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Peptides in the mammalian cardiovascular system

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Summary. Ample immunocytochemical evidence is now available demonstrating that several peptides are present in the mammalian cardiovascular system where they are localised to nerve fibres and myocardial cells. The neuropeptides (neuropeptide Y, calcitonin gene-related peptide, tachykinins and vasoactive intestinal polypeptide) are localised to large secretory vesicles in subpopulations of afferent or efferent nerves supplying the heart and vasculature of several mammals, including man. Although they often exert potent pharmacological effects on the tissues in which they occur their physiological significance has still to be established. They may act directly via specific receptors and/or indirectly by influencing the release and action of other cardiovascular transmitters. In marked contrast, atrial natriuretic peptide is produced by cardiac myocytes and considered to act as a circulating hormone.
Key words. Peptides; cardiovascular system; immunocytochemistry; neuropeptide Y; calcitonin gene-related peptide; tachykinins; substance P; vasoactive intestinal polypeptide; atrial natriuretic peptide.

Introduction

It is now recognised that in addition to classical sympathetic (noradrenaline) and parasympathetic (acetylcholine) transmitters, the subpopulations of nerve fibres supplying the cardiovascular system also contain other putative transmitters including several so-called regulatory peptides. Considerable advances have been made in our knowledge of cardiovascular innervation following the application of histochemical and ultrastructural methods[26, 95] but it is the recent use of immunocytochemical techniques which has allowed us to demonstrate the presence of peptides and transmitter synthesising enzymes in cardiovascular nerves and thus distinguish between different autonomic nerve types. In the future several other immunocytochemical markers maybe of value in studies of cardiovascular innervation. These include two membrane proteins, synapsin and synaptophysin, specifically associated with the small secretory vesicles that store classical transmitters in nerve terminals[146, 153, 154, 218]; and the neuronal cytoplasmic protein, protein gene product 9.5 (PGP 9.5), which was originally extracted from human brain[107] and is present throughout the cardiovascular innervation[95].

In this article we review the immunocytochemical and pharmacological evidence concerning the localisation and actions of regulatory peptides in the mammalian heart and blood vessels. Of the peptides identified to date in cardiovascular nerves the most widely distributed are neuropeptide Y, calcitonin gene-related peptide, the tachykinins and vasoactive intestinal polypeptide.

Neuropeptide Y

Neuropeptide Y (NPY) is a 36 amino acid peptide, originally extracted from porcine brain and chemically characterised as having a C-terminal tyrosine amide group[192]. It belongs to a group of peptides which have a high degree of sequence homology, including pancreatic polypeptide (PP) and peptide YY (PYY)[68]. Sequence analysis of the cDNA encoding human NPY has revealed that the prepro-NPY molecule consists of 97 amino acids and its predicted post-translational processing yields three peptides corresponding to the signal peptide (28 amino acids), NPY (36 amino acids) and the C-flanking peptide of NPY (CPON, 30 amino acids)[148]. CPON immunoreactivity occurs naturally in mammalian tissues[7] and has an identical distribution to NPY in both the nervous system and adrenal medulla[93]. NPY/CPON-immunoreactive nerve fibres appear to be the most abundant of all the peptide-containing nerve populations identified to date in the mammalian cardiovascular system. High concentrations of both peptide sequences are found in the heart[7, 8, 92] where they occur in nerve fibres associated with the endocardium, myocardium, and coronary vessels and in epicardial nerves. The number of immunoreactive fibres tends to be greater in the atria than the ventricles and higher in the right atrium than the left. NPY/CPON-immunoreactive nerve fibres are also distributed around arteries (elastic and muscular) throughout the vascular system, forming an outer network of nerve bundles containing preterminal axons, running mainly parallel to the vessel and a perivascular plexus of fine, mainly varicose fibres and fascicles running around the vessel at the adventitial-medial border[61, 62, 67, 93, 149, 202]. The density of the perivascular plexus varies in different species, as well as with vessel size and site. The immunostained nerve fibres are usually confined to the adventitial-medial border of systemic vessels, however, nerve fibres are known to penetrate the media of some large arteries in a number of species[26, 85]. We have observed NPY/CPON-immunoreactive nerve fibres in the outer media of the pig elastic pulmonary artery, running in a circular direction, in association with both the vasa vasorum and smooth muscle cells between the elastic laminae.

Most of the published studies concerning the distribution of NPY-immunoreactive nerves in the vascular system have used rats, guinea pig and cat tissues[61, 62, 67, 91, 93, 134, 143, 149], but the presence of NPY-immunoreactivity has also been noted in human omental[54], mesenteric[67], skin[108] and cerebral vessels[6]. We have localised NPY/CPON immunoreactivity to nerves around human spinal (fig. 2), coronary, pulmonary (fig. 5), renal, gastric, splenic and mesenteric blood vessels. These immunoreactive nerves occur in a perivascular plexus around both arteries and veins, the plexus being less dense in the latter, and represent a subpopulation of the total innervation which displays PGP 9.5-immunoreactivity (fig. 1).

Combined immunocytochemical and denervation studies have demonstrated that the distribution of NPY-immuno-

reactive nerve fibres in the mammalian cardiovascular system is very similar to that of nerves containing the catecholamine synthesising enzymes (tyrosine hydroxylase and dopamine-beta-hydroxylase) and the majority at least appear to represent noradrenergic, postganglionic, sympathetic neurones[61, 62, 67, 91, 93, 134, 143, 149]. Thus, the removal of the stellate ganglia results in an almost complete loss of NPY- and tyrosine hydroxylase-immunoreactive nerve fibres in the guinea pig heart[42] and superior cervical ganglionectomy produces a marked depletion of NPY-containing perivascular sympathetic nerve fibres in the upper respiratory tract, oral mucosa, dental pulp, thyroid, iris and around cerebral vessels[61, 62, 66, 67, 202]. NPY immunorectivity is also depleted from cardiovascular noradrenergic nerve terminals in the rat and guinea pig after chemical sympathectomy with 6-hydroxydopamine and following reserpine treatment[3, 8, 67, 95, 134, 137, 138, 149]. The amount of NPY immunoreactivity which is lost from cardiovascular nerves following 6-hydroxydopamine and reserpine treatment varies between different populations of sympathetic neurones and does not necessarily accompany the depletion in noradrenaline levels[137, 138, 149]. While it has been suggested that this could be due to variations in the number of terminal and preterminal axons in given tissues[149] it may also indicate the different sources and storage sites for NPY and noradrenaline in these neurones. Unlike noradrenaline, NPY is synthesised in the neuronal cell body and reaches nerve terminals via axonal transport. Furthermore, subcellular fractionation studies in the cat spleen[77] and rat vas deferens[76] have suggested that noradrenaline occurs mainly in the small vesicles whereas NPY is contained, together with some noradrenaline, in the less numerous large vesicles in sympathetic nerves. We have recently confirmed this proposed subcellular localisation of NPY in samples of human atrial appendage obtained from patients undergoing coronary artery by-pass grafts. Post-embedding, immunogold labelling techniques were employed at the ultrastructural level to demonstrate that both NPY and CPON immunoreactivity are localised to large electron dense secretory vesicles (diameter 70–100 nm) in nerve terminals which also contain numerous smaller sized vesicles (diameter 40–60 nm) and are presumed to represent sympathetic nerves (figs 7–8). Thus while NPY and noradrenaline coexist in sympathetic cardiovascular terminals their localisation in two distinct subcellular stores could enable a differential release of noradrenaline or NPY, the latter being preferentially released at high stimulation frequencies[135]. The noradrenergic neurone blocker guanethidine inhibits the release of both noradrenaline and NPY[133].

Although NPY and noradrenaline coexist in sympathetic cardiovascular nerves it should be remembered that not all noradrenergic nerves contain NPY[29] and not all NPY-immunoreactive nerve fibres are noradrenergic[80]. Although most NPY-containing nerve fibres in the heart seem to represent extrinsic sympathetic nerves there is immunocytochemical evidence to indicate that some intrinsic cardiac neurones may also contain NPY immunoractivity[42, 91, 100].

The functional significance of the coexistence of NPY and noradrenaline has yet to be established, but NPY may influence sympathetic vascular control in at least three ways, having both direct and indirect (pre- and post-junctional) effects. NPY exerts a direct, non-adrenergic, calcium dependent vasoconstrictor action on coronary[4, 74, 171, 173] and cerebral vessels[55, 61–63, 99] from a number of species. In man, NPY induces a direct vasoconstrictor response in some vessels, which is characteristically slow in onset and long lasting. This response has been demonstrated in arteries and veins in the human forearm in vivo[162], as well as in renal and submandibular arteries and mesenteric veins in vitro, but not in mesenteric arteries[54, 67, 136]. Variable, generally poor responses to NPY also occur in different vessels in experimental animals[65, 99, 161, 209]. In addition to a direct action, NPY may enhance the post-junctional vasoconstrictor effect of noradrenaline, as well as other transmitters, and inhibit its pre-junctional release[41, 67, 99, 137, 161, 208, 209]. Furthermore, the findings of recent studies using pithed guinea pigs indicates that noradrenaline is also capable of reducing the neuronal release of NPY by a pre-junctional α-$_2$-adrenoceptor mediated mechanism[40].

In the isolated rabbit heart NPY was originally reported to have a negative inotropic effect and reduce coronary perfusion[4] whereas positive inotropic and chronotropic effects were observed in the isolated guinea pig atrium[74, 132]. However, in other heart preparations from the dog, rat, cat, guinea pig and man, NPY has been found to have no inotropic or chronotropic effects, the only action being a vasoconstrictor one[5, 72]. Very little is known about the nature and distribution of NPY receptors in the cardiovascular system, but they have been reported to occur in vascular smooth muscle in the rabbit and guinea pig kidney[124]. Thus, although there are numerous NPY/CPON-immunoreactive nerves in mammalian myocardium the presence of NPY receptors in cardiac muscle is uncertain. None the less, NPY could influence cardiac function by means of its ability to produce vasoconstriction and effect pre-junctional noradrenaline release as well as other types of autonomic transmission[132], including the inhibition of acetylcholine from parasympathetic nerves in the heart[164].

Calcitonin gene-related peptide

Alternative processing of primary transcripts from the calcitonin gene leads to the expression of different mRNA's, encoding either calcitonin or the 37 amino acid peptide, calcitonin gene-related peptide (CGRP) which is predominantly expressed in the nervous system[11, 172]. Two forms of CGRP (alpha- and beta-) have been identified in both the rat[11] and human[186]. Immunocytochemical studies have demonstrated that CGRP immunoreactivity is widely distributed in sensory neurones and nerve fibres in the viscera and cardiovascular system of several species[87, 89, 94, 98, 121, 130, 142, 144, 150, 172, 179, 196, 201, 210, 216]. CGRP-immunoreactive nerve fibres are particularly numerous in guinea pig blood vessels and heart where they are prominent in the endocardium, pericardium and around coronary vessels and are also present in the myocardium and epicardium. Significant concentrations of CGRP immunoreactivity are found in the guinea pig cardiovascular system, with the highest levels occurring in the superior mesenteric artery, inferior vena cava, pulmonary trunk, carotid artery and aortic arch. However, the findings in the guinea pig are only partially representative of those in other animals, there being marked regional and species variations regarding the distribution of CGRP-immunoreactive nerve fibres[216]. In contrast to the guinea pig, relatively few nerve fibres in the human heart and

Figures 1–3. Whole mount preparations of a human anterior spinal artery, obtained at post mortem and immunostained for PGP 9.5 (fig. 1), NPY (fig. 2) and CGRP (fig. 3). A network of varicose and non-varicose fibres and fascicles is demonstrated in the adventitia using antisera to PGP 9.5 and NPY. In contrast, only a few, fine fibres are immunostained with an antiserum raised against synthetic alpha-CGRP.

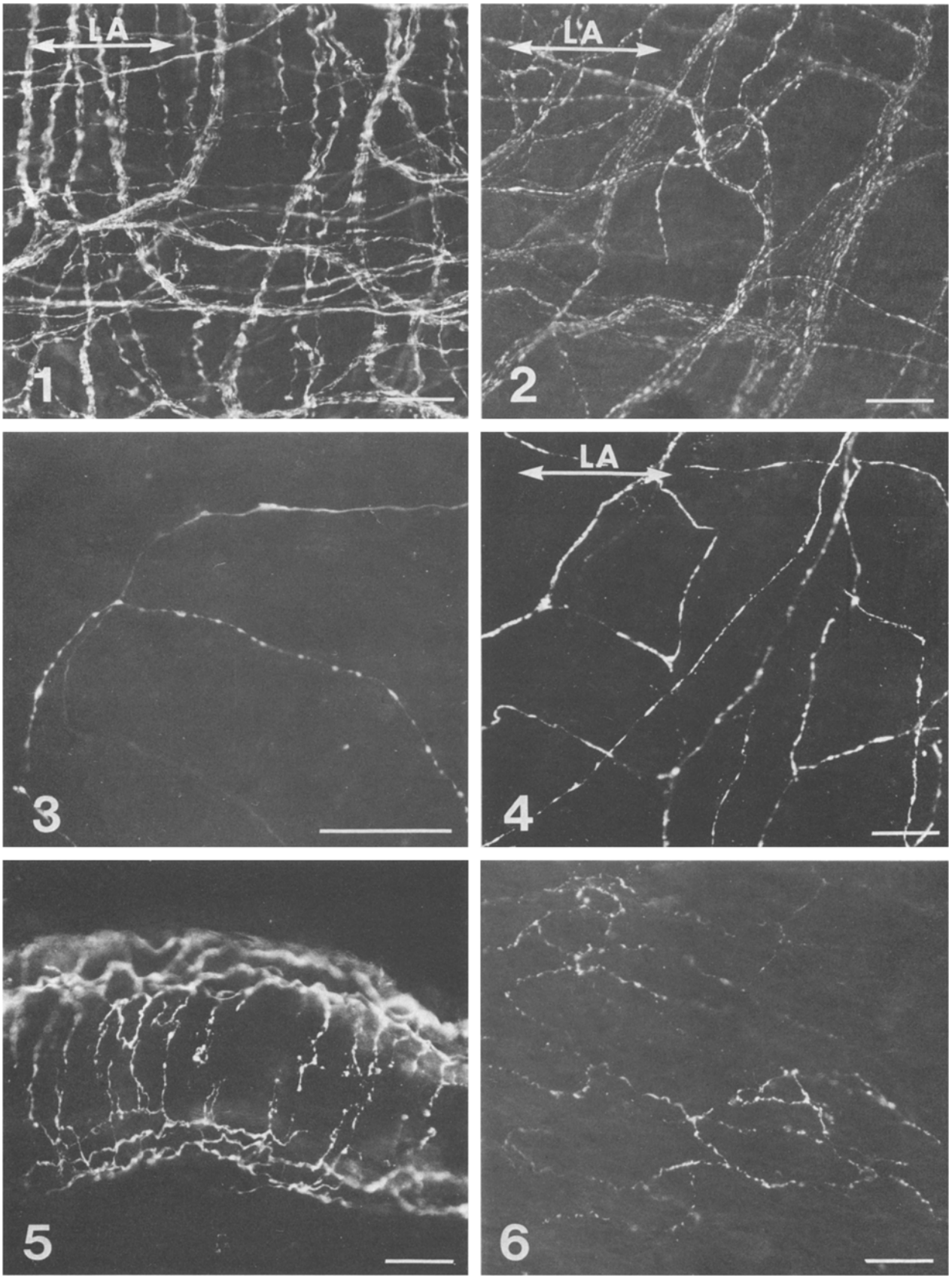
Figure 4. Tyrosine hydroxylase immunoreactivity localised to nerve fibres in a whole mount preparation of a human anterior spinal vein.

Figure 5. Fine perivascular fibres displaying CPON immunoreactivity running around a small artery of the adventitial vasa vasorum in a whole mount preparation of a human pulmonary artery obtained at surgery.

Figure 6. A whole mount preparation of a human mesenteric artery which contains a network of varicose fibres displaying VIP immunoreactivity. LA, longitudinal axis of vessel. Bar = 50 μm.

vasculature (fig. 3) appear to display CGRP immunoreactivity when immunostained with antisera raised against human or rat alpha-CGRP.

The distribution of CGRP and substance P immunoreactivities is very similar and several studies have now demonstrated that they are co-localised in a population of sensory



neurones[87, 89, 121, 122, 142, 210]. The degree of coexistence appears to be very high in the guinea pig where some 90% of all CGRP-immunoreacative sensory neurones display substance P immunoreactivity[87] and both peptides are invariably found together in the same perivascular fibres. Furthermore, recent immunoelectron microscopical studies in our laboratory have revealed that this coexistence extends to the subcellular level, the two immunoreactivities being present in the same secretory vesicles in both the varicosities of perivascular fibres (fig. 9) and in sensory perikarya of the guinea pig[94].

The sensory origin of CGRP and substance P immunoreactive nerve fibres in the cardiovascular system has been substantiated by using the selective neurotoxin capsaicin. Systemic capsaicin treatment of guinea pigs and rats leads to a marked loss of substance P and CGRP immunoreactivity in the heart and vasculature[51, 52, 79, 81, 150, 151, 160, 206]. Cardiac and perivascular nerves containing VIP and NPY immunoreactivities appear to be unaffected[42, 46, 51, 95].

Capsaicin treatment of adult guinea pigs produces an 88–99% depletion of CGRP immunoreactivity in the cardiovascular system, together with a parallel loss of substance P immunoreactivity, but a more varied dose dependent response is observed in rats treated neonatally[216]. In the rat, CGRP immunoreactivity appears to occur in two populations of sensory neurons, one of which also contains substance P and is sensitive to the action of capsaicin whereas the other possesses only CGRP and is resistent to the neurotoxin[142]. Surgical denervation has also been used to demonstrate the sensory origin of CGRP-immunoreactive nerves supplying the cardiovascular system. Thus, destruction of the trigeminal ganglion in the rat[142] and lesions of the trigeminal nerve in the cat[144] result in a loss of perivascular nerve fibres containing CGRP and substance P immunoreactivity around cerebral blood vessels.

CGRP has been shown to exert a potent vasodilatory action, both in vivo and in vitro, on cerebral, coronary and other peripheral blood vessels from several animals, including rat, guinea pig, rabbit, cat and man[18, 21, 22, 59, 98, 144, 145, 201]. The effect is dose dependent and not modified by adrenergic, cholinergic, histaminergic or neuronal blockade. CGRP binding sites have been reported to occur in both the media and intima of rat coronary arteries and aorta[185]. The vasodilatory response could be partly mediated by an endothelium dependent mechanism[22, 111] and partly via a direct action on the media with the activation of adenylate cyclase[59, 98, 207], but there may be regional and/or species variations. Recent studies using cultures of human umbilical vein endothelial cells have shown that CGRP produces a dose-dependent increase in adenylate cyclase activity and the release of prostacyclin[37]. It is uncertain whether any correlation exists between the density of the CGRP-immunoreactive innervation of blood vessels and their responsiveness to exogenous CGRP. CGRP may potentiate the effect of tachykinins and other agents which induce plasma extravasation in a inflammatory reaction[23, 82] and this might be brought about at least in part, by inhibiting substance P degradation[118] and potentiating substance P release[158].

In addition to its effect on coronary vessels, CGRP has direct inotropic and chronotropic actions on the isolated rat and guinea pig heart. Unlike substance P, it also mimics the non-adrenergic, non-cholinergic excitatory response induced by transmural nerve stimulation and capsaicin[75, 101, 130, 176, 180]. The effect of capsaicin on the heart appears to be mediated by the release of CGRP from afferent cardiac nerve fibres. CGRP has also been shown to have a positive inotropic effect on the isolated human atrium, but in contrast to the guinea pig capsaicin did not stimulate the contractility of the preparation[72]. This species variation in response to capsaicin may reflect the lower density of CGRP-containing nerves in the human heart compared to that found in the guinea pig.

Tachykinins

The tachykinins are a group of biologically active peptides possessing a common C-terminal amino acid sequence. Substance P is one of the best characterised neuropeptides and until recently was the only tachykinin known to occur in the mammalian nervous system, but at least two other tachykinins, neurokinin A (NKA) and neurokinin B (NKB), have now been identified[109, 110, 147]. The primary structure of two bovine preprotachykinins has also been determined, one (beta-prepro-tachykinin) containing sequences homologous to both substance P and NKA and the other substance P alone[155].

A considerable amount of immunocytochemical evidence is now available demonstrating that substance P occurs in primary sensory neurones which have peripheral branches associated with the cardiovascular system. These immunoreactive nerve fibres are generally sensitive to capsaicin treatment and have been identified in the heart[79, 81, 159, 160, 170, 206, 212, 214], around both large conducting arteries and veins and smaller vessels supplying many vascular beds in a variety of mammals[16, 52, 57, 81, 126, 127, 152, 160, 175, 203]. Substance P-immunoreactive cardiovascular nerves are particularly numerous in the guinea pig where, as indicated above, there is extensive coexistance with CGRP immunoreactivity. Immunostaining of the guinea pig cardiovascular system for either peptide probably demonstrates an identical population of sensory nerves, the density of the perivascular plexus being about half that of the noradrenergic network[48].

Recent immunocytochemical investigations have revealed that in addition to substance P other tachykinins are also present in guinea pig capsaicin sensitive nerves[103]. These nerves may therefore contain several bioactive peptides, comprising at least two tachykinins (substance P and NKA) and CGRP. In comparison to the guinea pig, the rat cardiovascular system contains significantly less substance P immunoreactivity and fewer substance P-immunoreactive nerve fibres. CGRP and substance P occur in a heterogenous subpopulation of rat sensory neurones with less than half of the CGRP-immunoreactive neurones containing substances P as well[121, 122, 142]. Substance P- and CGRP-immunoreactive cardiovascular nerves in the rat also shown marked variations in their sensitivity to capsaicin[142, 175, 216].

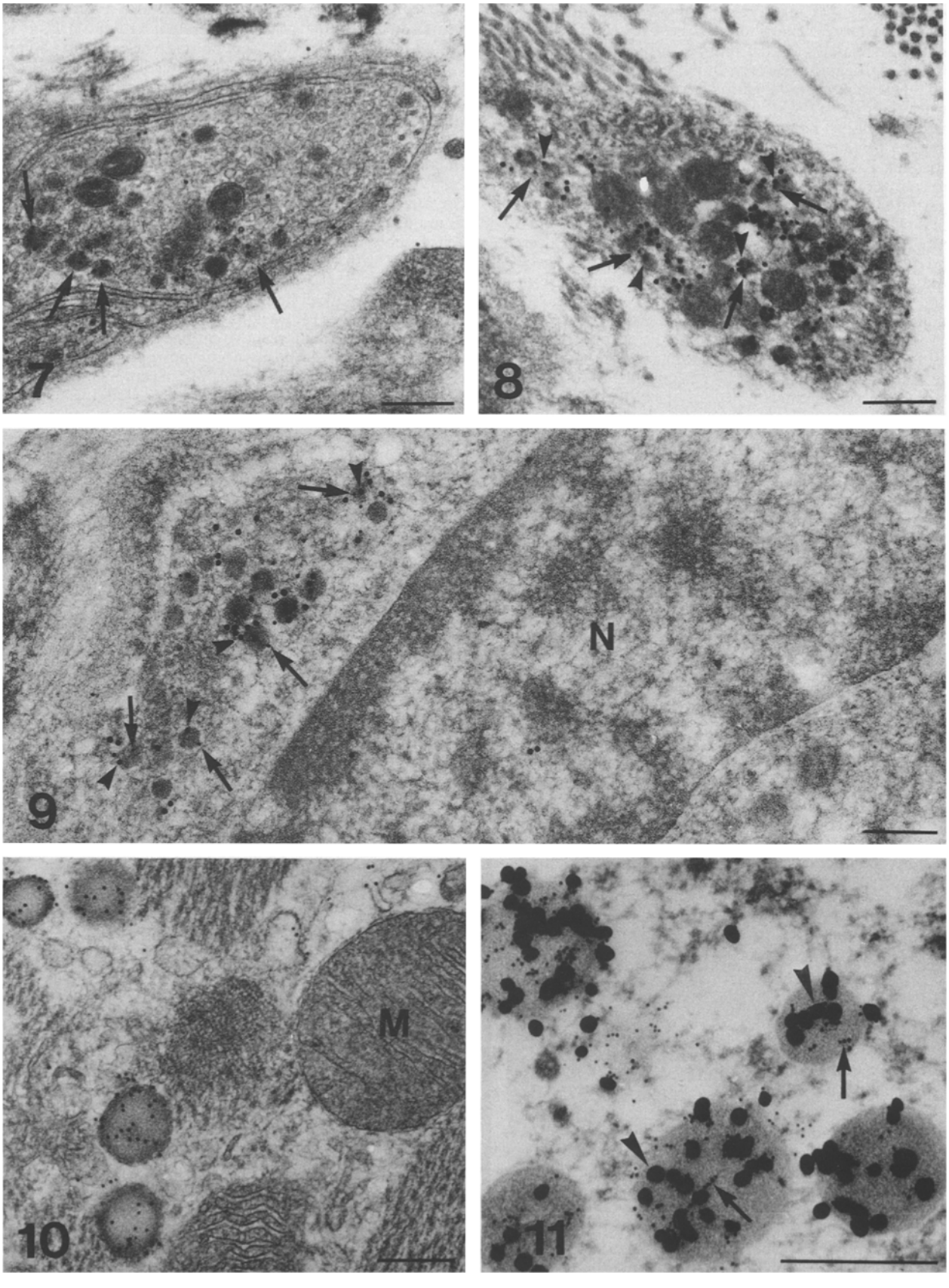
Substance P is considered to be a putative sensory neurotransmitter mediating nociceptive and neurogenic

Figures 7 and 8. Axon varicosities in the human right atrial appendage containing NPY (figs 7 and 8) and CPON immunoreactivity (fig. 8). The large granular vesicles (arrows), but not the small vesicles, display NPY-immunogold labelling (fig. 7). Using a double immunogold staining procedure, NPY and CPON are localised to the same secretory vesicles (fig. 8). NPY, 10-nm gold particles (arrows). CPON, 15-nm gold particles (arrowheads). Bar = 200 nm.

Figure 9. An axon varicosity in the adventitia of a guinea pig superior mesenteric artery. Co-localisation of substance P and CGRP immunoreactivities to the same secretory vesicles, as demonstrated by a double immunogold staining procedure. Substance P, 10-nm gold particles (arrows). CGRP, 15-nm gold particles (arrowheads). N, Schwann cell nucleus. Bar = 200 nm.

Figures 10 and 11. Ultrastructural localisation of alpha-ANP (figs 10–11) and cardiodilatin 1–16 (fig. 11) in human right atrial appeandage. ANP immunoreactivity localised to secretory vesicles in an atrial myocyte by immunogold staining. ANP, 10-nm gold particles (fig. 10). ANP and cardiodilatin immunoreactivities are co-localised to the same secretory vesicles (fig. 11). ANP, 5-nm gold particles (arrows). Cardiodilatin, 20-nm gold particles (arrowheads). M, mitochondria. Bar = 200 nm.

vasodilatory responses and plasma extravasation[83, 123, 178]. In addition to modulating peripheral vasodilatory processes substance P has a vasodilator action in a number of blood vessels[64, 96, 128, 177]. Substance P and other vasoactive agents, such as acetylcholine, bradykinin, ATP and related purines, require the presence of an intact endothelium to exert all or



part of their effect on arteries, this being mediated by the release of an endothelial derived relaxing factor[14, 27, 39, 59, 78]. Substance P binding sites have been identified by in vitro autoradiographic mapping in blood vessels in the guinea pig and human lung[32], rat thymus[184], dog carotid artery[187] and dog renal artery[188]. The binding in these vessels appears to be mainly associated with the intimal surface and requires an intact endothelium; however, in the bovine coronary artery and rat thoracic aorta substance P seems to bind to the media rather than the intima[188]. It is now thought that there are three tachykinin receptors, NK-1, NK-2 and NK-3, each having a preferential affinity for the respective tachykinins, substance P, NKA and NKB[168]. These receptors have a heterogenous distribution in the vascular wall of different species and this may be responsible for the various effects which the tachykinins exert in the mammalian cardiovascular system. Thus, the guinea pig basilar artery, dog carotid artery and rabbit pulmonary artery are all thought to possess NK-1 type receptors with substance P inducing the release of an endothelial derived relaxing factor[39, 60, 168]. On the other hand, NKA and NKB may induce a direct contractile response in the rabbit pulmonary artery and rat portal vein, acting via NK-2 and NK-3 tachykinin receptors located on the vascular smooth muscle[39, 168]. In contrast to CGRP, substance P does not mimic the actions of capsaicin on the heart, apparently lacking any direct effect on the contactility of the mammalian heart[28, 72, 128, 130].

Vasoactive intestinal polypeptide (VIP)

Vasoactive intestinal polypeptide (VIP) is a 28 amino acid peptide which was originally isolated from porcine intestine and recognised for its potent vasodilatory effect[174]. Another peptide, PHI-27 (*p*eptide with N-terminal *h*istidine and C-terminal *i*soleucine), has also been isolated from porcine intestine[191, 193] and found to have a distribution similar to that of VIP[19, 34, 139]. The human form of this peptide, PHM (C-terminal *m*ethionine), is derived from the same pre-promolecule as VIP[20, 106]. Both peptide sequences are present in cardiovascular nerves, these generally being presumed to represent post-ganglionic parasympathetic neurones. Perivascular nerve fibres displaying VIP and PHI/PHM immunoreactivity tend to occur more frequently around vessels in regional vascular beds than in association with larger conducting vessels[46, 199]. As with the other types of peptide-containing cardiovascular nerves there are species variations in the distribution of VIP-immunoreactive nerves, the perivascular plexus usually being more dense in the cat and pig than in other animals (e.g. rat, guinea pig and dog). In man, the density of the VIP-immunoreactive perivascular nerves appears to be between that of nerves displaying NPY/CPON and CGRP or substance P immunoreactivities, whereas in the guinea pig it is the least dense of all these networks[48]. VIP- and PHI/PHM-immunoreactive perivascular nerves are reported to be relatively numerous in many tissues including the gastrointestinal (fig. 6)[19, 25, 36, 183], genito-urinary[9, 10, 140, 215] and respiratory tracts[47, 131], salivary glands[129, 198, 217] and the eye[195, 200]. They also occur frequently around the cerebral vasculature, with anterior vessels in the circle of Willis receiving a more dense supply than those in the posterior circulation[53, 56, 58, 88, 105, 115]. At the ultrastructural level, VIP immunoreactivity has been localised to large secretory vesicles in nerve terminals and axons of cat cerebral vessels[120]. The immunoreactivity persisted after long term sympathetic denervation indicating its presence in nonadrenergic nerves.

VIP has a direct vasodilatory action on cerebral, pulmonary, coronary and other systemic vessels from several species, both in vivo and in vitro[17, 24, 50, 58, 104, 115, 116, 120, 205]. The extent to which the arterial vasodilatory response to exogenous VIP is coupled with the density of the VIP-containing perivascular network is uncertain[189], but the response is not dependent on the presence of an intact endothelium[50, 120, 207]. Specific VIP-binding sites have been identified in the mammalian lung and localised to the media of pulmonary blood vessels[17, 31, 125, 181]. Similar receptors have also been demonstrated in the media of bovine cerebral arteries[165, 190]. It is thought that VIP may mediate non-adrenergic, non-cholinergic, neurogenic vasodilation in a number of vascular beds, this having been most extensively studied in the cat submandibular gland[129]. In addition to VIP, PHI and PHM are also known to have vasoactive properties, although they may be less potent vasodilatory agents than VIP[56, 116, 157, 189].

In contrast to the numerous cardiac nerve fibres displaying NPY/CPON immunoreactivity it seems that relatively few contain VIP-like material. VIP immunoreactivity has been found in the guinea pig, rat, dog, cat, monkey and human heart where it is reported to occur mainly in nerve fibres associated with the atria, conduction system and coronary vessels[24, 46, 167, 212, 213]. The presence of some VIP-immunoreactive neuronal cell bodies in intracardiac ganglia has also been demonstrated, at least in the dog suggesting an intrinsic origin for VIP-immunoreactive cardiac nerve fibres[212, 213].

VIP exerts a direct positive chronotropic and inotropic effects on the heart[45, 72, 205] and VIP receptors, coupled to adenylate cyclase, have been found in atrial and ventricular membrane preparations of the dog, monkey[33] and human heart[194]. PHI receptors were also identified in the human preparations, VIP and PHI having a similar capacity to stimulate adenylate cyclase activity. Finally, VIP has been implicated in pathophysiological aspects of haemorrhagic shock[35], hypertension and heart failure[71, 204].

Other neuropeptides

In addition to the peptides described above there are a number of others which have also been detected in mammalian cardiovascular nerves, including somatostatin, enkephalin and neurotensin, but in general they appear to have a relatively limited distribution.

Somatostatin-like immunoreactivity has been extracted from the human, guinea pig and rat heart[43] and localised to both nerve fibres in the myocardium and conduction system and to local, presumably parasympathetic ganglion cells in the atrium[43, 73]. In the isolated guinea pig atrium, somatostatin has a negative inotropic action on both basal and electrically stimulated contractions[49] whereas in human atrial preparations it only inhibits the positive inotropic action of noradrenaline[72, 73]. It is suggested that the negative inotropic effect of somatostatin could be due to its ability to reduce Ca^{2+} influx in the atrium and this may also explain its actions on atrioventricular nodal function and its antiarrhythmic properties[90, 211].

The rat heart is reported to contain the mRNA encoding preproenkephalin[102] and there is indirect evidence to suggest that enkephalin immunoreactivity in the guinea pig heart is associated with sympathetic nerves, there being a significant reduction in the amount of cardiac enkephalin following 6-hydroxydopamine treatment[113]. Neurotensin-like immunoreactivity also occurs in extracts of the guinea pig heart and has been localised to cardiac nerves in several mammals[169, 212] where it has coronary vasoconstrictor actions and may be a positive inotropic and chronotropic agent.

Atrial natriuretic peptide

In contrast to the neuropeptides found in cardiovascular nerves, atrial natriuretic peptide (ANP) is synthesised in cardiac myocytes and may act as a circulating hormone. Following the observation of de Bold and co-workers[44] that intravenous injections of rat atrial extracts induced diuresis and

natriuresis in donor rats there has been an intense effort to isolate and characterise the factor responsible. The resulting literature has been comprehensively reviewed elsewhere[13, 15, 69, 86] and here we shall only briefly consider the localisation of ANP in the heart and the possible influence of the cardiovascular innervation on the release and actions of ANP.

Like other so-called regulatory peptides, ANP is synthesised as a larger prepromolecule which in man comprises 151 amino acids. The C-terminal 28 amino acids represents alpha-ANP and this is probably the major circulating form of the peptide in man. The rest of the precursor contains a signal peptide and N-terminal sequence homologous to a peptide having vasodilatory properties which was isolated from the porcine heart and termed cardiodilatin[70]. The anatomical localization of ANP to atrial myocytes has been confirmed in several mammals including man[12, 30, 141], where it is present in secretory vesicles (fig. 10) together with cardiodilatin immunoreactivity (fig. 11). Tension of the atrial wall[2, 114, 119, 182] and atrial contraction frequency[166, 182] are thought to be the main factors regulating the secretion of ANP. The role of the autonomic nervous system[38, 112, 163] and the possible influence of cardiac neuropeptides, such as NPY, in this process is uncertain. On the other hand, there is evidence to suggest that ANP stimulates vagal afferent nerve endings in the heart[1, 197] and it may attenuate the vasopressor actions of angiotensin II and noradrenaline[220]. Attention has focused on the atria as the site of ANP production in the mammalian heart, but it is now appararent that ventricular myocytes are also capable of synthesising ANP, at least in the rat[84, 97, 156], and this extra-atrial expression of the ANP gene is increased by volume loading[117].

Conclusion

Several neuropeptides are present in the mammalian cardiovascular system where they are localised to specific subpopulations of efferent and afferent nerves, but exhibit both regional and species variations in their distribution pattern. The immunocytochemical evidence indicates that these peptides often occur in the same nerve fibres as other putative or classical transmitters. While this coexistence may also extend to the subcellular level it appears that peptide precursors are localised exclusively to the large secretory vesicles in axon terminals, whereas the classical transmitters noradrenaline and acetylcholine are thought to occur mainly in the smaller sized vesicle population. The significance of this localisation and the physiological role(s) of neuropeptides in the cardiovascular system have still to be established, but they may functions as 1) hormones; 2) transmitters acting via their own receptors, 3) neuromodulators influencing the release and/or action of transmitters and 4) long term (trophic) agents.

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Regulatory peptides in the respiratory system

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Summary. Many regulatory peptides have been described in the respiratory tract of animals and humans. Some peptides (bombesin, calcitonin, calcitonin gene-related peptide) are localised to neuroendocrine cells and may have a trophic or transmitter role. Others are localised to motor nerves. Vasoactive intestinal peptide and peptide histidine isoleucine are candidates for neurotransmitters of non-adrenergic inhibitory fibres and may be cotransmitters in cholinergic nerves. These peptides may regulate airway smooth muscle tone, bronchial blood flow and airway secretions. Sensory neuropeptides (substance P, neurokinin A and B, calcitonin gene-related peptide) may contract airway smooth muscle, stimulate mucus secretion and regulate bronchial blood flow and microvascular permeability. If released by an axon reflex mechanism these peptides may be involved in the pathogenesis of asthma. Other peptides, such as galanin and neuropeptide Y, are also present but their function is not yet known.
Key words. Neuropeptide; vasoactive intestinal peptide; substance P; neurokinins; calcitonin gene-related peptide; asthma.

Introduction

Recently, a large number of regulatory peptides have been identified in the respiratory tract of several species, including humans (table). Many of these peptides have potent effects on several aspects of airway function, including bronchomotor tone, airway secretions and the bronchial circulation. The precise physiological role of all these peptides is obscure, although some clues are provided by their localisation and functional effects. The purpose of this chapter is to discuss what is known of these peptides, particularly in human airways, and to speculate about their possible pathophysiological role.

Many neuropeptides have been isolated from the gut, where they are involved in regulation of gut motility, sphincters and secretion. There is convincing evidence that these neuropeptides are neurotransmitters or neuromodulators, and appear to be involved in the complex integrative regulation of the gastrointestinal tract. Since the airways are derived embryologically from the foregut it is not surprising that similar peptides are also to be found in lung[7,76]. As in the gut, these peptides are localised either to nerves or to neuroendocrine cells.

Neuroendocrine cells

Specialised cells containing neurosecretory granules are present in the respiratory tract of several species, including man, and are prominent in the fetal and neonatal lung. Because these cells disappear during maturation they may