Review article

Cardiovascular effects of neuropeptide Y: receptor interactions and cellular mechanisms

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In the classical textbook description of the hormonal regulation of cardiovascular function, the effects of catecholamines and acetylcholine are the ones most often considered. These transmitters have not only a postjunctional effect on vascular or cardiac cells, but also prejunctional effects on neurones resulting in modulation of the release of transmitters. It is now known that adrenergic and cholinergic substances act in concert with other agonists and antagonists at a multitude of receptors present on the target cells. Some of these other effectors are neurotransmitters themselves and may be coreleased with adrenergic and cholinergic substances. The picture becomes even more complex when it is considered that the number and sensitivity of receptors and also the components of the cellular machinery for signal transmission, for example GTP binding proteins (G proteins), are subject to regulation under physiological or pathological conditions.

Furthermore, the autonomic neural control of cardiovascular function involves a number of neuropeptides. In the heart, neuropeptide Y is the most abundant among these neuropeptides. Others are vasoactive intestinal polypeptide, peptide histidine isoleucine, calcitonin gene related peptide, neurotensin, and substance P. Apart from its existence in peripheral tissues, neuropeptide Y is also present in many central nervous structures where it was first identified.[1]

In this overview the current state of knowledge about the peripheral effects of neuropeptide Y in the cardiovascular system is summarised. Several excellent reviews covering this subject have been published over the past five years with accent on localisation,[2] functionality and cardiovascular regulation,[3 4] receptor subtypes,[5 6] and signal transduction systems.[4 5] The major emphasis of this review is to assess what is known of the vascular and cardiac effects of neuropeptide Y in the light of the most recent findings, focusing particularly on the receptor interactions and cellular mechanisms by which the peptide exerts its vasoconstrictor and inotropic response. Because the development and utilisation of neuropeptide Y analogues and fragments have been fundamental in the recent advances made in characterisation of neuropeptide Y receptor subtypes and their physiological significance, the present knowledge of the structure of neuropeptide Y and its partial peptides is summarised as a basis for discussion of their actions at the level of vascular smooth muscle cells and cardiomyocytes, and in isolated vascular and cardiac tissues. Finally, our conclusion is based on examining what understanding exists regarding the involvement of neuropeptide Y in normal and pathological states of the cardiovascular system.

Structure

Amino acid composition

The strong conservation of the amino acid sequence of neuropeptide Y indicates that this peptide subserves evolutionarily old and important functions. Clones of DNA encoding chick and goldfish neuropeptide Y variants display only one and five differences, respectively, with respect to human neuropeptide Y.[7] Probes for chick neuropeptide Y have been used to isolate clones from the shark genomic library. The shark peptide displays only two differences by comparison to the sequence of the goldfish peptide and three to that of the human peptide. The impressive similarity of shark and human forms of neuropeptide Y makes this peptide one of the most highly conserved known, even more so than insulin.[7] To date, the sequence of neuropeptide Y isolated from the pig,[1] ox,[8] guinea pig, sheep,[9] and rabbit[10] have been characterised by amino acid analysis. The structures of the neuropeptide Y molecules obtained from human, guinea pig, rabbit, and adult rat have been deduced from cDNA sequences.[10]

All mammalian forms of neuropeptide Y consist of 36 amino acid residues, including five tyrosine residues in each molecule and a C terminal amide structure.[1] Human, rat, rabbit, and guinea pig forms of neuropeptide Y are identical and they possess a methionine residue at position 17, which is readily oxidised.[10] Two other structural forms of neuropeptide Y are known to exist. Porcine and bovine neuropeptide Y variants are identical to the mammalian forms described above except that the methionine residue in the latter at position 17 is replaced by a leucine residue which is not oxidised.[1 8] Neuropeptide Y isolated from the sheep differs from all the earlier characterised mammalian forms by having an asparagine residue instead of a glutamine residue at position 10. At position 17 it has a leucine residue, as do both porcine and bovine forms of neuropeptide Y.[9]

It is believed that the neuropeptide Y released from nerve endings is in the non-oxidised form, since occasionally reduced neuropeptide Y has been found in media and cellular extracts.[10] These findings have been substantiated by the detection in dog plasma of non-oxidised neuropeptide Y

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during and immediately after cardiac sympathetic stimulation, whereas under basal conditions, the oxidised peptide predominates.[11] The oxidation of neuropeptide Y may be important in determining the biological activity of the peptide, since it is known that the synthetic form of human neuropeptide Y loses activity when stored in solution, whereas porcine neuropeptide Y is stable.[10]

Three dimensional structure

The *x* ray crystallographic data for avian pancreatic polypeptide (PP)[12] has been used to define the three dimensional structure of neuropeptide Y by computer aided modelling techniques (see fig 1 and[13 14]). As is the case with pancreatic polypeptide, neuropeptide Y contains two helical limbs running antiparallel to each other and stabilised by hydrophobic interactions involving proline at positions 2, 5, and 8. Residues (15-31) of neuropeptide Y contain the basic hydrophilic sequence called the PP-fold.[15] The N terminal portion of the molecule constitutes a left handed type II polyproline helix (residues 1-13) followed by a β-turn region (14-18) which is connected to an α helix (19-32). A hydrophilic chain or helical limb occurs at the C terminal (33-36). Both the PP-fold and the helical limb are highly conserved among members of the pancreatic polypeptide family.[16] However, the structure of porcine neuropeptide Y in solution determined by nuclear magnetic resonance techniques has been found to differ substantially from one based on the crystal structure of avian pancreatic polypeptide.[17 18] While this approach indicated a strong hydrophobic interaction between one face of the α helix and the N terminal polyproline-like helix, there does not appear to be a close connection between these two parts of the molecule in solution, and the N terminal appears to have no regular structure.[17 18] It does appear, however, that as with the arrangement in the crystal structure of avian pancreatic polypeptide, a dimeric organisation of neuropeptide Y exists in solution.[17 18]

Functional aspects of partial peptides

Structure-activity studies have indicated that the C terminal tetrapeptide, that is NPY(33-36), is essential for receptor recognition.[19] The C terminal amide group with an aromatic side chain is essential for the binding and biological effects of neuropeptide Y.[20] On comparison with its related peptide, pancreatic polypeptide, high conservation of the C terminal segment (32-36) is observed, four out of the five C terminal residues being identical. Residue 34 determines the specificity of the peptides among the neuropeptide Y receptors. This is confirmed by the fact that pancreatic polypeptide may be converted into a high affinity ligand for the Y_2 receptor by exchanging the proline for a glutamine residue which is found in neuropeptide Y at this position. Although the tetrapeptide NPY(33-36) is important, it is not the only factor primarily involved in determining receptor specificity as the tetrapeptide per se does not bind to the receptor. It must therefore be assumed that N terminal extension may lead to conformational stabilisation.[19] ^{32}Threonine, which links the C terminal tetrapeptide and helical part of the molecule, is an important site. Substitution of residue 32 with its D-enantiomer leads to loss of biological activity.[19]

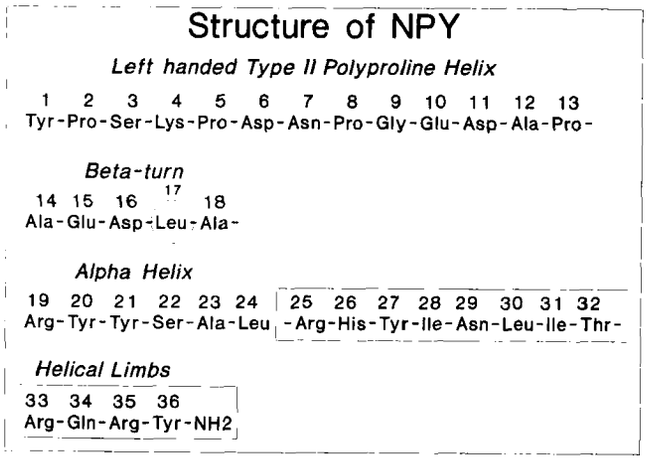

Figure 1 *Amino acid composition and summary of the three dimensional structure of neuropeptide Y (NPY). Bovine and porcine NPY possess a leucine residue in position 17. Human, rat, rabbit and guinea pig forms of NPY are identical except that the leucine residue in position 17 is replaced by a methionine residue (see text). Maintenance of the conformation and low configurational entropy of the 25-36 region of NPY favour both receptor binding and biological activity.*

The overall three dimensional structure of the peptide is also necessary for interaction with the neuropeptide Y receptor.[21] Centrally truncated analogues of neuropeptide Y such as [D-Cys^7-Aoc$^{8-17}$-Cys^{20}]pNPY, which is selective for the Y_1 receptor, maintain the three dimensional structure of neuropeptide Y, particularly around the binding site, and so retain binding capacity and biological activity.[22] [D-Cys^7-Aoc$^{8-17}$-Cys^{20}]pNPY is a analogue designed specifically to maintain the relative conformation of the two helices while further stabilising the molecule with an intrachain disulphide bond. The fact that [D-Cys^7-Aoc$^{8-17}$-Cys^{20}]pNPY maintains biological activity at the Y_1 receptor, while the C terminal fragment NPY(13-36) does not, indicates that the N terminal portion of neuropeptide Y plays an important role in the interaction of the peptide with Y_1 receptors and subsequent signal transduction.[22]

NPY(28-32) is part of the α helix and this segment is essential for receptor recognition and especially biological activity. The C and N terminal fragments of neuropeptide Y are important for receptor binding and therefore analogues which link the terminals stabilise the α helical conformation of NPY(25-32). Molecular modelling has revealed that N terminal amino acids (1-4) are in close proximity to the C terminal residues (25-31). Therefore, discontinuous analogues which lack the natural sequence (5-25) have been developed.[23] These compounds, for example NPY-(1-4-Ahx-25-36), are comprised of only 50% of the amino acids of the native hormone, but the right handed helical structure is maintained, as is its receptor binding which is almost as high as that for neuropeptide Y. The N terminal aromatic ring also seems to be mainly responsible for increased activity.[23]

Subtypes of receptor for neuropeptide Y

Two receptor populations of neuropeptide Y, tentatively designated as Y_1 and Y_2 subtypes, were first separated on the basis of the differential action of the neuropeptide Y fragment (13-36), which appeared to be selective at prejunctional sites by comparison with postjunctional sites.[24] Precise separation of prejunctional and postjunctional effects on the basis of the actions of the (13-36) fragment has not been obtained. For example, in vagotomised anaesthetised rats, NPY(13-36) was found to produce changes in cardiac vagal action and blood pressure indicating that the fragment has both prejunctional and postjunctional activities.[25] Subsequently, of particular importance has been the development

of the selective agonist of the Y_1 receptor [Leu^{31},Pro^{34}]-NPY, the efficacy of which was tested with respect to transient increases in cytoplasmic concentrations of free Ca^{2+} in a human neuroblastoma cell line expressing only Y_1 receptors.[26] The analogue also increased blood pressure in anaesthetised rats, indicating that [Leu^{31},Pro^{34}]-NPY binds postjunctionally. The analogue did not attenuate cardiac vagal action and this was taken as indicative of a lack of prejunctional activity.[27] Similarly, in the same in vivo model, [Pro^{34}]-NPY had no significant effect on vagal action, but the analogue increased blood pressure to a similar extent as neuropeptide Y, indicating that [Pro^{34}]-NPY had selective postsynaptic activity. Also, centrally truncated analogues of neuropeptide Y selective for the Y_1 receptor, such as [D-Cys^7-Aoc$^{8-17}$-Cys^{20}]pNPY, have been developed to maintain the three dimensional structure of neuropeptide Y, particularly around the binding site, and so retain binding capacity and biological activity.[21] The fact that [D-Cys^7-Aoc$^{8-17}$-Cys^{20}]pNPY maintains biological activity at the Y_1 receptor, while the C terminal fragment NPY(13-36) does not, indicates that the N terminal portion of neuropeptide Y plays an important role in the interaction of the peptide with Y_1 receptors and subsequent signal transduction.[22] (25-36) NPY is the minimum length required to activate the Y_2 receptor.[15] Also of major interest has been the development of NPY(18-36), which has both agonistic and antagonistic actions depending on the experimental model used. This fragment is a partial agonist in human erythroleukaemia cells.[28] In cardiac ventricular membranes from the rat, however, it is the first competitive antagonist to be described at the neuropeptide Y receptor.[29] More recent has been the development of fragment NPY(17-36), which has a biphasic effect on adenylate cyclase activity in the heart[30] and acts as a potent antagonist at neuropeptide Y receptors mediating myocyte contraction.[31]

Based on the use of neuropeptide Y and peptide YY analogues and C terminal fragments, single subpopulations of neuropeptide Y receptors have been identified, particularly in transformed cell lines.[5 32] In cardiovascular tissues, also, there appears to be selective distribution of receptors in some cell types (see table I) and binding sites have been characterised in a number of cases. Receptors on porcine aortic smooth muscle cells appear to be of the Y_1 subtype since NPY(13-36), an agonist with some selectivity for Y_2 receptors, was about 300 times less effective than porcine neuropeptide Y in displacing [^{125}I]NPY binding.[33] The binding characteristics of radiolabelled neuropeptide Y to this single population of sites were a K_d of 1.1 nM and a B_{max} pmol·mg^{-1} protein.[33] Similar data were obtained using membrane fractions of porcine aortic smooth muscle, in which the K_d was 0.99 nM and the B_{max} was 0.35 fmol·mg^{-1} protein.[34] A similar affinity of sites (K_d 1.1 nM) has been found in rabbit aorta.[35] A homogeneous subpopulation of Y_1 receptors on rat aortic smooth muscle cells has also been demonstrated by Shen *et al*,[36] since [Leu^{31}, Pro^{34}]-NPY, the selective Y_1 type receptor agonist, had a binding constant similar to neuropeptide Y in the region of 1 nM, whereas NPY(13-36) showed two or three orders of magnitude lower affinity. This result was confirmed by Grundemar *et al*,[37] using the analogue [Pro^{34}]-NPY, which also acts as a selective Y_1-type agonist. Thus, there is a wealth of data to support the fact that the aorta possesses a single population of receptors. Binding sites for neuropeptide Y/peptide YY in vascular smooth muscle of the rat pancreas have been localised using electron microscopic autoradiography and binding sites identified with affinity one order of magnitude higher[38] than those characterised in aorta.[33–35] The rat pancreatic receptor had a high affinity for [Leu^{31},Pro^{34}]NPY and a much lower affinity for the NPY(13-36) peptide, so that the binding site was characterised as being of the Y_1 subtype.[38] In other vascular smooth muscle cells, however, a mixed population of Y_1 and Y_2 receptors exists, as for example in the caval vein.[37] Binding sites for neuropeptide Y in rat platelets, tentatively designated as of the Y_2 subtype, have been characterised, with a K_d of 0.8 nM and a B_{max} of 470 fmol·mg^{-1} protein.[39]

In rat cardiac ventricular membranes, binding sites of both high and low affinities for neuropeptide Y have been identified, which have apparent K_d values of 0.3 and 22 nM and B_{max} values of 7.13 and 261 fmol·mg^{-1} protein, respectively.[40] NPY(13-36) can bind with a lower affinity than neuropeptide Y, indicating initially that the cardiac receptor for neuropeptide Y belonged to the Y_2 subtype.[40] Both Y_1 and Y_2 receptors characterised to date have nearly equal affinity to both peptide YY and neuropeptide Y.[5] In the ventricular myocardium, however, the neuropeptide Y receptors discriminate between these two related peptides. Thus it has been suggested that these binding sites may belong to another class, Y_3.[40] It remains to characterise the binding sites for neuropeptide Y on isolated and purified cardiomyocytes, which might help to clarify the nature of the postjunctional receptors in heart muscle.

Table I *Distribution of neuropeptide Y receptors in the cardiovascular system based on selectivity of neuropeptide Y analogues and fragments for receptor subtypes.*

Bioassay system		*Rank order*	*NPY receptor subtype*	*Reference*
		IC_{50} values in radioligand binding studies		
Vascular smooth muscle:	Porcine aorta	NPY<<NPY(13-36)	Y_1	Mihara *et al*[33]; Shigeri *et al*[34]
	Rat aorta	NPY=[Leu^{31},Pro^{34}]-NPY<<NPY(13-36)	Y_1	Shen *et al*[36]
	Rat aorta	NPY=[Pro^{34}]-NPY<<NPY(13-36)<NPY(18-36)	Y_1	Grundemar *et al*[37]
	Rat caval vein	NPY=[Pro^{34}]-NPY<NPY(13-36)	Y_1/Y_2	Grundemar *et al*[37]
	Rat pancreas	NPY=[Leu^{31},Pro^{34}]-NPY<<NPY(13-36)	Y_1	Sheikh *et al*[38]
Platelets:	Rat blood	NPY<<[Leu^{31},Pro^{34}]-NPY	Y_2	Myers *et al*[39]
Ventricular membranes:	Rat heart	NPY(13-36)<<NPY<<PYY	Y_3?	Balasubramaniam *et al*[40]
		EC_{50} values of pharmacological effect		
Arterial pressure:	Anaesthetised rat	NPY=[Pro^{34}]-NPY	Y_1	Grundemar *et al*[37]
		NPY=[Leu^{31},Pro^{34}]-NPY	Y_1	Potter and McCloskey[27]
Cardiac vagal action:	Vagotomised anaesthetised rat	NPY(13-36)>NPY=[Leu^{31},Pro^{34}]-NPY=[Pro^{34}]-NPY	Y_2	McCloskey and Potter[25] Potter and McCloskey[27]
Coronary resistance:	Langendorff rat heart	NPY=[Pro^{34}]-NPY	Y_1	Grundemar *et al*[37] Potter and McCloskey[27]
Vasoconstriction:	Isolated caval vein	[Pro^{34}]-NPY<NPY	Y_1/Y_2	Grundemar *et al*[37]

Further evidence for multiple cardiac receptors comes from recent work using NPY(17-36), which had a dual effect on adenylate cyclase activity in rat cardiac ventricular membranes. In the concentration range of 0-300 pM, the fragment had high affinity for neuropeptide Y receptors and inhibited adenylate cyclase activity through an inhibitory G (G_i) protein. At concentrations >300 pM, the fragment had a low affinity for neuropeptide Y receptors and stimulated adenylate cyclase acting through a G_s protein.[30] In isolated ventricular cardiomyocytes, NPY(17-36) also produced a biphasic effect on basally contracting cells,[31] but the profile of the concentration-contractile response curve was opposite to that observed for the adenylate cyclase response. NPY(17-36) at concentrations up to 10^{-8} M stimulated contraction of cardiomyocytes, whereas at higher concentrations, the stimulated contractile response decreased (fig 2B). Although the observations made previously regarding the effects of neuropeptide Y in rat ventricular tissue[29] and isolated cells[41] were similar, it appears that the receptor population or signal transduction machinery mediating the effects of the NPY(17-36) fragment is different in these experimental systems. It is of interest that a biphasic response to NPY(17-36) was noted also in experiments using the perfused mesenteric arterial bed of the rat.[42] In this model of the vascular neuroeffector junction, prejunctional and postjunctional actions were judged by effects of periarterial nerve stimulation induced release of noradrenaline and increase in perfusion pressure, respectively. Whereas neuropeptide Y produced a concentration dependent decrease in the evoked release of noradrenaline, the fragment NPY(17-36) increased release at concentrations up to 10^{-9} M, but inhibited release at higher concentrations. The action of the NPY(17-36) on the postjunctional response was a concentration dependent reduction in perfusion pressure, whereas neuropeptide Y initially decreased, but at concentrations greater than 10^{-8} M increased, perfusion pressure. The postjunctional effects of neuropeptide Y and NPY(17-36) observed in this vascular model[42] and in the isolated heart cell model[31] are totally different and may be a consequence of distinct receptor distributions in these systems.

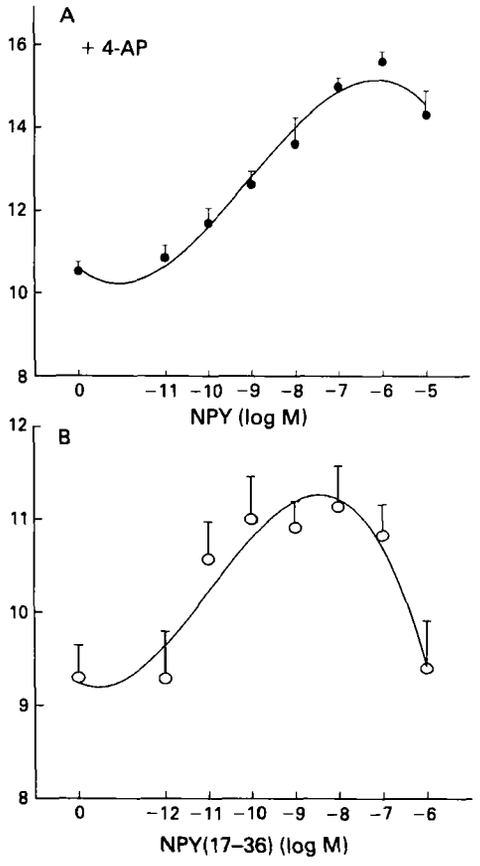

Figure 2 *(A) Stimulation of contractile response by various concentrations of neuropeptide Y (NPY) in the presence of 4-aminopyridine (+4-AP, 0.5 mM). Values are means, bars=SEM, n=5 experiments. (B) Stimulation of contractile response by various concentrations of NPY (17-36). Values are means, bars=SEM, n=4 experiments. Cardiomyocytes were stimulated at 0.5 Hz in the presence of adenosine deaminase (5 U·ml^{-1}). Contractile response is expressed as percentage of maximum shortening relative to fully extended length.*

Multiple subtypes of the cardiac neuropeptide Y receptor in ventricular cells may also be inferred from studies in rat cardiomyocytes (see table II).[43] It has been shown that the negative effect of neuropeptide Y on the contractile response is due, primarily, to stimulation of the transient outward current and this action may be attenuated by pertussis toxin and NPY(18-36). Interestingly, in the absence of isoprenaline but in the presence of a blocking agent of the transient outward current, 4-aminopyridine, neuropeptide Y exerts a positive contractile response (fig 2A) which cannot be abolished by pretreatment with pertussis toxin or attenuated by NPY(18-36). The positive effect is, however, abolished by treatment with the calcium antagonist, verapamil, and also the peptide fragment, NPY(17-36).[31] We propose, therefore, that two postjunctional subtypes of the neuropeptide Y receptor exist on rat ventricular cardiomyocytes. Since NPY(18-36) has antagonistic effects at receptors with Y_1 characteristics,[44] it may be that Y_1 receptors are present on cardiomyocytes, but it remains to be established if the receptors which mediate the positive contractile response to neuropeptide Y have characteristics of Y_3 receptors or if another receptor subtype is involved.

Recently, the molecular cloning of a human neuropeptide Y receptor of the Y_1 subtype has been reported.[45 46] Sequence analysis has indicated strongly that this Y_1 receptor belongs to the G protein coupled receptor superfamily. In the region spanning the transmembrane domains, the Y_1 receptor displays only 21% homology to the bovine neuropeptide Y receptor clone, the characteristics of which could be defined in terms of the existing subtypes.[47] The functional expression of the cloned human neuropeptide Y receptor was typical of the mammalian Y_1 receptor in that calcium influx was accelerated and forskolin stimulated accumulation of cyclic AMP attenuated.[46] It has been demonstrated, however, that expression of the cloned human Y_1 receptor in terms of coupling to second messenger systems can vary in different cell types.[45]

Functional effects of neuropeptide Y in the cardiovascular system

Using immunohistochemical techniques, the presence of neuropeptide Y has been demonstrated in most parts of the

Table II *Postjunctional subtypes of the neuropeptide Y receptor on adult heart muscle cells.*

Contractile response	*Negative*	*Positive*
Effector pathway	Inhibition of cyclic AMP[a]	
Channel	Transient outward[b]	L-type calcium[b]
Sensitivity to pertussis toxin	Sensitive[a,c]	Insensitive[b]
Antagonist	NPY(18-36)[b]	NPY(17-36)[d]

From [a] Millar *et al*[41]; [b] Millar *et al*[43]; [c] Piper *et al*[62]; [d] Millar *et al*[31]

vascular tree, including vessels of the heart.[2] The functional significance of the coexistence of neuropeptide Y and noradrenaline has yet to be established fully, but neuropeptide Y released at nerve endings may influence sympathetic cardiovascular control in at least three ways, having both direct and indirect (prejunctional and postjunctional) effects. Neuropeptide Y may enhance the postjunctional vasoconstricting effect of noradrenaline and other transmitters and can inhibit its prejunctional release by an α_2 adrenoceptor mediated mechanism.[48]

Among the signal transduction mechanisms of neuropeptide Y so far identified in the cardiovascular system are modulation of adenylate cyclase,[29 41 49–54] modulation of calcium channels,[43 55 56] transplasmalemmal influx of calcium,[52 57 58] and mobilisation of intracellular calcium.[59] Activation of the inositol phosphate signalling system by neuropeptide Y in the cardiovascular system has not been demonstrated conclusively. Investigations of the possible involvement of G protein mechanisms in coupling of neuropeptide Y receptors in cardiovascular tissue have utilised different approaches, including assessment of the guanosine triphosphate (GTP) dependency of ligand binding using the GppNHp analogue[40 60] and most commonly using pertussis toxin as a probe for involvement of the inhibitory GTP binding (G_i) protein in producing a second messenger response[29 41 51 54 59 61] or functional effect.[58 62 63]

EFFECTS ON BLOOD VESSELS

Localisation

Particularly dense staining for neuropeptide-Y-like immunoreactivity has been noted in the aorta and coronary vessels.[64 65] Dense plexuses of neuropeptide Y immunoreactivity have been identified around cerebral arteries of cat, guinea pig, rat, and human.[66–70] Neuropeptide Y is widely distributed in the gastrointestinal tract, in smooth muscles, in muscularis mucosa, and in surrounding blood vessels.[71] Numerous nerve fibres, immunoreactive for neuropeptide Y, have been shown to be associated with vascular smooth muscle in the respiratory tract and neuropeptide Y immunoreactivity has also been found in arteries and arterioles of the lung.[72 73] The peptide is widely distributed within the male and female reproductive systems. Immunoreactive neuropeptide Y fibres have been identified in the human fallopian tube, supplying vascular and non-vascular muscle.[74] In the urinary system, neuropeptide-Y-like immunoreactivity has been found throughout the ureter in the vicinity of blood vessels.[75] The peptide is also associated with blood vessels supplying the pancreas.[76] Nerve fibres immunoreactive for neuropeptide Y surround the blood vessels and follicles of the thyroid gland of several mammalian species.[77] Neuropeptide Y is colocalised with noradrenaline in some postganglionic neurones innervating arteries and arterioles, but the immunoreactive neuropeptide Y nerves which innervate the islets and the exocrine parenchyma seem to be non-adrenergic.[78] Non-adrenergic neurones containing neuropeptide Y are also found in the heart (see below). Immunohistochemical localisation of neuropeptide Y has always shown strong positive staining in conjunction with nerve fibres. This does not preclude neuropeptide Y from also being produced and stored in other cell types, such as cardiomyocytes.[79 80]

Neuropeptide-Y-like immunoreactivity has also been identified in platelets, which are by far the largest potential source of circulating neuropeptide Y in the rat.[39] Furthermore, it has been reported recently that neuropeptide-Y-like immunoreactivity is present in endothelial cells of the rabbit ear artery after long term electrical stimulation of the perivascular nerves,[81] but the functional significance of this finding has yet to be established.

Direct (postjunctional) effects

Postjunctional effects of neuropeptide Y on blood vessels in mammals differ according to localisation of the vessel and mammalian species. In experiments in which tissues are treated with exogenous neuropeptide Y it is not always clear whether the reported effects are truly postjunctional, since the influence on transmitter release from the nerve endings within the tissues cannot be excluded. With the exception of the study by Tseng,[82] postjunctional effects of neuropeptide Y on blood vessels were either found to be vasoconstrictive or non-existent, but not vasorelaxant. In some vascular beds, neuropeptide Y exerts a direct vasoconstrictive effect, for example in coronary and cerebral arteries the peptide exerts a concentration dependent constrictive effect. In other vascular beds the peptide remains ineffective when given alone but potentiates the effect of other vasoconstrictive agents.

Strong vasoconstriction in the presence of neuropeptide Y has been observed in coronary arteries from man,[82] dog,[83–86] pig,[87] rabbit,[61 88] guinea pig,[89] and rat.[90] In man, the epicardial sections of coronary arteries are less sensitive or unresponsive to neuropeptide Y compared to the more distal branches. Intracoronary infusion of neuropeptide Y in patients caused only small angiographic changes in the diameter of epicardial coronary arteries, but increased coronary resistance distinctly.[91 92] This shows that the primary vasoconstrictive effect in the coronary system is localised in small arteries. In rat coronary arteries, the neuropeptide Y induced vasoconstriction was significantly smaller in proximal epicardial than in distal intramural coronary arteries.[93] It is believed that the coronary vasoconstrictor effect of neuropeptide Y is the result of a direct action on vascular smooth muscle, as this action is resistant to blockade of α adrenergic[85 87] and serotonergic receptors.[85] Based on the fact that neuropeptide Y constricts coronary vessels directly, it has been hypothesised that the peptide can provoke coronary vasospasm in vivo.[83]

In the cerebral circulation, neuropeptide Y is also a potent vasoconstrictor, as shown for dog,[94] cat,[67] rat,[68 70 95] and rabbit.[96] This vasoconstrictive response is not affected by blockade of adrenoceptors or antagonists at receptors for 5-hydroxytryptamine.[97] In some other vascular beds, a direct constrictor effect of neuropeptide Y has also been reported, for example in the small arteries of gluteus maximus muscle from man,[98 99] in human forearm resistance vessels,[100] in the iliac and femoral vein of the guinea pig and rat,[101] in the uterine artery from guinea pig,[63] and in pig spleen artery.[99] These cases represent a minority, however, compared to the many examples of vessels isolated from vascular beds other than coronary and cerebral circulation which do not respond to neuropeptide Y when it is given as a single stimulant.

Potentiating (postjunctional) effects

In some peripheral vessels neuropeptide Y potentiates the effect of other vasoconstricting agents. This behaviour has been observed in saphenous vein from dog,[102] in ear,[103 104] pulmonary,[105] and femoral arteries from rabbit,[66] and in tail arteries[106 107] and mesenteric arteries from rat.[108] In these vessels the response to other vasoconstrictors was enhanced in the presence of neuropeptide Y. It has been shown that neuropeptide Y is able to potentiate the effect of a multitude of vasoconstricting agents, including agonists at α adrenergic receptors, histamine, angiotensin II, 5-hydroxytryptamine,

prostaglandin $F_{2\alpha}$, and endothelin.[66] The mechanism by which neuropeptide Y potentiates the effect of other vasoconstrictors has not yet been identified. A phenomenon related to the potentiating actions of neuropeptide Y is that in some peripheral arteries which have been electrically stimulated the peptide enhances contraction,[66 109] but it is ineffective on non-stimulated vessels. The vasoconstrictive response to field stimulation can be abolished[78] by phentolamine, an antagonist at the α adrenoceptor, which can reduce the release of adrenergic transmitters by a presynaptic action, or guanethidine, which is toxic to sympathetic nerves.[110] From these findings it may be hypothesised that neuropeptide Y potentiates the effect of noradrenaline which is released endogenously in electrically stimulated vessel sections.

In some vessels the potentiating effect of neuropeptide Y on vasomotor tone appears to be mediated by endothelium and is not a direct action on the smooth muscle cells. In the rabbit ear artery and canine saphenous vein, the potentiation of noradrenaline-induced contraction was found to be dependent on an intact endothelium,[102] whereas contractions induced by neuropeptide Y in small human skeletal muscle arteries and pig splenic arteries[99 111] and rat tail artery[112] were endothelium independent. It is not clear at present whether endothelium dependence of neuropeptide Y potentiation is due to a primary action of neuropeptide Y on the endothelial cells or to release of endothelial factors which in turn condition smooth muscle cells for a constrictive response, for example through an endothelium derived constricting factor. A recent study shows that such a factor could not be identical to endothelin-1, since exogenous action of endothelin-1 did not permit neuropeptide Y induced responses in the absence of endothelium in rat tail arteries.[107]

In contrast to those vascular beds in which an intact endothelium was found necessary for a vasoconstrictor response to neuropeptide Y, in other vascular beds presence of an intact endothelium can reduce the vasoconstrictor response. In the study of Prieto *et al*,[93] removal of the endothelium from epicardial conduit coronary arteries from rat increased the vasoconstrictor effect of neuropeptide Y. In small intramural arteries the action of neuropeptide Y was not attenuated by the presence of endothelium. Similar findings were reported for mesenteric resistance arteries from rat.[113] These results suggest that the release of vasorelaxant agents from the endothelium can antagonise the action of neuropeptide Y. It is not known whether in such vessels neuropeptide Y specifically stimulates the release of such agents from the endothelium, for example prostacyclin or endothelium dependent relaxing factor. In endothelial cultures it has been shown that neuropeptide Y can stimulate the production of prostacyclin[114] and increase intracellular calcium ion concentration.[115]

In summary, most peripheral vessels show no direct response to neuropeptide Y, but in many of these sites, neuropeptide Y can potentiate the sensitivity to vasoconstrictors. The potentiation of vasoconstrictor effects by neuropeptide Y appears in some cases to be dependent on endothelium and in others the presence of an intact endothelium may even attenuate the vasoconstrictor effect.

Prejunctional effects

A presynaptic effect of neuropeptide Y on release of noradrenaline is indicated by experiments in which adrenergic nerves are stimulated by trains of electrical stimuli and release of noradrenaline from these becomes reduced when exogenous neuropeptide Y is present. This has been demonstrated in small human arteries from colonic mesentery, gluteus maximus muscle, and kidney cortex,[98] rabbit ear artery,[103] rat femoral artery and portal vein,[109 116 117] rat and mouse vas deferens,[118-121] and heart tissues from guinea pig.[83 122-126]

There have also been reports about effects of sympathetic nerve stimulation that could not be modulated by neuropeptide Y. In the rat heart, tachycardia provoked by stimulation of sympathetic nerves was attenuated in the presence of neuropeptide Y, whereas the stimulated neuronal release of noradrenaline was not.[127] In another study on rat heart,[128] tachycardia provoked in a similar manner could only be influenced in an indirect way in the presence of neuropeptide Y. In these tachycardia hearts the attenuating effect of xylazine, an agonist at α_2 adrenoceptors, was enhanced, however, when neuropeptide Y was present. This indicates that neuropeptide Y can potentiate the presynaptic response of α_2 adrenoceptor stimulation.

In anaesthetised dogs, high frequency stimulation of the cardiac sympathetic nerves is followed by a period of attenuated vagal activity, an effect that can be mimicked by exogenously applied neuropeptide Y.[129] The vagal inhibition seems to be the result of a corelease of neuropeptide Y from the stimulated sympathetic nerves which in turn causes presynaptic inhibition of vagal nerve terminals.[3 11 130] This long lasting inhibition of vagal activity could function as a "memory effect" for a brief, intense sympathetic stimulation, since it prolongs a dominance of sympathetic influence on the heart.

In summary, in many adrenergic nerve endings, neuropeptide Y can reduce the release of noradrenaline by a prejunctional action. This can either be a direct action of neuropeptide Y or an indirect one, through potentiation of the action of other effectors on prejunctional receptors. Inhibitory prejunctional effects of neuropeptide Y may function as a feedback mechanism preventing excessive depletion of transmitter reserves during prolonged sympathetic stimulation.[103] In the heart, the spillover of neuropeptide Y coreleased from adrenergic neurones could be the cause for sustained vagal attenuation after intense stimulation of sympathetic nerves.

Signal transduction in smooth muscle

Some of the data reported regarding the signal transduction systems in vascular smooth muscle are difficult to interpret mechanistically, since several of the studies were carried out in whole vessel preparations, in which neuropeptide Y might also exert indirect effects on the smooth muscle cells. For example, in the rabbit pulmonary artery, neuropeptide Y potentiates the effect of vasoconstrictors, most of which are known to cause contraction of smooth muscle cells, by stimulation of phosphoinositide hydrolysis and subsequent increase in cytosolic Ca^{2+} levels.[131] This may be an indirect effect, since in cultured smooth muscle cells derived from rabbit pulmonary artery, neuropeptide Y had no effect on this signal transduction pathway.[53]

The possible coupling of receptors for neuropeptide Y on cultured porcine aortic smooth muscle cells to intracellular calcium concentration ($[Ca^{2+}]_i$) was investigated by Mihara *et al*.[59] Neuropeptide Y increased $[Ca^{2+}]_i$ in a dose dependent manner by a mechanism involving a G_i protein. It was suggested that the increase in $[Ca^{2+}]_i$ in these cultured smooth muscle cells was primarily due to neuropeptide Y induced mobilisation of Ca^{2+} from internal storage sites. The intracellular messenger substances, inositol 1,4,5 triphosphate or cyclic adenosine 3',5' monophosphate (cAMP),

seemed not to be involved. In contrast, Reynolds and Yokota[53] found that in primary cultures of smooth muscle cells from rabbit pulmonary artery, neuropeptide Y inhibited forskolin stimulated adenylate cyclase but had no effect on noradrenaline induced hydrolysis of phosphoinositide or increase in $[Ca^{2+}]_i$. In vascular smooth muscle and renin producing cells in the kidney, it was found that neuropeptide Y induces renal vasoconstriction and inhibition of renin release by inhibition of adenylate cyclase activity.[50] The vasoconstrictive action of neuropeptide Y given to dogs by intracoronary injection was atttenuated by Ca^{2+} channel blocking agents,[132] indicating that the contraction of vascular smooth muscle cells in the coronary artery is dependent on the influx of Ca^{2+} from the exterior space. The vasoconstrictor response in the cerebral circulation of the cat can be attenuated by lowering the extracellular Ca^{2+} concentration or by the addition of Ca^{2+} channel blocking agents,[97] again suggesting a role for the influx of Ca^{2+}. In rat mesenteric arterioles, neuropeptide Y, at a concentration which produced no effect by itself, markedly enhanced Ca^{2+} entry dependent responses elicited either by the addition of Ca^{2+} to depolarised vessels or by the addition of the calcium agonist, BAY K 8644.[57 58] These effects are consistent with partial depolarisation and subsequent influx through voltage dependent calcium channels. Abel and Han[133] reached similar conclusions from experiments using rabbit cerebral arteries. The action of neuropeptide Y on contraction in rat mesenteric arterioles induced by BAY K 8644 was abolished following treatment with pertussis toxin, indicating again that in its coupling to Ca^{2+} influx the peptide acts through a G protein.[58] The situation is even more complicated since in many arteries neuropeptide Y has no vasoconstrictor effect of its own but potentiates the effect of other vasoconstricting agents. It was shown that in the presence of either nitrendipine or diltiazem the potentiation by neuropeptide Y of the vasoconstriction of rat mesenteric arterioles induced by α_1 adrenoceptor agonists was abolished.[57] The potentiating effect of neuropeptide Y was also blocked by nifedipine in rat femoral arteries.[109] In contrast to these results, in rabbit vessels neither the presence of the Ca^{2+} antagonists nifedipine, verapamil, or diltiazem, nor the acute withdrawal of Ca^{2+} abolished the potentiating effects of neuropeptide Y.[105] These latter findings suggest that in rabbit arteries, transplasmalemmal Ca^{2+} fluxes are not involved in the potentiating effect of neuropeptide Y.

From the results reported so far, a common mechanism for the positive contractile effect of neuropeptide Y on vascular smooth muscle cells has not been identified. The current picture suggests, rather, that different mechanisms may work in vascular smooth muscle cells from different origins. It appears that the effects of neuropeptide Y on some vascular tissues causing inhibition of accumulation of cyclic AMP,[134] mobilisation of cytosolic calcium,[59] and promotion of calcium influx into the cell,[58] or eliciting contraction[63] are mediated by a pertussis toxin sensitive G protein. This may not be an inhibitory G protein coupled to adenylate cyclase but one coupled to an ion channel. It is known from the work of Nelson *et al*[135] that vascular smooth muscle cells possess a tight electromechanical coupling with a strong voltage dependency of the Ca^{2+} channel. It is conceivable therefore that neuropeptide Y would cause or potentiate vasoconstriction by inducing a depolarisation of the plasma membrane of vascular smooth muscle cells. This has been demonstrated in smooth muscle cells of rabbit cerebral arteries, in which neuropeptide Y was shown to produce a significant decrease in the intracellular membrane potential.[133] In the same study, neuropeptide Y was found to contract arterial ring segments, to potentiate agonist induced contraction, and to inhibit vasodilator induced relaxation. These effects could be mediated, therefore, at least partly by neuropeptide Y induced depolarisation.[134] It has been shown, also, that the effect of neuropeptide Y in vascular smooth muscle cells is not always mediated by inhibitory G protein-receptor coupling.[133] Rather, inhibition of isoprenaline stimulated accumulation of cyclic AMP in rat aortic cells by neuropeptide Y, at concentrations in excess of approximately 5 μM, is mediated by a non-receptor-mediated pathway.[136] This was proposed on the basis that this action of neuropeptide Y at high concentrations results from an alteration of the cell membrane bilayer structure.[136]

EFFECTS ON THE HEART

Localisation

The hearts of several species such as dog, man, cat, guinea pig, rat, and mouse have been shown to contain high levels of neuropeptide Y immunoreactivity.[2] The distribution of neuropeptide Y has been characterised most extensively in the heart of the guinea pig in which the peptide was identified in all regions of the organ.[65 69] Highest concentrations of neuropeptide Y immunoreactivity were found in nerve fibres around coronary vessels and in the atria, particularly near the sinus and atrioventricular node conductive tissues, and in the endocardial layer. The peptide is also observed in rat,[137 138] cat, pig,[139] and human hearts.[64 140–142] Neuropeptide Y immunoreactive nerve fibres are distributed throughout the human myocardium, being more abundant in the atria than in the ventricles, and are concentrated around small arteries and arterioles at the adventitial-medial border.[140] Neuropeptide Y immunoreactive nerves in the adult human heart are similar numerically and in their pattern of distribution to tyrosine hydroxylase immunoreactive nerves.[141 142] The loss of these nerves in transplanted human tissue indicates that they are of extrinsic origin.

In the heart, the distribution and nature of nerve fibres containing neuropeptide Y seem to vary with age and mammalian species. In six week old rats, average concentrations of neuropeptide Y were over three times higher than in four month old, that is, adult rats.[143] About half the neuropeptide Y in the heart of the adult rat is not co-stored with noradrenaline in sympathetic nerves, but contained in intrinsic myocardial neurones.[143–145] In the guinea pig, these intrinsic non-sympathetic neurones seem to be rare or absent and most of the neuropeptide Y is stored in adrenergic neurones.[65 83 146] In man, the existence of intrinsic neuropeptide-Y-containing neurones in the heart has not been established. Intrinsic neuronal cell bodies in the adult human heart appear to lack neuropeptide Y immunoreactivity,[140] but this has been identified in a small proportion of intracardiac neurones of human fetal origin in culture.[141] The anatomical projections and the functional role of intrinsic neuropeptide-Y-containing neurones are still obscure. In investigations of the cardiac septum in chemically sympathectomised rats, no neuropeptide-Y-containing fibres were found around the large septal coronary artery, whereas these were present around smaller arteries.[143]

In summary, nerve fibres containing neuropeptide Y extend throughout all regions and tissues of the heart. It seems possible, therefore, that neuropeptide Y released from these fibres exerts functional effects on all parts of the heart. At the ultrastructural level, it has been demonstrated that neuropeptide Y immunoreactivity is localised in large

granular vesicles in sympathetic nerve terminals.[140] As such, neuropeptide Y and noradrenaline have distinct subcellular compartments and exhibit differential release, in which case the peptide is preferentially released at high frequencies of stimulation.[147]

Inotropic and chronotropic effects

The documented actions of neuropeptide Y on cardiac contraction vary depending on the species and preparation of tissue used. In studies using whole isolated heart from rabbit,[61] guinea pig,[89 123] and rat[148] perfused at constant pressure, infusion of neuropeptide Y reduced contractile force and blood flow. Others failed to demonstrate inotropic affects of neuropeptide Y in papillary muscles from cat, guinea pig,[83] and rat.[28 83] In isolated atria or strips of atrial tissue, neuropeptide Y showed negative inotropic effects in dog[149] and rat,[148] but positive effects in guinea pig.[123 125] Isolated right atrial appendages from human hearts were unresponsive to neuropeptide Y.[149]

The significance of these results is, however, often questionable. This is because, in studies conducted using whole perfused hearts, the secondary contractile effects of ischaemia caused by the strong vasoconstrictor action of neuropeptide Y[61 89 123 127] cannot be excluded. Also, in myocardial strip preparations, inadequate diffusion may bias the results, such that the concentration of the peptide at molecular sites of action and therefore the inotropic response would be diminished. In all tissue preparations, release of transmitters from contained nerve endings cannot be excluded, and this may also modify the result. In addition, the inotropic state of the preparation may determine whether a particular effect is manifest, such that a positive response might not be observed when the level of stimulation is high, or vice versa.

In recent studies the direct action of neuropeptide Y on the myocardial cells has been investigated. Adult ventricular cardiomyocytes have been used to avoid the problem of secondary effects. At the cellular level, the contractile effect of neuropeptide Y was predominantly inhibitory in the rat heart[43 62] but both inhibitory[56] and stimulatory effects[43] have been observed in the guinea pig heart.

Postjunctional chronotropic effects of neuropeptide Y have been studied in isolated spontaneously beating hearts. In isolated preparations of the spontaneously beating atrium from the guinea pig, some investigators found a positive chronotropic effect, shown by an increase in the spontaneous beating frequency.[125 150] In another preparation of the guinea pig heart,[68 113] and in hearts from dog,[130 149] cat,[83] rabbit,[61] and rat,[83 148 151] neuropeptide Y did not influence spontaneous beating frequency. Neuropeptide Y can, however, exert presynaptic effects on chronotropy, as it has inhibitory actions both on noradrenaline release from sympathetic nerve endings and on acetylcholine release from parasympathetic nerve endings. Theoretically, therefore, neuropeptide Y could have either a predominantly negative or a predominantly positive chronotropic effect, depending on the relative activities of the opposing innervations. In innervated heart preparations the release of endogenous neuropeptide Y, coupled to the release of noradrenaline from sympathetic nerves, has either no effect or a positive chronotropic effect, based on vagal inhibition.[129 152 153]

Signal transduction in heart cells

In cardiomyocyte cultures derived from atrial tissue of the rat, neuropeptide Y was found to inhibit isoprenaline stimulated adenylate cyclase activity,[51] indicating that it has an antiadrenergic effect as demonstrated in cardiac myocytes from ventricular tissue of the rat.[41] In both cases, the functional antagonism was effected through an inhibitory G protein. In neonatal rat ventricular cardiomyocytes, however, neuropeptide Y was found to exert a dual action on modulation of levels of cAMP.[54] Following stimulation of cAMP levels in neonatal rat ventricular myocytes by noradrenaline, forskolin, or cholera toxin, neuropeptide Y attenuated the cAMP response, in each case through a pertussis toxin sensitive mechanism.

The effect of neuropeptide Y on contracting adult rat cardiomyocytes in the presence of isoprenaline is inhibitory and mediated through a receptor linked to G_i protein.[62] Furthermore, the negative effect of neuropeptide Y can be completely abolished by blocking the transient outward current using 4-aminopyridine.[43] The mechanism by which neuropeptide Y exerts a negative contractile effect on isoprenaline stimulated cardiomyocytes from the rat differs from that of other agents which act similarly to reduce contraction through G_i protein linked pathways. For example, the action of the selective agonist of A_1 adenosine receptors, R-phenylisopropyladenosine, is not influenced by 4-aminopyridine, and the inhibitor has only a partial effect on the response to oxotremorine, the non-selective agonist of muscarinic cholinoceptors.

In rat ventricular cardiomyocytes, a positive effect of neuropeptide Y can be unmasked in the presence of 4-aminopyridine (fig 2A). This positive effect can be abolished using verapamil, but not by pertussis toxin.[43] In the cited study from our group, we found that in ventricular cells from the guinea pig heart, which lacks the transient outward current, neuropeptide Y induces a positive contractile response, consistent with only the L-type Ca^{2+} channel being activated.[43] The results obtained using guinea pig cardiomyocytes are in contrast to those reported by Bryant *et al*[56] for cardiomyocytes from the same species. In their preparation of cells, neuropeptide Y reduced the contractile response both in the absence of and in the presence of isoprenaline, and the L-type Ca^{2+} current was decreased in the presence of neuropeptide Y. The variance in the results obtained using isolated ventricular cardiomyocytes from guinea pigs may be explained by the use of different experimental conditions. The only obvious distinction between the two studies is that the negative contractile effects were observed at high concentrations of neuropeptide Y (10^{-6}-10^{-4} M),[56] whereas the positive contractile effect was reported for a lower concentration (10^{-7} M).[43]

In summary, there is general support for the hypothesis that neuropeptide Y exerts its inhibitory postjunctional actions in the heart through G_i proteins. There is now also evidence for postjunctional mechanisms which are not linked to pertussis toxin sensitive G proteins, which can be inhibitory[136] or stimulatory.[43]

Possible physiological and pathophysiological role of neuropeptide Y in the cardiovascular system in vivo

VASCULAR EFFECTS

The ubiquity and strength of the local effects of neuropeptide Y in the cardiovascular system indicate a physiological function in the peripheral regulation of blood pressure. Systemically administered neuropeptide Y causes increased arterial blood pressure and reduced cardiac output. The pressor response is resistant to α and β adrenoceptor blockade,[72 125 154 155] but can be reversed by the Ca^{2+} channel blocking agent, nifedipine.[128] This systemic pressor effect of

exogenous neuropeptide Y is less potent but longer lasting than that of noradrenaline and angiotensin II when compared at equimolar concentrations.[156] Since the onset of action is slow, the difference in potency may only be apparent, caused by differences in diffusion. It is not clear, however, whether experiments with systemic application of neuropeptide Y have any physiological significance, since in the neurohormonal control of the cardiovascular system, circulating neuropeptide Y may be of only minor importance. In normal human subjects, plasma concentrations of neuropeptide Y are low, on average 20 pmol·litre^{-1} measured using a radioimmunoassay.[157 158] Physical exercise, for example, a physiological condition of sympathetic activation, raised these levels to 80 pmol·litre^{-1}. Increased levels of plasma neuropeptide Y have been reported for a variety of cardiovascular disease states, including hypertension, angina pectoris, myocardial infarction, and heart failure.[159–161] A causal role of circulating neuropeptide Y in these disease states has not yet been identified. In most patients with increased plasma concentrations of neuropeptide Y, these did not exceed 100 pmol·litre^{-1}.[161] In vitro such concentrations have only weak if any vasoconstrictor effects, since the EC_{50} values are in the nanomolar range. In fact, a 30-fold increase of plasma neuropeptide Y through intravenous neuropeptide Y infusion did not produce any detectable cardiovascular effects in healthy volunteers.[98] The most consistent finding regarding neuropeptide Y levels in cardiovascular disease states seems to be a correlation between plasma concentrations of the peptide and the severity of left heart failure.[161]

The cause for an increase in plasma neuropeptide Y in patients with cardiovascular disease seems to be predominantly the spillover from neuronal release. It has been suggested that increased neuronal transmitter release may be the cause of the depletion of neuropeptide Y and noradrenaline in failing human myocardium.[162] However, a strict correlation between plasma noradrenaline and neuropeptide Y concentrations in patients with heart failure was not found.[161] This could be due to the difference between the half lives of neuropeptide Y and noradrenaline. At levels of neuropeptide Y below 100 pmol·litre^{-1}, the half life of the peptide was determined as 20 min, in contrast to 1-2 min for noradrenaline.[158] Another reason may be that neuropeptide Y is also released from non-sympathetic sources.

According to present knowledge, a pathogenic role of raised plasma levels of neuropeptide Y cannot be assumed. It has in fact been argued that the pathophysiological role of increased systemic levels of neuropeptide Y may even be a beneficial one. It has been shown that neuropeptide Y can interfere with the renin-angiotensin system,[152] which plays a major role in the progression of heart failure.[163] In the isolated rat kidney, neuropeptide Y inhibits renin release by a mechanism not dependent on intrarenal haemodynamics.[164] This effect has been used to explain the fact that continuous infusion of neuropeptide Y in the rat can suppress the rise in plasma renin levels and blood pressure in response to unilateral renal artery clipping.[165] In rats, neuropeptide Y was also found to increase plasma levels of atrial natriuretic factor, which antagonises the effect on blood pressure of activation of the renin-angiotensin system.[166] Even though extrapolation of these results to human pathophysiology is at present hypothetical, it seems conceivable that raised plasma levels of neuropeptide Y may actually be beneficial for the heart.

Apart from its role on vasomotion, neuropeptide Y may also exert other effects in the intraluminal vascular space. It is stored in blood platelets and released from them during platelet aggregation.[167] One may speculate that this release plays a vital role in haemostasis due to the local vasoconstrictive effect of neuropeptide Y, supporting the action of thromboxane. While this is a possible acute intraluminal effect of neuropeptide Y, exposure of vascular endothelial cells to neuropeptide Y for several hours has been shown to upregulate adhesiveness of endothelial cells for leucocytes[168] and, in other experiments, endothelial production of prostacyclin.[114] So far these effects are single observations, awaiting evaluation of the vascular effects of neuropeptide Y in a larger scheme. Many peptide hormones have been shown to possess growth factor properties, stimulating phenotypic changes or proliferation of their target cells during long term exposures. Vasoconstrictors like angiotensin II[169] or endothelin I[170] stimulate growth or proliferation of smooth muscle cells in vitro. A common denominator in the actions of these agonists is the increase of cytosolic levels of Ca^{2+}. In many cell types, including smooth muscle cells, increased cytosolic Ca^{2+} levels can lead to the activation of protein kinase C which then initiates a change in transcriptional regulation. One may hypothesise, therefore, that neuropeptide Y acts also as a growth factor on the vessel wall.

CARDIAC EFFECTS

Cardiovascular effects of normal and pathologically raised plasma levels of neuropeptide Y on cardiac function are uncertain, since these levels remain in the subnanomolar range. In mammalian hearts perfused with neuropeptide Y in vitro, subnanomolar concentrations are nearly ineffective. High concentrations infused into the coronary arteries cause coronary spasm in man, but these concentrations may not be physiologically relevant. It seems possible that continuous administration of low levels of neuropeptide Y may alter the cellular responsiveness to other stimuli. It was shown recently by Sun *et al*[171] that chronic conditioning of cardiomyocytes from postnatal rat in culture with low concentrations of neuropeptide Y altered their response to the α adrenoceptor agonist, phenylephrine, from positive to negative chronotropy. This conditioning effect of neuropeptide Y was already maximal at nanomolar concentration. The results suggest that neuropeptide Y can alter the phenotypic differentiation of the myocardial cell. A growth factor function of neuropeptide Y may thus also be discussed for the myocardial cell. Interestingly, it has recently been reported that ventricular cardiomyocytes isolated from adult rat contain neuropeptide Y immunoreactive material.[80] Also, neuropeptide Y immunoreactive material and NPY-mRNA have been identified in primary cultures of atrial cardiomyocytes from neonatal rats.[79]

Experiments using isolated ventricular cardiomyocytes from rat and guinea pig have unequivocally demonstrated acute and direct effects of neuropeptide Y on the ventricular cardiomyocyte. As neuropeptide Y seems to exert multiple effects on ion channels in cardiomyocytes and as these differ between species, the overall effect of neuropeptide Y on cardiomyocytes could be one of positive or negative inotropy, and it is also conceivable that the net effect is nil. The contractile response to neuropeptide Y of human ventricular cardiomyocytes is not yet known. It is a yet unanswered question whether neuropeptide Y directly influences the activity of cardiac pacemaker tissues. It has been shown histochemically that the sinus node region is well supplied with nerve fibres containing neuropeptide Y.[64 65 139] Experiments using isolated pacemaker structures have not yet been reported and therefore one may still doubt

whether the failure in many studies to demonstrate direct chronotropic effects on denervated heart preparations represents true results. An indirect chronotropic effect of neuropeptide Y has been demonstrated in the innervated canine heart in situ.[129-131] The vagal actions on heart rate were attenuated after brief periods of intense sympathetic stimulation. These effects were long lasting, not inhibited by adrenergic receptor blockade, decayed in parallel with overflow of neuropeptide Y from the heart, and could be mimicked by exogenous neuropeptide Y.

Little is known about the pathophysiological role of neuropeptide Y in the heart. As in other sympathetically innervated organs, neuropeptide Y is stored in the heart in adrenergic nerve endings and is released from these together with noradrenaline upon sympathetic nerve stimulation by an exocytotic mechanism.[122] Under energy depleting conditions such as anoxia and ischaemia, the energy dependent mechanism of exocytosis of transmitter-containing vesicles is impaired. Then noradrenaline may be released by a non-exocytotic mechanism, but not neuropeptide Y.[124 172] This non-exocytotic release of noradrenaline is not modulated by presynaptic receptor agonists.[173] It seems possible, therefore, that local release of neuropeptide Y in the heart contributes to the initiation of ischaemia by provoking vascular spasm, but within the ischaemic area a major role for neuropeptide Y is unlikley. In acute myocardial infarction, plasma levels of catecholamines and frequently of neuropeptide Y[161] are increased, due to reflex activation of the sympathetic system. It has frequently been hypothesised that the raised catecholamine levels may cause arrhythmias. The levels of circulating catecholamines and neuropeptide Y do not normally exceed those reached by physical exercise and therefore an arrhythmogenic effect of these levels per se seems unlikely.[161 173]

An interesting question about the role of intrinsic non-sympathetic neuropeptide-Y-containing neurones in the heart has recently been highlighted by Corr *et al.*[143] These investigators speculated that neuropeptide Y from such neurones may contribute to coronary spasm in denervated transplanted hearts. In a rat model, they showed that chemical sympathectomy does not eradicate this population of neurones. These neurones seem to project to small arteries, in which spasm is observed in transplanted hearts, rather than to conduit vessels. Whether the human heart contains a sufficient number of such intrinsic neuropeptide-Y-containing neurones is at present not known.

In conclusion, several local postjunctional effects of neuropeptide Y on vasomotion and isolated cardiac cell function have been identified. Vascular effects promote constriction, but are not homogeneous in mechanism. There are direct constrictor, potentiating, and possibly endothelium dependent effects which vary according to vascular regions and species. In contrast to such local effects, systemic effects of circulating neuropeptide Y are uncertain. An increase in plasma neuropeptide Y in various cardiovascular disease states seems in most cases to be due to an increased sympathetic drive leading to spillover of neuropeptide Y into the circulation. It seems improbable that the increased plasma levels of neuropeptide Y observed under such pathological circumstances are sufficient to influence haemodynamics. They may be sufficient, however, to interfere with other hormonal control systems, for example the renin-angiotensin system, or to exert trophic effects on vascular and myocardial cells. At the present time, the role of neuropeptide Y in the cardiovascular system is only partially understood.

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Key terms: neuropeptide Y; cardiovascular system; heart, cardiomyocytes; smooth muscle; endothelial cells; pathophysiology; prejunctional action; postjunctional action.

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