Peripheral cardiac sympathetic hyperactivity in cardiovascular disease: role of neuropeptides

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Shanks J, Herring N. Peripheral cardiac sympathetic hyperactivity in cardiovascular disease: role of neuropeptides. *Am J Physiol Regul Integr Comp Physiol* 305: R1411–R1420, 2013. First published September 4, 2013; doi:10.1152/ajpregu.00118.2013.—High levels of sympathetic drive in several cardiovascular diseases including postmyocardial infarction, chronic congestive heart failure and hypertension are reinforced through dysregulation of afferent input and central integration of autonomic balance. However, recent evidence suggests that a significant component of sympathetic hyperactivity may also reside peripherally at the level of the postganglionic neuron. This has been studied in depth using the spontaneously hypertensive rat, an animal model of genetic essential hypertension, where larger neuronal calcium transients, increased release and impaired reuptake of norepinephrine in neurons of the stellate ganglia lead to a significant tachycardia even before hypertension has developed. The release of additional sympathetic cotransmitters during high levels of sympathetic drive can also have deleterious consequences for peripheral cardiac parasympathetic neurotransmission even in the presence of β-adrenergic blockade. Stimulation of the cardiac vagus reduces heart rate, lowers myocardial oxygen demand, improves coronary blood flow, and independently raises ventricular fibrillation threshold. Recent data demonstrates a direct action of the sympathetic cotransmitters neuropeptide Y (NPY) and galanin on the ability of the vagus to release acetylcholine and control heart rate. Moreover, there is a strong correlation between plasma NPY levels and coronary microvascular function in patients with ST-elevation myocardial infarction being treated with primary percutaneous coronary intervention. Antagonists of the NPY receptors Y₁ and Y₂ may be therapeutically beneficial both acutely during myocardial infarction and also during chronic heart failure and hypertension. Such medications would be expected to act synergistically with β-blockers and implantable vagus nerve stimulators to improve patient outcome.

autonomic nervous system; cardiac; hypertension; myocardial infarction; neuropeptide Y

THE “TEXTBOOK” VIEW of cardiovascular control by the autonomic nervous system is that brain stem autonomic outflow merely responds to changes in afferent feedback from peripheral arterial, cardiopulmonary and renal baro- and chemoreceptors. The sympathetic and parasympathetic efferent output therefore act to oppose changes in circulatory disturbance and maintain adequate arterial blood pressure and perfusion of organ systems. It is becoming increasingly apparent that this system is not “hard-wired,” and integration and plasticity exists at many anatomical sites in the cardiac-vascular-renal neural axis (42). In this article we review recent evidence that a significant component of the sympathetic hyperactivity associated with hypertension arises at the level of the postganglionic neuron, and that cotransmitters released during high levels of sympathetic drive can cross talk and impair vagal neurotransmission and control of heart rate and coronary blood flow.

Importance of Autonomic Balance in Cardiovascular Disease

High cardiac sympathetic drive promotes myocyte calcium influx, increases the inotropic state of the heart, and increases myocardial oxygen demand. This can exacerbate the harmful effects of preexisting cardiac ischemia and precipitate life-threatening ventricular arrhythmias (63, 79). This is particularly prevalent when there is an abnormal structural phenotype to support reentrant pathways, such as an ischemic or dilated cardiomyopathy, hypertrophic cardiomyopathy, or arrhythmogenic right ventricular cardiomyopathy (121). High sympa-
Peripheral Sympathetic Hyperactivity Precedes the Development of Hypertension in the Spontaneously Hypertensive Rat

The distribution of blood pressure in the human population is continuous and negatively skewed without a clear bimodal pattern to help define a subset of hypertensive patients. There is evidence linking impairment of the kidney, vasculature, and the autonomic nervous system in many patients with higher blood pressure who we define as being hypertensive. In addition clinically severe hypertension also results in renal and vascular end-organ damage thereby exacerbating the condition. Increased sympathetic activity and reduced vagal tone are now well established as major contributing factors to the pathophysiology of human hypertension (2, 24, 34, 36, 95, 99) and that observed in the SHR (17, 38, 57, 59, 61). Hypertension is associated with increased muscle sympathetic nerve activity (2, 36), increased levels of plasma norepinephrine (NE) (31, 95), and increased renal NE spillover (30, 95). Evidence for sympathetic hyperactivity has also been inferred from spectral analysis of heart rate variability in borderline and hypertensive patients where a linear correlation with blood pressure has been demonstrated (64). Increased muscle sympathetic nerve activity in response to mental stress has also been documented within normotensive offspring from families with a history of hypertension (78). This suggests that sympathetic activation may be an early marker of hypertension in those who are genetically predisposed to the disease.

In a recent series of papers we studied the SHR at 16 wk of age, when hypertension is well established and the animal is tachycardic compared with its Wistar-Kyoto (WKY) control (40), and also at 4 wk of age when it is phenotypically normotensive with no evidence of left ventricular hypertrophy (59). There are multiple methods of measuring blood pressure in rats at 4 wk of age including tail-cuff, radiotelemetry, and implanted devices with most previous studies investigating the 4-wk-old SHR consistently finding them to be normotensive (49, 50, 109). It is also worth noting that the SHR is often used as a model of attention deficit hyperactivity disorders, which may complicate these measurements. We therefore have also measured blood pressure at rest under general anesthesia with...
a large sample number (SHR, \( n = 18 \); WKY, \( n = 20 \)) to confirm these findings (103).

**Dysregulation of intracellular calcium handling in SHR cardiac sympathetic neurons.** Studies on cultured sympathetic neurons from the stellate ganglion and superior cervical ganglion from the SHR at 16 wk of age have demonstrated that the depolarization-induced rise in intracellular free \( \text{Ca}^{2+} \), measured using fura-2-acetoxymethyl ester, is significantly larger and the decay of the \( \text{Ca}^{2+} \) transient also faster in the SHR compared with age-matched WKY controls (59) (see Fig. 1A). The in vitro analysis of calcium handling by cultured stellate ganglion cells is used as a surrogate for the behavior of terminal varicosities in vivo. Endoplasmic reticulum (ER) \( \text{Ca}^{2+} \) content and caffeine-induced \( \text{Ca}^{2+} \) release were significantly higher in the SHR, with lower protein levels of phospholamban and increased ryanodine receptor mRNA (59). A lower mitochondrial membrane potential was also observed, resulting in impaired mitochondrial \( \text{Ca}^{2+} \) uptake and release (59). Depletion of ER \( \text{Ca}^{2+} \) stores with caffeine and thapsigargin (to prevent SERCA \( \text{Ca}^{2+} \) uptake and release), did not alter the differences of evoked \( \text{Ca}^{2+} \) transient and decay time observed, whereas removing mitochondrial buffering did (59). Interestingly this calcium handling phenotype is also seen in stellate and superior cervical neurons of the SHR before the development of hypertension (in both neonates and at 4 wk of age) (59). A rise in intracellular \( \text{Ca}^{2+} \) in response to cellular depolarization also stimulates the exocytotic vesicular release of NE in sympathetic neurons (16, 90). Cardiac NE release from the atria can be measured using a \(^3\)H-labeling technique (40). Significantly higher \([\text{H}]\)NE release is observed in the SHR compared with age-

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**Fig. 1.** The peripheral cardiac sympathetic phenotype of the spontaneously hypertensive rat (SHR). **A:** calcium transients (measured using Fura 2-AM) within cultured neurons from the stellate ganglia in response to cellular depolarization (100 mmol/l KCl) are larger in the SHR compared with age-matched Wistar-Kyoto (WKY) controls. This can be observed at 4 wk of age when the SHR is normotensive and without left ventricular hypertrophy and when hypertension is well established at 16 wk of age [modified from Li et al. (60) with permission]. **B:** increased release of \([\text{H}]\)norepinephrine (NE) from isolated atria during stimulation at 5 Hz is also observed within the SHR compared with WKY at both developmental stages [from Shanks et al. (103) and republished from Herring et al. (41) with permission from Elsevier]. **C:** a reduction in the activity of the NE transporter (NET), slowing the rate at which NE is cleared from the synaptic cleft is observed in the SHR at both 4 and 16 wk of age. NET activity is measured using a novel fluorescent assay in cultured neurons from the stellate ganglia. Desipramine (DMI) can block all fluorescence increase confirming uptake specificity to NET [from Shanks et al. (101)]. **D:** heart rate response to stimulation of the right stellate ganglia in isolated atrial preparations is significantly greater in the SHR compared with age matched WKY at both 16 wk (1–7 Hz) and 4 wk of age (5 and 7 Hz) [from Shanks et al. (103) and republished from Herring et al. (41) with permission from Elsevier].
Impairment of NE reuptake transporter in SHR cardiac sympathetic neurons. In addition to the neuronal calcium signal, the synaptic NE concentration is also regulated by the kinetics of NE degradation and removal from the cleft. The majority of released NE (>90%) is taken up into the sympathetic nerve terminal by the NE reuptake transporter (NET), with less than 5% being metabolized within the extracellular space (93). The increased concentration of cardiac NE release observed within the prehypertensive SHR might also be due to impairment of the NET. Previous studies into the role of NET in hypertension have produced inconclusive or conflicting results. In human hypertension the putative role of NET has been reported to be either reduced (31, 91, 95) or unaltered (47). This apparent disparity is likely to be due to the indirect methods used to measure NET, including traditional radioisotope overflow measurements (68), and the diverse range of tissues studied (15, 68). A novel fluorescent assay has been recently developed to temporally monitor NET dynamics within intact tissue and isolated sympathetic nerve preparations (80, 101). The technique uses a fluorescent NET substrate in a masking dye solution. When the substrate is transported intracellularly by NET and separates from the masking dye, the rate of increase of intracellular fluorescence then directly reflects the activity of NET (Fig. 1C). Using this assay, we have recently demonstrated impaired NET activity in cultured sympathetic neurons from the cardiac stellate in both the depolarized and resting state. This could be observed both in hypertensive and prehypertensive SHRs compared with age-matched WKY (101). No difference in NET activity was observed within sympathetic neurons isolated from the superior cervical ganglia, which predominantly innervates the head and neck region (8), nor sympathetic neurons isolated from renal sympathetic ganglia (101). Interestingly, the difference in [3H]NE release from isolated atria between prehypertensive SHRs and WKYs could be normalized using the NET inhibitor desipramine (102).

NE release can also be autoinhibited by presynaptic α2-adrenoreceptors (α2-AR) as part of a negative feedback mechanism. In the adult SHR, expression of α2B-AR mRNA assessed by RT-PCR is increased in the stellate ganglion, and inhibition of these receptors with yohimbine produces a greater increase in cardiac [3H]NE release in the adult WKY compared with the SHR (123). However, at 4 wk of age cardiac [3H]NE release remains significantly greater than the WKY (102) suggesting that autoinhibition does not contribute to increased sympathetic neurotransmission before the onset of hypertension.

The increased sympathetic neurotransmission in the SHR also appears to be functionally significant in terms of changes in heart rate. In isolated atrial preparations with the right stellate ganglion still intact, the tachycardic response to direct stimulation of the stellate is significantly greater in the 16-wk SHR compared with WKY control at a range of stimulation frequencies (40), and this is also observed at 4 wk of age at higher stimulation frequencies despite the heart rate response to exogenous NE being no different from the WKY (102) (Fig. 1D). Interestingly a small, but significant, resting tachycardia can also be measured in vivo in the SHR under general anesthesia at this developmental stage and others have confirmed this using radioisometry data (50). Despite this the intrinsic rate of the isolated hearts is no different between 4-wk-old SHRs and WKYs reinforcing the idea that dysregulation of the sympathetic nervous system may be one of the early hallmarks of the hypertensive phenotype (103).

Indirect Consequences of Sympathetic Hyperactivity: Sympathovagal Cross Talk

The idea that nerve cells may release more than one neurotransmitter with distinct postsynaptic targets and actions was postulated by Burnstock in 1976 (12). This went against the traditional view based on Dale’s principle that nerves of the same class only utilize one neurotransmitter (107). To date cotransmitters have been identified within most major nerve populations both centrally and peripherally. However, the role of different cotransmitters varies greatly between different species, disease, and developmental state (13). The release of cotransmitters is highly dependent on the level of neuronal activity and they are usually slowly diffusing molecules. Cotransmitters and their metabolites often have a longer biological half-life than the main neurotransmitter, although there are notable exceptions such as ATP (9).

Cross talk between different neuronal populations by cotransmitters has been extensively studied within the central nervous system and identified as an important modulator of neural activity (10, 14, 54, 70, 119). However, increasing evidence is emerging that cross talk between neuronal populations in the heart is also important in autonomic control.

The phenomenon that high levels of cardiac sympathetic stimulation is coupled to a subsequent long-lasting inhibition of cardiac vagal function in vivo was first observed by Potter as early as 1982 (84, 87). Interestingly, this phenomenon is only observed in vitro with direct stimulation of the sympathetic and vagus nerves rather than replacing neuronal stimulation with exogenous application of one or both of the respective neurotransmitters (39) (Fig. 2C). This suggests that the phenomenon is dependent on interneuronal signaling rather than convergence at the level of myocyte second messenger pathways. High sympathetic drive and reduced vagal tone is the characteristic phenotype associated with congestive heart failure and myocardial infarction. La Rovere in 1998 commented “…in ischemic patients a reduction in vagal activity, which is almost always accompanied by a concomitant increase in sympathetic activity, is sufficient to facilitate cardiac death by several mechanisms” (53). This may occur at least in part due to the release of sympathetic cotransmitters acting locally on cardiac cholinergic nerves to reduce acetylcholine release, in a process known as “sympathovagal cross talk” (43).

Sympathetic nerves throughout the autonomic nervous system contain cotransmitters such as ATP, neuropeptide Y (NPY), and galanin in addition to NE (62) (39, 52, 115). NE itself is not thought to regulate cardiac vagal acetylcholine release within humans or guinea pigs (67, 98), although there is evidence of a short-lived inhibition on acetylcholine release due to NE within rat atria via α-adrenoceptors (118). ATP also appears to be unable to alter acetylcholine release in the human or guinea pig heart (67, 98).

Levels of sympathetic stimulation identical to those that impair vagal function have also been shown to release NPY into the perfusate of isolated atria in vitro (39) (Fig. 2A) as well
as into coronary sinus blood in vivo (116). The release of galanin can also be detected in vitro but at lower levels than that observed for NPY (39). Evidence for galanin release as a cotransmitter under conditions of high sympathetic stimulation has also been reported within the liver and the gut (7, 51) Antagonists of the Y2 receptor and GALR1 can reverse the inhibition of vagal bradycardia inhibition postsympathetic stimulation in vitro (39) and in vivo (104) as can genetic knockout of these receptors in vivo (88, 105, 106). Immunohistochemical labeling has localized the NPY Y2 receptor (41) as well as the galanin GalR1 receptor (39) to vagal neurons in the right atria and around the sinoatrial node providing the anatomical basis for the sympathovagal cross-talk model.

We have recently shown that both exogenous NPY and galanin can reduce the heart rate response to vagus nerve stimulation in vitro, while being unable to alter the bradycardic response to the stable analogue to acetylcholine, carbachololine (39, 41). At the same concentration range both NPY and galanin can also reduce [3H]acetylcholine release (Fig. 2B). Intravenous administration of NPY and galanin are also able reduce the magnitude of the vagal bradycardia in vivo, but these experiments may be complicated by potential changes in haemodynamics and circulating factors (86, 106, 114). In our atrial preparation with intact right vagus nerve, inhibitors of protein kinase C completely abolish the action of both NPY and galanin, while protein kinase A inhibitors have no effects. Conversely, a protein kinase C activator can mimic the effects of these cotransmitters and reduce [3H]acetylcholine release (39). The exact mechanisms linking GALR1 and Y2 receptor binding, protein kinase C signaling, and the release of acetylcholine have not yet been elucidated.

Galanin is a 29/30 amino acid neuropeptide, first isolated from the porcine gut (111), while NPY is a 36-amino acid peptide that was first isolated from the porcine brain (110). NPY within the heart is the most abundant neuropeptide and was first identified in intramural sympathetic nerves in close association with myocytes and coronary blood vessels (1). In addition to its action as a neuromodulator of parasympathetic control of heart rate, significant evidence points toward a role for NPY in controlling vascular function during the development and maintenance of hypertension (69, 117). NPY has also been implicated in the development and progression of left ventricular hypertrophy (LVH), as the peptide is a hypertrophic factor (69) with increased plasma levels of the neuropeptide during hypertension correlating to the severity of LVH (28, 44).

Indirect Consequences of Sympathetic Hyperactivity: Sympathovascular Cross Talk

Independent of changes in heart rate, the cardiac vagus nerve can also increase myocardial perfusion (55, 89) and improve microcirculatory flow in patients with coronary artery disease (120). During conditions of high sympathetic drive such as during myocardial infarction, it is therefore conceivable that cotransmitter release could impair parasympathetic mediated vasodilation and exacerbate on-going ischemia. Several sympathetic cotransmitters are also directly vasoactive. ATP re-
leased either as a cotransmitter from sympathetic nerves or from the vascular lumen in response to changes in blood flow or hypoxic stress can dilate or contract vessels (11). Within the vascular endothelium it is thought to predominantly act through the P2Y1 and P2Y2, Gq/11-coupled receptors, although the half-life of ATP is short and sympathetic released ATP is not thought to have a significant effect on the coronary microcirculation (29). While the breakdown product of ATP, adenosine, is a predominantly vasodilating metabolite, its half life is also short and the main action of sympathetic stimulation remains coronary vasoconstriction. NPY has a far longer half-life and intracoronary infusion causes microvascular constriction in humans and can precipitate angina and ischemic electrocardiographic changes (18). NPY can also constrict isolated human coronary arteries in vitro (112). This may be in part by inhibition of acetylcholine release through a Y2 receptor mechanism but could also occur via a direct action on vascular smooth muscle to cause vasoconstriction or by potentiating the actions of other vasoconstrictors such as NE, angiotensin II, serotonin, endothelin-1, and prostaglandins (27) via a Y1 receptor mechanism. Of note, the vasoconstrictive action of NPY as well as its potentiating effect on other vasoconstrictors is lost in the Y1 receptor knockout mouse (82). The evidence for NPY being as a potent vasoconstrictor released during sympathetic stimulation is reviewed in detail elsewhere (69).

NPY gene polymorphisms are linked to an increased risk of early-onset atherosclerosis (100), and NPY can influence angiogenesis and ventricular remodelling, effects linked to the Y2 and Y5 receptors (85, 124). Animal studies suggest that cardiac NPY is released from sympathetic nerves during experimentally induced myocardial infarction (37) and several studies published over 20 years ago demonstrate that plasma NPY levels are elevated following acute coronary syndromes and during left ventricular failure in humans where they correlate positively with the severity of heart failure and 1-yr mortality (44, 113).

![Epicardial coronary artery](image1)

Epicardial coronary artery

Sympathetic varicosity

Cardiac myocyte

**Angiographic reflow post PPCI**

- Angiographic No Reflow (n=16)
- Angiographic Reflow (n=48)

**Microvascular function post PPCI**

- Coronary Flow Reserve < 1.5 (n=32)
- Coronary Flow Reserve > 1.5 (n=32)

**ECG resolution post PPCI**

- No complete ST-Resolution (n=30)
- Complete ST-Resolution (n=34)

![Graphs](image2)

Fig. 3. Sympathovascular cross talk during ST-elevation myocardial infarction (STEMI) treated with primary percutaneous coronary intervention (PPCI): a role of neuropeptide-Y (NPY)? A: schematic diagram of an epicardial coronary artery stent, inserted during emergency PPCI treatment of a STEMI. Once the occluded epicardial coronary artery (white arrow, bottom left) has been opened and stented (shown in the angiogram in the bottom right), blood flow is not always completely restored. High plasma levels of the vasoconstrictor NPY can be detected pre and post-PPCI and for up to 48 h in those patients where angiographic reflow is not restored. B: high plasma NPY levels are also associated with low coronary flow reserve (<1.5) and a high index of microcirculatory resistance (>33) post-STEMI. C: ST elevation on the electrocardiogram (shown in bottom) remains despite opening and stenting of the epicardial coronary artery in those patients with higher plasma NPY levels at all time points post-PPCI [republished from Cuculi et al. (22) with permission from BMJ Publishing Group].

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Contemporary treatment of ST-elevation myocardial infarction (STEMI) involves emergency primary percutaneous coronary intervention (PPCI) to reopen the occluded epicardial coronary artery (Fig. 3A). Microrcirculatory “no-reflow” can be identified in nearly one-third of patients and correlates with persistent ST elevation, larger infarctions, and poor ejection fraction and prognosis (21, 22, 74). Distal atherothrombotic embolization from the ruptured plaque during PPCI may contribute to this phenomenon, but there is also an important functional vasoactive component that is poorly understood. Several possible candidates have been implicated including endothelin-1 (76), thromboxane A2 (75), and B-type natriuretic peptide (45). Working with collaborators, we have recently explored the possibility that NPY may play a key role in this important clinical entity (22).

In 64 patients presenting throughout the 24-h cycle of clinical activity to a University hospital heart center being treated with PPCI for STEMI, plasma NPY levels sampled from peripheral blood were significantly elevated in the first 6 h from presentation before plateauing but remaining elevated compared with the normal range. Before, immediately after PPCI, and at several time points during the first 48 h of presentation, plasma NPY levels were significantly higher in those patients with no angiographic reflow (Fig. 3A) and strongly correlated with coronary flow reserve and the index of microrcirculatory resistance measured using a coronary pressure wire and thermodilution techniques (Fig. 3B). Importantly, plasma NPY levels also correlated with the resolution of ST elevation (Fig. 3C) and the size of the infarct assessed by total troponin release (22). We did not directly measure cardiac NPY release via a coronary arterial/sinus concentration difference, because clinically this is difficult to obtain compared with a peripheral blood sample and can only be performed at the time of PCI limiting its utility. The strong correlations with plasma NPY levels we observe lead us to speculate that maintained microvascular vasoconstriction post-PPCI by NPY contributes to larger myocardial infarctions and that Y1 receptor antagonism may be beneficial at the time of revascularization in PPCI to improve angiographic reflow and prognosis.

Perspectives and Significance

High levels of sympathetic drive in several cardiovascular diseases including postmyocardial infarction, during chronic congestive heart failure, and hypertension are reinforced through dysregulation of afferent input and central integration of autonomic balance. However, recent evidence suggests that a significant component of sympathetic hyperactivity may also reside peripherally at the level of the postganglionic neuron. This has been studied in depth in the animal model of genetic hypertension. The spontaneously hypertensive rat, therefore be a useful therapeutic strategy both acutely during myocardial infarction and also during chronic heart failure and hypertension. Such medications would be expected to act synergistically with β-blockers and new technologies targeting efferent parasympathetic outflow such as implantable vagus nerve stimulators (97) to improve patient outcome.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Author contributions: J.S. and N.H. performed experiments; J.S. and N.H. analyzed data; J.S. and N.H. interpreted results of experiments; J.S. and N.H. prepared figures; J.S. and N.H. drafted manuscript; J.S. and N.H. edited and revised manuscript; J.S. and N.H. approved final version of manuscript; N.H. conception and design of research.

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