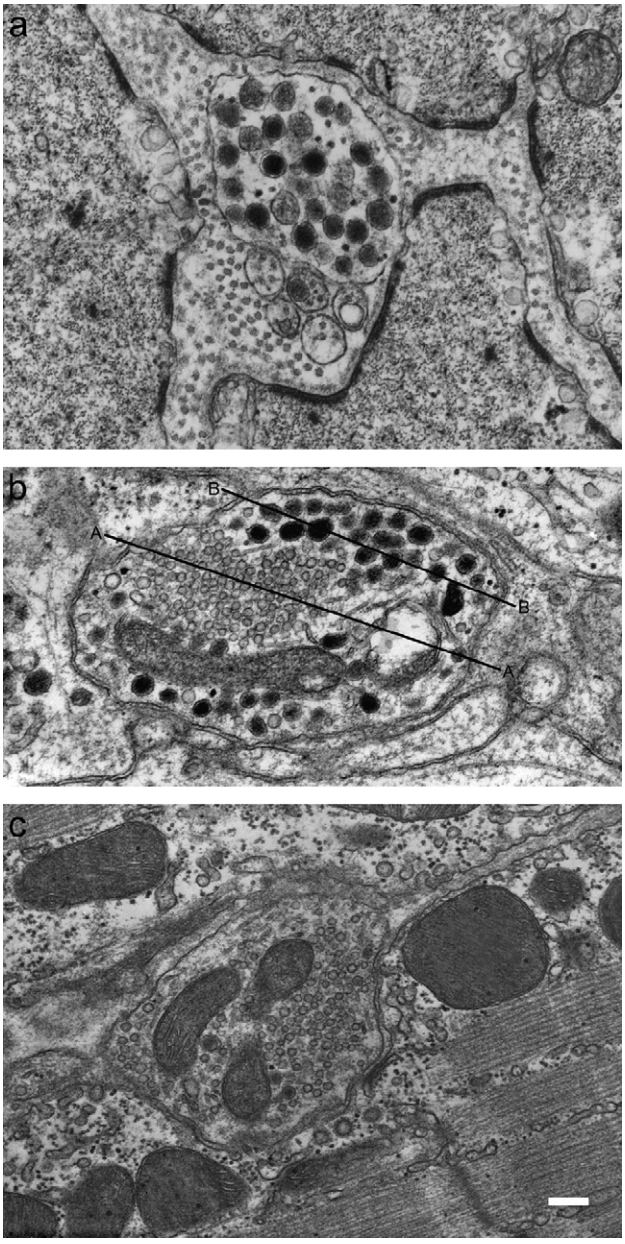

Fig. 4. (a) The axon varicosity from guinea pig taenia coli illustrated in this micrograph shows a predominance of 'large opaque vesicles' (100–200 nm). These vesicles differ from the 'large granular vesicles', which are found in small numbers in both adrenergic and cholinergic nerves, in that they are usually larger and have a less prominent halo between the granular matrix and the vesicle membrane. Such profiles may represent non-adrenergic, non-cholinergic nerves that are present in the gastrointestinal tract and lung. This preparation was fixed 54 h after injection of the animal with 6-hydroxydopamine (which destroys adrenergic nerve terminals) and with 5,6-dihydroxytryptamine (which destroys mono-aminergic nerve terminals including those containing 5-hydroxytryptamine). Note the accompanying profiles through intervaricose regions of terminal nerve fibres. Glutaraldehyde fixation (reproduced from Uehara et al. (1976), with permission from Hodder Arnold). (b) A nerve profile from the guinea pig ileum containing two different vesicle types, perhaps representing cotransmitters. If this profile had been sectioned in plane (A–A, or B–B) then it might have appeared to contain predominantly agranular vesicles or predominantly large granular vesicles (reproduced from Cook and Burnstock (1976), with permission from Springer). (c) Cholinergic nerve terminals in mouse atrium containing predominantly agranular vesicles and several large granulated vesicles 100 nm. Glutaraldehyde fixation (reproduced from Uehara et al. (1976), with permission from Hodder Arnold). Scale bar for a–c = 0.2 μm.

prevent action potential discharge in spontaneously active smooth muscle and thus cause relaxation.

3.1. The multiplicity of neurotransmitters in autonomic nerves

Acceptance of a substance as a neurotransmitter required satisfaction of the following criteria (Eccles, 1964): (1) the presynaptic neuron synthesizes and stores the transmitter; (2) the transmitter is released in a calcium-dependent manner; (3) there should be a mechanism for terminating the activity of the transmitter, either by enzymatic degradation or by cellular uptake; (4) local exogenous application of the substance should mimic its effects following release due to electrical nerve stimulation; and (5) agents that block or potentiate the endogenous activity of the transmitter should also affect local exogenous application in the same way.

The classical view of autonomic nervous control as antagonistic actions of NA and acetylcholine (ACh) causing either constriction or relaxation, depending on the tissue, was changed in the early 1960s when clear evidence of a non-adrenergic, non-cholinergic (NANC) system was presented (Burnstock, 2006b). About a decade later, studies of autonomic neurotransmission revealed a multiplicity of neurotransmitters in the autonomic nervous system. Neurally released substances, including monoamines, amino acids, neuropeptides, adenosine 5′-triphosphate (ATP) and nitric oxide (NO) were identified (Milner et al., 1999).

3.2. Cotransmission

The concept of cotransmission was first formulated by Burnstock (1976) incorporating hints in the earlier literature from both vertebrate and invertebrate systems. It is now well established (Burnstock, 1990a, 2004a; Kupfermann, 1991; Lundberg, 1996). Immunohistochemical evidence of coexistence of more than one neurotransmitter should not necessarily be interpreted as evidence of cotransmission since in order for substances to be termed cotransmitters it is essential to show that postjunctional actions to each substance occur via their own specific receptors. For example, many neuropeptides have slow trophic actions on surrounding tissues and this may be their primary role or they may act as neuromodulators. The relative contribution of each transmitter to neurogenic responses is dependent on the parameters of stimulation. For example, short bursts (1 s) of electrical stimulation of sympathetic nerves at low frequency (2–5 Hz) favor ATP release whereas longer periods of nerve stimulation (30 s or more) favor NA release.

Peptides, purine nucleotides and NO (identified by localisation of nitric oxide synthase) are often found together with the classic neurotransmitters, NA and ACh. In fact, the majority, if not all, of nerve fibres in the autonomic nervous system contain a mixture of different neurotransmitter substances that vary in proportion in different tissues and species and during development and disease. The widespread use of double and triple immunohistochemical labelling

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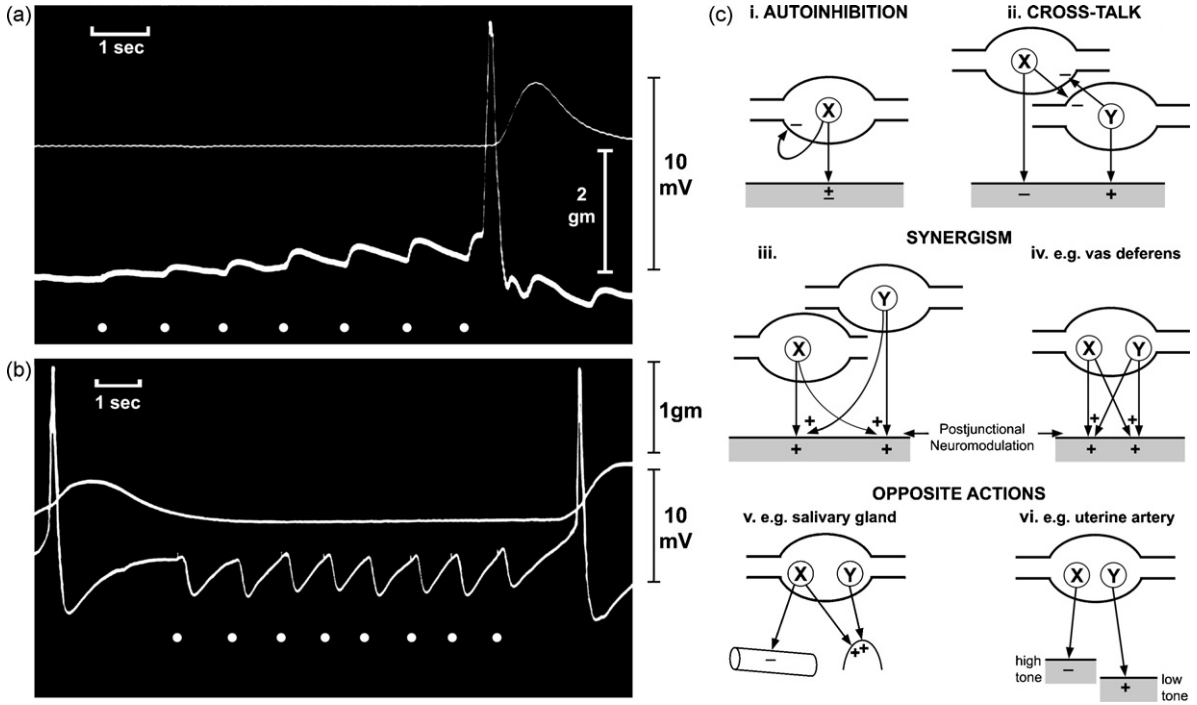

Fig. 5. Transmission at autonomic neuromuscular junctions. Changes in membrane potential (bottom trace) and contraction (top trace) recorded with a sucrose-gap method. The junction potentials recorded with this method are qualitatively similar to those recorded with intracellular microelectrodes. (a) Excitatory junction potentials (EJPs) recorded in smooth muscle of the guinea pig vas deferens in response to repetitive stimulation of postganglionic sympathetic nerves (*white dots*). Note both summation and facilitation of successive EJPs. At a critical depolarization threshold, an action potential is initiated that results in contraction. (b) Inhibitory junction potentials (IJPs) recorded in smooth muscle of the atropinised guinea pig taenia coli in response to transmural repetitive stimulation (*white dots*) of the intramural nerves remaining after degeneration of the adrenergic nerves by treatment of the animal with 6-hydroxydopamine (250 mg/kg intraperitoneally for 2 successive days) 7 days previously. Note that the IJPs in response to repetitive stimulation results in inhibition of spontaneous spike activity and relaxation (a and b reproduced from Burnstock (1973), with permission from Blackwell Publishing). (c) Neurochemical organisation of the autonomic nervous system. Schematic representation of different types of interactions between two neurotransmitter substances, X and Y released from non-synaptic varicosities. Examples of: (i) autoinhibition, where, in addition to the neurotransmitter (X) acting postjunctionally to either contract (+) or relax (−) the muscle, it acts on prejunctional receptors (usually of a different subclass) to form a negative feedback system that inhibits release of the transmitter; (ii) cross-talk, where transmitters X and Y in separate varicosities not only act on receptors in the muscle but also on prejunctional receptors on each other's terminals to modulate transmitter release; (iii) synergism, where transmitters X and Y in separate varicosities have the same contractile (+) action on the muscle cell and potentiate each other's action by the process of postjunctional neuromodulation; (iv) synergism, where transmitters X and Y, released as cotransmitters from a single varicosity, potentiate each other's action on the postjunctional effector; (v) opposite actions, where cotransmitters X and Y have opposite actions on different effector sites and; (vi) opposite actions depending on the tone of the effector tissue (from Burnstock (1987b), with permission from Lippincott, Williams and Wilkins).

techniques has been critical to the demonstration of colocalisation of potential cotransmitters within the same nerve fiber and has been invaluable when combined with electron microscopy. Different neurotransmitters within the same varicosity may be localised in the same or separate vesicular populations (Fig. 4b). In the gastrointestinal tract many neurons contain multiple transmitters. The main neurotransmitters/neuromodulators utilized by sympathetic nerves are NA, ATP and neuropeptide Y (NPY) (Burnstock, 1990a). ATP is a cotransmitter with calcitonin gene-related peptide and substance P (SP) in many sensory-motor nerves (Burnstock, 1993), ACh, ATP and vasoactive intestinal peptide (VIP) in parasympathetic nerves and ATP, NO and VIP in enteric NANC inhibitory nerves (Burnstock, 2001). Transmitters with seemingly diverse and opposing effector action are sometimes colocalised in the same neuron, but generally they act in the same way and usually synergistically (Fig. 5c).

Ultrastructural studies of the enteric nervous system offered the first suggestion that there were several different neurotransmitters in autonomic nerves; at least nine distinguishable types of axon profile were described in the guinea pig myenteric plexus (Cook and Burnstock, 1976). The precise combinations of neurotransmitters (and neuromodulators) contained in individual enteric neurons was termed 'chemical coding' by Furness and Costa (1987).

There are increasing examples in the literature of cross-talk between sensory-motor, sympathetic and parasympathetic nerves, physiological events, which are facilitated by the nature of non-synaptic neuroeffector junctions (Fig. 5c; Burnstock, 2004b). Altered expression of cotransmitters in autonomic nerves during development, ageing, following trauma, surgery, chronic exposure to drugs, and in disease have been reported (see Abbracchio and Burnstock, 1998; Burnstock, 2006a).

3.3. Neuromodulation

Some substances stored and released from nerves do not have direct actions on effector muscle cells but alter the release and/or the actions of transmitters; these substances are termed

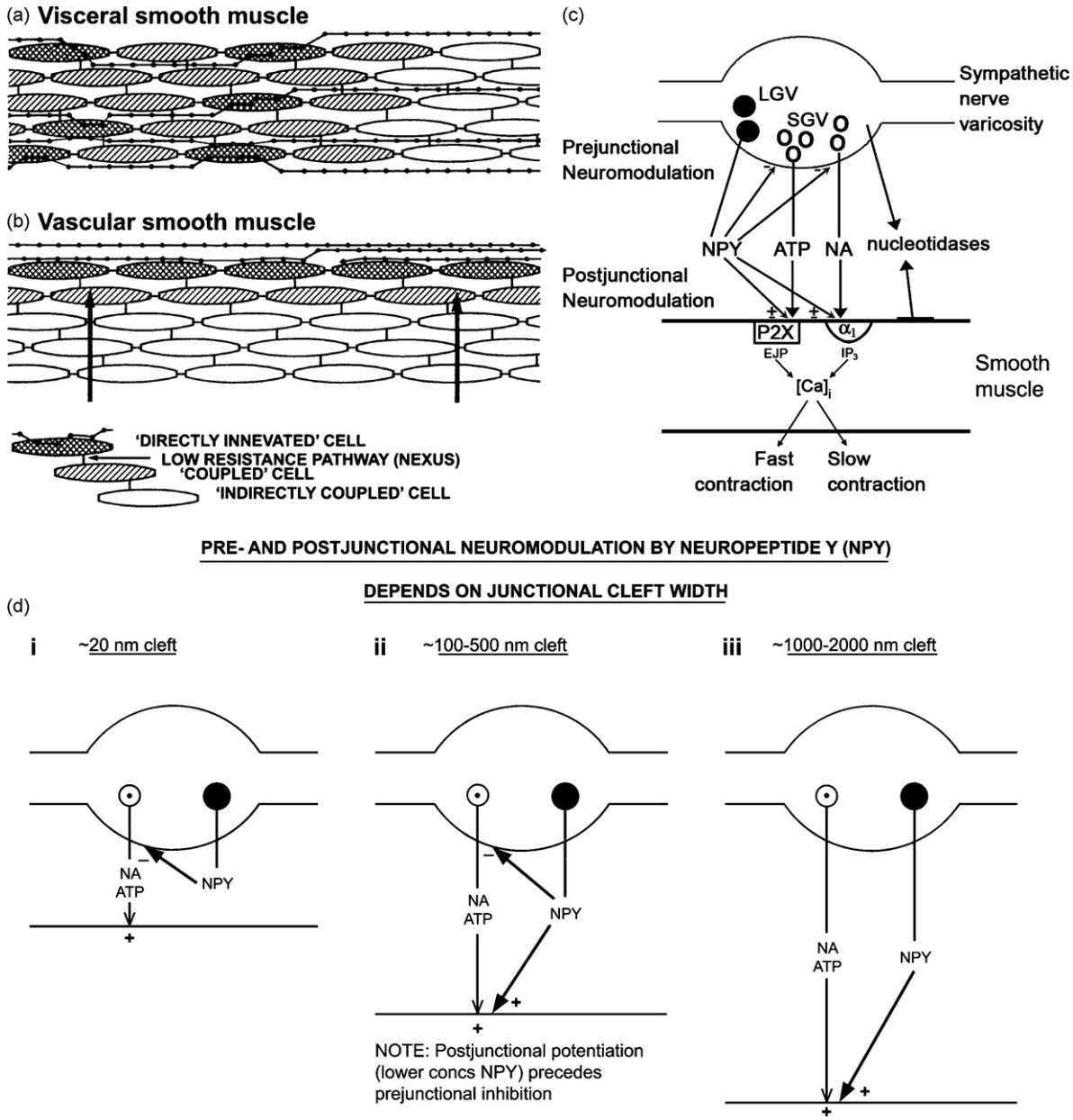

Fig. 6. (a) Schematic representation of the innervation of visceral smooth muscle. ''Directly innervated'' cells (*cross-hatched*) are those that are directly activated by neurotransmitter; ''coupled cells'' (*hatched*) are those where junction potentials spread from ''directly innervated'' cells. When a sufficient area of the muscle effector bundle is depolarized, a propagated action potential will activate the ''indirectly coupled'' cells (*white*). (b) Schematic representation of control of vascular smooth muscle by perivascular varicose nerves in the adventitia (●) and endothelial factors (*arrows*) (a and b, modified from Burnstock and Costa (1975), with permission from CRC Press). (c) Schematic of sympathetic cotransmission. ATP and NA released from small granular vesicles (SGV) act on P2X and α1 receptors on smooth muscle, respectively. ATP acting on inotropic P2X receptors evokes excitatory junction potentials (EJPs), increase in intracellular calcium ($[Ca^{2+}]_i$) and fast contraction; while occupation of metabotropic α1 adrenoceptors leads to production of inositol triphosphate (IP3), increase in $[Ca^{2+}]_i$ and slow contraction. Neuropeptide Y (NPY) stored in large granular vesicles (LGV) acts on release both as a prejunctional inhibitory modulator of release of ATP and NA and as a postjunctional modulatory potentiator of the actions of ATP and NA. Soluble nucleotidases are released from nerve varicosities, and are also present as ectonucleotidases (reproduced from Burnstock (in press), with permission from Elsevier). (d) A diagram illustrating that pre- and/or postjunctional modulation of sympathetic cotransmission by NPY depends on the junctional cleft width. (i) Close (20 nm) anterior neuromuscular cleft as seen, for example, in the vas deferens, where prejunctional inhibition of transmitter release by NPY is dominant. (ii) Medium-sized junctional cleft (100–500 nm) characteristic of many blood vessels, where postjunctional potentiation of transmitter action occurs with low concentrations of NPY and, later, prejunctional modulation as the concentration of NPY in the cleft increases during transmission. (iii) Wide (1000–2000 nm) cleft typical of large elastic arteries, where postjunctional modulation by NPY is dominant (reproduced from Burnstock (1990b), with permission from Blackwell Publishing).

neuromodulators. Many other substances (e.g. circulating neurohormones, locally released agents such as prostanoids, bradykinin, histamine and endothelin and neurotransmitters from nearby nerves) are also neuromodulators in that they modify the process of neurotransmission. Many substances that are cotransmitters are also neuromodulators. The wide and variable cleft characteristic of the non-synaptic autonomic neuroeffector junctions makes them particularly amenable to

the modulatory mechanisms of neural control mentioned above. For example, the geometry of sympathetic neuromuscular junctions appears to influence the type of neuromodulation produced by NPY (Fig. 6c); with wide junctional clefts postjunctional potentiation by NPY dominates, while narrow clefts favour prejunctional inhibition by NPY (Burnstock, 1990b) (Fig. 6d). The plasticity of expression of neural substances co-coordinated to environmental cues allows rapid and precise matching of neurotransmission to altered demands. Several neurotransmitters/neuromodulators are themselves trophic molecules, with mitogenic or growth-promoting/-inhibiting properties.

4. Autonomic neuroeffector transmission to immune epithelial and endothelial cells

Many non-excitable effector cells are innervated, albeit transiently, by nerves. This is because, as described above, the autonomic neuroeffector junction is not a fixed structure with post-junctional specialisation as is seen at the skeletal neuromuscular junction or neuronal synapses. Rather, when varicosities in extensive terminal autonomic nerve fibres, which are actively moving, form close relationships with effector cells, the cotransmitters released are within striking distance of the receptors expressed for these transmitters on effector cells (Burnstock, 2002b, 2004b).

4.1. Immune cells

Cells of the immune system were not considered for many years to be innervated, since neural boutons could not be found on their surface membranes. However, in accordance with the definition of the non-synaptic autonomic neuroeffector junction, close contact of nerve varicosities with effector cells in effect constitutes innervation, albeit of a transient nature. Also there is increasing recognition that nerves can influence the immune system and the field of neuroimmunology is growing (Elenkov et al., 2000; Serafeim and Gordon, 2001; Bienenstock et al., 2003). Cells of the immune system consist of a large family, including lymphocytes, mast cells, macrophages, neutrophils, eosinophils, thymocytes, dendritic and haematopoietic cells as well as microglia and osteoclasts. The sympathetic nervous system innervates immune organs and releases its cotransmitters NA and ATP in the vicinity of immune cells (Haskó and Szabó, 1998).

Mast cells were the first immune cell type to be shown to be innervated (see Williams et al., 1995). For example, antidromic stimulation of sensory nerves was shown to increase degranulation of mast cells in the skin and to be mimicked by ATP by Kiernan (1974). Electron microscopic studies showed close opposition of nerve varicosities containing small and large vesicles and mast cells in the mucosa of intestine (Newson et al., 1983; Bienenstock et al., 1991) and in cerebral blood vessels (Dimitriadou et al., 1987; see Fig. 7). The activities of synovial mast cells that contribute to inflammation in joints were shown to be influenced by both unmyelinated afferent and sympathetic efferent nerves (Levine et al., 1990).

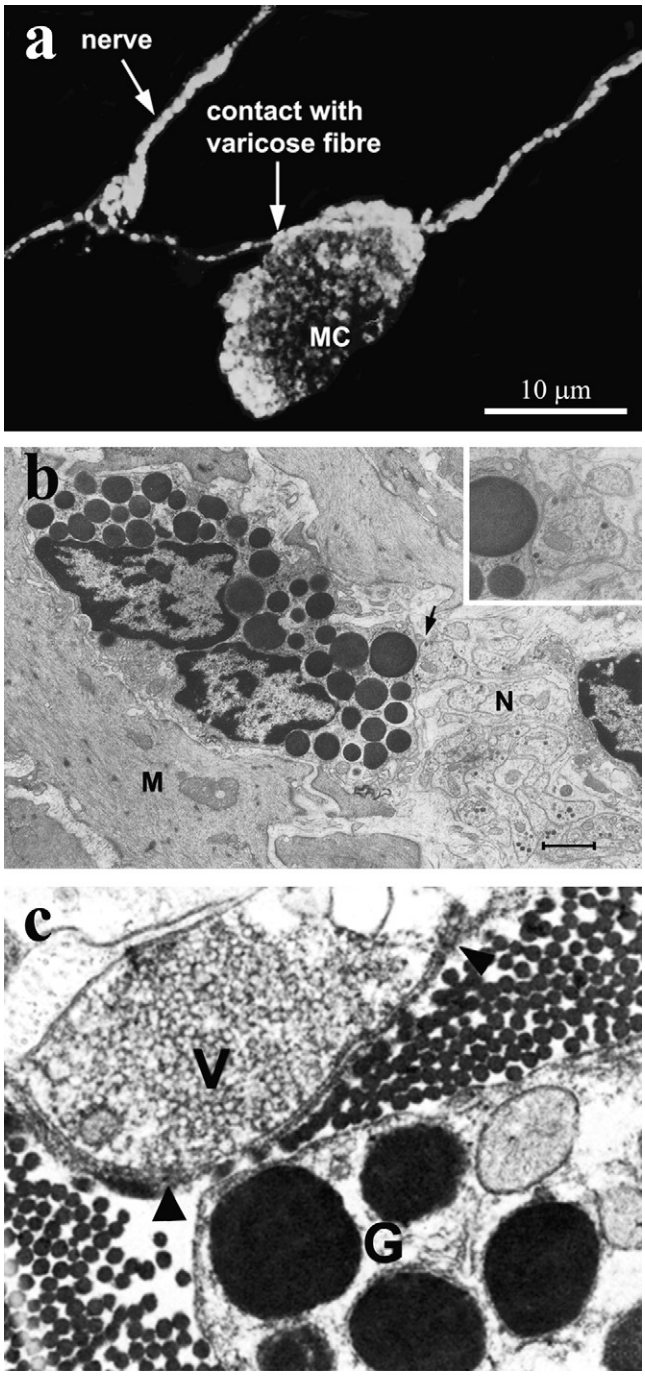

Fig. 7. (a) Close apposition between rat mast cell protease 1 immunoreactive and calcitonin gene-related peptide immunoreactive nerve fibres observed by confocal microscopy (reproduced from Dimitriadou et al. (1997), with permission from Elsevier). (b) Electron micrograph of a mast cell in the muscularis propria of the small intestine of the rat 6 weeks after *Nippostrongylus brasiliensis* infection. Nonmyelinated nerves (N) with electron-dense vesicles and empty vesicles are seen very near the mast cell. The arrow indicates very close approximation (and possible contact) between the mast cell and the neural process, shown at higher magnification in the inset. The photomicrograph also includes smooth muscle cells (e.g., M). Bar = 1.0 µm (reproduced from Arizono et al. (1990), with permission from the United States and Canadian Academy of Pathology). (c) Ultrathin section of rabbit middle cerebral artery showing granular cells (G) separated by a distance of less than 200 nm. V, varicosities; arrowheads, basement membranes. Magnification, ×29,374 (Reproduced from Dimitriadou et al. (1987), with permission from Elsevier).

Sympathetic as well as trigeminal sensory nerve fibres influence rat dural mast cells and play a role in the oedema pathophysiology of vascular headache (Keller et al., 1991). Electrical stimulation of the vagus nerve modulates the histamine release from mast cells in the rat jejunal mucosa (Gottwald et al., 1995).

There have been few investigations of the influence of nerves on non-mast cell immune cells, but the possibility that varicose nerve fibres form transient close relationships with some of these cell types too, cannot be discounted. For example, electron micrographs showing close association of nerves with eosinophils have been described (Arizono et al., 1990). The sympathetic nervous system has been shown to modulate macrophage function (Chelmicka-Schorr et al., 1992) and alterations in T and B lymphocyte proliferation and differentiation have been described following chemical sympathectomy (Madden et al., 1994). Close contacts between enteric nerves and lymphocytes in mouse intestinal mucosa and submucosa have been reported (Crivellato et al., 1998; Genton and Kudsk, 2003).

4.2. Epithelial cells

Stimulation of parasympathetic nerves produces increased production of saliva from parotid and submandibular glands (Ekström et al., 1998). The autonomic innervation of parotid ducts occurs on the basal side of epithelial cells where muscarinic receptors are located (Takemura and Horio, 2005). The coordinated roles of VIP and ACh in parasympathetic neurotransmission were demonstrated in an elegant study of the cat exocrine salivary gland innervation (Lundberg, 1981). This showed that VIP and ACh were stored in separate vesicles in the same nerve varicosities, and were both released upon transmural nerve stimulation, but with different stimulation parameters. ACh was released during low frequency stimulation to increase salivary secretion from acinar cells and to elicit some minor dilatation of blood vessels in the gland. At high stimulation frequencies, VIP was released to produce marked dilatation of the blood vessels in the gland and to act as a neuromodulator postjunctionally on the acinar gland to enhance the actions of ACh, and prejunctionally on the nerve varicosities to enhance the release of ACh. The control of pancreatic exocrine function is complex and regulated by both neural and hormonal factors (Owyang and Logsdon, 2004; Noble and Liddle, 2005). There is a rich innervation of the lacrimal gland by both sympathetic and parasympathetic varicose nerve fibres (Gromada et al., 1995).

Epithelial cells in airways, liver, kidney, gut, gall bladder, adipose tissue and uterus express multiple receptors to neurotransmitters involved in cytosolic calcium regulation of chloride and fluid secretion, sodium transport and ciliary and mucociliary clearance (Braunstein and Schwiebert, 2003). Sympathetic innervation of epithelial cells in proximal and distal renal tubules has been described, particularly the ascending limb of Henle's loop (see DiBona, 1989; McLachlan and Luff, 1992).

The thyroid gland is extensively innervated by sympathetic, parasympathetic and sensory nerves (Melander et al., 1975; Grunditz et al., 1988) and sympathetic control of thyroid hormone secretion has been reported (Green, 1987). In the thymus, varicose sympathetic nerves run in septa in close connection to subcapsular and perivascular thymic epithelial cells. Cotransmitters NA and ATP from sympathetic nerves have a co-stimulatory effect on synthesis of interleukin-6 that is an important factor for thymocyte differentiation and proliferation (von Patay et al., 1999). The sympathetic innervation of the testis shows a predominant supply to blood vessels, but there is also ultrastructural evidence for sympathetic innervation of Leydig and interstitial cells. The mammalian ovary is directly innervated by sympathetic nerves, which appear to play major roles in regulating ovarian functions, such as follicular maturation, steroid secretion and ovulation. There is a rich innervation of pancreatic islets by both sympathetic and parasympathetic nerves (Miller, 1981). Electron microscopic studies have shown autonomic axons supplying adrenal cortical tissue, which sometimes penetrate the basal lamina of the cortical cells and come into close (200 nm) contact with their plasma membranes (Unsicker, 1971; Robinson et al., 1977).

The presence of nerve fibres arising from intrinsic neurons in the enteric plexuses controlling secretion in mucosal epithelial cells has been recognised for a long time, with both cholinergic and non-cholinergic secreto-motor neurons involved (Scratcherd and Grundy, 1984). In general, extrinsic parasympathetic activity increases intestinal secretion, while inhibition occurs with sympathetic stimulation. The liver is supplied by sympathetic, parasympathetic and sensory nerves, which contribute to the regulation of hepatic carbohydrate metabolism. The sympathetic cotransmitters NA and ATP stimulate hepatic glycogenolysis (Buxton et al., 1986) and suppress the secretion of very low-density lipoprotein (Yamauchi et al., 1998). The extrahepatic biliary tract is innervated by dense networks of extrinsic and intrinsic nerves that regulate both smooth muscle tone and epithelial cell function (Balemba et al., 2004). The metabolism, proliferation and thermogenesis of adipose tissue are controlled by the sympathetic nervous system (see Himms-Hagen et al., 1990; Rayner, 2001).

The mammalian choroid plexus is a highly vascularized villous structure, covered with a single layer of cuboidal epithelial cells. It is present in all four ventricles in the brain and plays a major role in the production and regulation of cerebral spinal fluid. The choroid plexus is supplied by a well-developed sympathetic and parasympathetic innervation and also probably by some nerve fibres originating in the brain stem reaching both the secretory epithelium and the blood vessels. Sympathetic stimulation evokes an inhibition of cerebral spinal fluid formation. Both sympathetic and parasympathetic nerve terminal fibres have been identified in the vicinity of ciliary epithelial cells in the eye. Sympathetic innervation regulates basement membrane thickening and pericyte number in rat retina (Wiley et al., 2005). Sympathetic nerves supply the nasal mucosa, but are probably largely involved in vasomotor control (Lacroix et al., 1994).

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4.3. Endothelial cells

There is dual control of vascular tone by cotransmitters released from perivascular sympathetic nerves to act on receptors that mediate smooth muscle contraction and transmitter substances, including ACh, ATP and SP, are released from endothelial cells during changes in blood flow (sheer stress) and hypoxia to act on endothelial receptors to release NO resulting in vasodilatation (Burnstock, 2002a; Yamamoto et al., 2006). In large to small muscular vessels, transmitters released from the perivascular nerves are unlikely to be active on endothelial receptors since they would be rapidly degraded before reaching the endothelial cells in the intima. However, in the microvasculature it is likely that transmitters released from varicosities in the perivascular nerve plexus would act on endothelial receptors. There are a few examples where this has been experimentally supported. For example, neurally released ATP has been shown to mediate endothelium-dependent hyperpolarisation in smooth muscle cells of hamster, rabbit and chicken small mesenteric arteries (Kakuyama et al., 1998; Thapaliya et al., 1999; Draid et al., 2005). Release of ATP from nerves and astrocytes has been considered to mediate endothelium-dependent vasodilatation of cerebral vessels (Albert et al., 1997). Inhibitory purinergic neurotransmission has been considered to be endothelium-dependent in pulmonary (Liu et al., 1992) and coronary (Simonsen et al., 1997) vessels. In addition to control of vascular tone, ATP and its breakdown product, adenosine and uridine 5′-triphosphate have important long-term (trophic) actions on endothelial and smooth muscle cell proliferation, differentiation and death (see Erlinge, 1998; Burnstock, 2002a). However, whether the source for these effects on endothelial cells, which are important in angiogenesis and restenosis, is perivascular nerves and/or endothelial cells has not been addressed yet.

5. Model of non-synaptic autonomic neuroeffector transmission

A model of the autonomic neuromuscular junction has been proposed on the basis of combined electrophysiological, histochemical, and electron-microscopical studies described earlier (Fig. 6a and b). The essential features of this model are that the terminal portions of autonomic nerve fibers are varicose, transmitter being released *en passage* from varicosities during conduction of an impulse, although EJPs and IJPs are probably elicited only at close junctions. Furthermore, the effectors are muscle bundles rather than single smooth muscle cells, which are connected by low-resistance pathways (gap junctions) that allow electrotonic spread of activity within the effector bundle. In blood vessels, the nerves are confined to the adventitial side of the media muscle coat, and this geometry appears to facilitate dual control of vascular smooth muscle by endothelial relaxing and contracting factors and perivascular nerves. Neuroeffector junctions do not have a permanent geometry with postjunctional specializations, but rather the varicosities are continuously moving and their special relation with muscle cell membranes changes with time. For example, it is likely to occur in cerebral blood arteries, where there is a continuously increasing density of sympathetic innervation during development until old age (Cowen et al., 1982) and in vessels that have been stimulated chronically *in vivo*, where there can be an increase in innervation density of up to threefold, including an increase in the number of varicosities per unit length of nerve from 10 to 20 per 100 μm to 30 per 100 mm.

The non-synaptic autonomic effector junctions appear to be particularly suitable for neuromodulation. A neuromodulator is defined as any substance that modifies the process of neurotransmission. It may achieve this either by prejunctional action that increases or decreases transmitter release or by postjunctional action that alters the time course or extent of action of the transmitter, or both. Further, the combination of the variety of neurotransmitters involved in autonomic neurotransmission and the interactions between sympathetic, parasympathetic, sensory-motor nerves, and those arising from intrinsic ganglia, via mechanisms of cotransmission and pre- and postjunctional neuromodulation, indicate the complexity of peripheral autonomic control and the variety of ways by which autonomic dysfunction can occur (Fig. 5c).

Finally, it should be emphasized that with this model of the autonomic effector junction, then the earlier emphasis on looking for images of specialized nerve endings (boutons) on effector cells is not appropriate; even if a varicosity has a passing close relation with a cell, releasing transmitter for which receptors are expressed on that cell (e.g., mast cells and epithelial cells), then, in effect, that cell is innervated.

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