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Nitric Oxide Regulation of Autonomic Function in Heart Failure

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Abstract

Nitric oxide (NO) functions at all levels of the autonomic nervous system to influence sympathetic and parasympathetic control of cardiovascular function. It modulates the excitability of peripheral sensory and motor neurons of cardiovascular reflexes and of the central neurons that integrate their function. Its effects within this system are diverse and site specific, and at many levels, not well defined. Overall, however, most evidence suggests that the neuromodulator's influence acts to restrain sympathetic outflow and facilitate parasympathetic outflow. In chronic heart failure (CHF), these functional effects of NO are impaired or downregulated and contribute to the state of sympathetic over-activation and parasympathetic deactivation characterized by the disease. The cellular and molecular mechanisms regulating NO production and signaling in the autonomic nervous system in the normal and CHF state are summarized and discussed in light of therapeutic implications. This review serves to emphasize important questions of the regulation of NO function in the ANS that remain unresolved.

Introduction

Nitric oxide (NO) is well recognized as a modulator of synaptic excitability of many types of neurons. Nitric oxide signaling exerts significant effects on autonomic control of cardiovascular function and contributes to the dysfunctional state of the sympathetic and parasympathetic nervous system in several cardiovascular diseases including chronic heart failure (CHF) [1].

CHF is characterized by a chronic elevation in sympathetic outflow to the heart and vasculature and an impairment of vagal control of the heart. This change in autonomic 'tone' is a major contributor to arrhythmias and cardiac decompensation, hastening mortality in patients with CHF [1,2]. Numerous neurohumoral alterations have been documented in the development of heart failure that impact autonomic function [3]. These effects, however, are exacerbated by derangements of NO signaling that impact the afferent, central and efferent neural pathways of both sympathetic and parasympathetic function. This review summarizes our current state of knowledge of the role of NO in altered autonomic function in CHF.

Basic Mechanisms of NO Regulation and Signaling in the ANS

Nitric oxide is produced by three isoforms of nitric oxide synthase (NOS): NOS1 (neural), NOS2 (inducible), and NOS3 (endothelial). All three isoforms have been shown to influence autonomic neural function in some manner (Table 1). NOS positive autonomic neurons express mainly NOS1 but NOS3 and NOS2 immunostaining have been found in neurons and astrocytes in the brainstem [4,5]. NOS1 and NOS3 are under tight transcriptional control and their protein levels can be markedly altered in a variety of conditions including CHF. In autonomic nerves and most vascular beds, NOS1 and NOS3 are downregulated in CHF [6,7], but there are exceptions such as in cardiac tissue [8]. The cellular signaling pathways and transcription factors responsible for changes in NOS expression in CHF are not well defined. In particular,

the NOS1 gene, unlike NOS3 and NOS2, is an extremely large and complex locus, giving rise to numerous diverse cDNA sequences [9]. Some of the known variant mRNA transcripts result in truncated proteins whose function is not well understood [9].

Post-translational regulation of NOS activity is extensive, making NOS one of the most highly regulated enzymes known, and conferring diverse signaling mechanisms by which cells can rapidly control NO production [10]. Of foremost importance, dimerization of NOS protein and activation of enzyme activity requires calcium (Ca^{++}) activated calmodulin binding. In central neurons, NOS1 is commonly activated by post-synaptic NMDA receptor activation, which allows Ca^{++} influx [11]. NOS1 protein possesses an amino acid sequence at the N-terminal region that allows binding to specific post-synaptic density or scaffolding proteins, which binds NOS1 to NMDA receptors to facilitate NOS activation. Interruption of NOS1 scaffolding with the NMDA receptor reduces NOS activity [9,11]. However, this model can be modified to include activation of NOS via Ca^{++} influx from voltage gated Ca^{++} (Ca_v) channels and transient receptor potential channels through other neuro-excitatory synapses, or phosphorylation of NOS1, which can enhance activity independently of Ca^{++} .

All NOS isoforms require binding of co-factor tetrahydrobiopterin (BH4) to produce NO. NOS activation without proper BH4 binding uncouples normal electron transfer to produce superoxide anion ($\text{O}_2^{\bullet-}$) rather than NO. NO production can then be further compromised by its reaction with the $\text{O}_2^{\bullet-}$ to generate peroxynitrite. BH4 bioavailability is impaired in endothelial cells in vascular disease including CHF [12], but conditions that affect BH4 function in neurons are poorly understood. A number of other proteins can interact with NOS1 to negatively impact its enzyme activity. These include CAPON (C-terminal PDZ domain ligand of neuronal nitric oxide synthase), PMCA (plasma membrane calcium/calmodulin-dependent calcium ATPase) and PIN (protein inhibitor of NOS) [10]. The relevance of these various protein interactions with NOS function in autonomic neurons is not known.

NOS protein also can be phosphorylated / dephosphorylated at specific residues to alter activity. Phosphorylation of specific serine loci in NOS1 by kinases, such as serine/threonine protein kinase B (Akt), can either greatly enhance NO production and reduce Ca^{++} dependency of the enzyme or inhibit activity [13]. These phosphorylation sites on NOS1 confer another level of enzyme regulation via kinase and phosphatase pathways within autonomic neurons that, as yet, are not well defined.

The implications of substrate availability on NOS function in the brain may be under-appreciated. The enzymes for de novo synthesis of l-arginine are undetectable in most brain regions [14]. Thus, factors that control the transport of arginine into central neurons and the metabolic recycling of citrulline may play important roles in substrate availability and control of NOS activity. Indeed, application of l-arginine at discrete autonomic nuclei in the medulla and hypothalamus evokes NO-mediated effects on neuronal excitability [15]. Thus the availability of endogenous l-arginine may limit NOS activity and NO signaling in autonomic neurons. Furthermore, in the face of l-arginine depletion, NOS activation leads to uncoupled $\text{O}_2^{\bullet-}$ production rather than NO [14]. This issue, which is generally overlooked, may be particularly important in studies with experimental over-expression of NOS or in autonomic regions in which NOS2 is activated in CHF.

Alternate pathways for metabolism of arginine exist in brain tissue, producing natural competitive antagonists for NOS, such as agmatine, N(G)-monomethyl L-arginine, and asymmetric dimethylarginine (ADMA) [14]. Plasma ADMA is elevated in CHF and may contribute to impaired endothelial NOS3 function [16]. Astroglial cells are capable of producing substantial amounts of agmatine [14], but the functional significance of these peptide inhibitors on autonomic neural function is not yet known.

A host of other factors influence NO bioavailability independently of NOS activity. NO readily binds to heme groups of globins, in fact the term 'NO receptor' has been coined for the heme moiety of guanylyl cyclase, which exhibits very high affinity and selectivity for NO. Other cytoglobins, however, may serve to inactivate NO, and influence NO signaling. Alternatively, in pro-oxidant and impaired antioxidant conditions, NO bioavailability can be quenched due to the high reactivity of NO with $O_2^{\bullet}$. The pro-oxidant shift that occurs in CHF due to elevated cytokines and angiotensin II (angII) and activation of NADPH oxidase- $O_2^{\bullet}$ production contributes to a decrease in functional NO in autonomic areas of the brain [1,17].

NO signals a change in cellular function by binding to the NO receptor in guanylyl cyclase to activate cGMP production, which remains the only fully recognized physiological signal transduction mechanism for NO. In central neurons, cGMP then can have diverse effects on neuronal excitability. Cyclic GMP can directly bind to and modulate cyclic nucleotide-gated ion channels (e.g. HCN, hyperpolarizing cation channel), bind to phosphodiesterases to impair cAMP hydrolysis, or most prominently, activate PKG which can directly or indirectly lead to phosphorylation of effector proteins on ion channels [11]. Other potential signaling effects have been reported, such as s-nitrosylation of protein thiol groups, and inhibition of mitochondrial cytochrome c oxidase [11]. This effect of NO to increase mitochondrial $O_2^{\bullet}$ production has been shown to influence autonomic control of sympathetic function with overexpression of NOS in the brainstem [18].

NO generated at nerve synapses diffuses in an autocrine and paracrine fashion to influence both presynaptic and postsynaptic events on both excitatory and inhibitory synapses. These mechanisms of action include altering the activity of NMDA and GABA_A receptors, altering membrane excitability via voltage gated K^+ and Ca^{++} channels, and altering presynaptic release of neurotransmitters, among others [11]. These diverse signaling effects of NO allow it to either enhance or inhibit neuronal transmission at specific synapses and exert opposing functional effects. Many of the known influences of NO on autonomic neurons are summarized in Table 1.

NO and Autonomic Afferent Pathways in CHF

Alterations in afferent reflex pathways play an important role in autonomic dysfunction in CHF. These include blunted sympatho-inhibitory baroreceptor function and enhanced sympatho-excitatory function from cardiac, skeletal muscle, and arterial chemoafferent neurons [19–21]. A marked impairment of baroreflex control of heart rate and sympathetic outflow in CHF has been known for many years. At the peripheral level, sensory afferent baroreceptor neurons in the aorta and carotid sinus and in the heart fail to respond adequately to changes in pressure, and thus lessen their ability to tonically restrain sympathetic outflow [22,23].

The cellular mechanisms responsible for baroreceptor desensitization in CHF may involve activation of a Na/K ATPase [23], but a role for NO has not been explored. Alternatively, NO derived from NOS3 exerts an inhibitory influence on carotid sinus baroreceptor activity, resulting from the s-nitrosylation and subsequent inhibition of Na^+ currents in the afferent baroreceptor neuron [24]. However in CHF, NOS3 is downregulated in the major vessels, which might be expected to enhance baroreceptor sensitivity. NOS1-containing varicose nerve fibers innervate the wall of the carotid sinus [25], but their influence on baroreceptors is not known.

While sympatho-inhibitory baroreflexes are impaired in HF, sympathoexcitatory reflexes are enhanced. The sensitivities of cardiac sympathetic afferent endings to chemical mediators [19] and of the somatic afferent endings to muscle contraction [20] are enhanced in CHF.

However, a relation of NO to enhanced cardiac afferent discharge in CHF could not be demonstrated [19]. The role of NO in somatic afferent function is not well defined.

On the other hand, NO has been shown to play a prominent role in modulating the sensitivity of arterial chemoreceptors to hypoxia [21]. Chemoreceptors in the carotid body exhibit enhanced afferent discharge in normoxic and hypoxic conditions in CHF and contribute to the sympathetic activation and breathing disturbances of CHF [21]. The carotid body expresses an abundance of NOS3 and NOS1 within the dense network of capillaries and neuronal processes, respectively. NO restrains chemoreceptor discharge under resting normoxic conditions. In fact, it is thought that the hypoxic activation of carotid body chemoafferents involves, in part, a decrease in NO production as oxygen levels fall [26].

In CHF, both NOS3 and NOS1 are markedly downregulated in the carotid body, leading to disinhibition of chemoreceptor afferent discharge [21]. NO acts upon the oxygen sensing glomus cells in the carotid body to enhance the activity of Ca^{++} dependent voltage gated K^+ (K_V) channels and to inhibit Ca_V channels, to impair depolarization and neurotransmitter release. The excitatory effect of NO on K_V channels in glomus cells is mediated by cGMP activation [21]; whereas NO inhibits L-type Ca_V channels in glomus cells, in part, by a cGMP-independent mechanism that may involve s-nitrosylation of the channel [26]. In carotid body glomus cells from CHF animals, K_V currents are suppressed and Ca_V currents enhanced due to decreased availability of NO.

It is not clear to what extent either NOS1 or NOS3 or both contribute to NO control of carotid body afferent function. Because both isoforms of NOS are downregulated in the CB in CHF, the issue remains unresolved. However, upregulation of NOS1 in the carotid body in CHF via gene transfer with adenoviral vector normalizes chemoafferent discharge and functionality and leads to a reduction in sympathetic outflow [27].

NOS3 in the carotid body is largely localized to endothelial cells, and its downregulation is likely to be related to mechanisms of endothelial dysfunction that occur in CHF, including increased oxidative stress and decreased shear stress [28]. Recent studies indicate that a chronic reduction in blood flow to the carotid body alone produces molecular and functional downregulation of both NOS1 and NOS3, similar to that observed in CHF [29]. These results suggest that impairments in vascular endothelial function in the carotid body contribute to its enhanced chemoafferent responsiveness in CHF, and implicate an important role for downregulation of NOS3 in these effects. These implications may be worthy of consideration for studies elucidating NO effects at other autonomic sites in CHF.

NO and Autonomic Efferent Pathways in CHF

The cardiac autonomic ganglia are an important site for NO modulation of heart rate and contractility. NOS1 is located within both intrinsic cardiac vagal and sympathetic stellate ganglionic neurons innervating the heart. However, the role of NO in autonomic control of the heart is complicated by the fact that both NOS1 and NOS3 are located within myocytes and exert significant NO effects on its contractile properties. NO facilitates bradycardia by enhancing vagal cholinergic and suppressing sympathetic β adrenergic neurotransmission [8]. In cholinergic vagal neurons, NO activates cGMP inhibition of phosphodiesterase PDE3 and promotes an increase in cAMP and PKA dependent phosphorylation of N-type Ca_V channels in the presynaptic terminal to enhance acetylcholine release. Conversely, a cGMP-PDE2 mediated action of NO at cardiac sympathetic varicosities reduces calcium influx through Ca_V channels to reduce norepinephrine release [8]. Additionally, NO facilitates accentuated antagonism or cholinergic suppression of beta-adrenergic signaling in myocytes, but the contributory roles of NOS1 and NOS3 are still debated.

Autonomic control of the heart is markedly altered in CHF. Studies in both humans and animal models indicate an increase in resting heart rate with reduced parasympathetic regulation in CHF [1,30]. In addition NO-mediated reflex cholinergic vasodilatation of coronary vessels is impaired [31]. These impairments in autonomic balance expose the myocardium to adverse arrhythmic episodes and enhanced deterioration of contractile function as evidenced by its correlation with adverse prognosis in CHF patients [30]. It is logical to suggest that an impairment of NO signaling in cardiac autonomic ganglia contributes to the altered autonomic control of cardiac function in CHF, but evidence that suppressed NO mediates these changes are not clear cut. In fact evidence suggests that NOS1 expression in cardiac ganglia and myocytes is increased, at least transiently following myocardial infarction [8]. It is thought that this may represent a compensatory protective mechanism to limit inotropic and chronotropic responses to sympathetic activation. The role of NO on cardiac autonomic control in CHF is further complicated by the induction of NOS2 via inflammatory cytokines activated with cardiac injury. Such uncertainties bring to light an important unknown concerning the time course of altered NO functionality through the development of CHF.

NO and Central Autonomic Pathways in CHF

Neurons immunopositive for nNOS are found within all areas of the brainstem and hypothalamus related to autonomic control [32]. In the hypothalamus, the most prominent localization of NOS containing neurons is in the paraventricular nucleus (PVN) and supraoptic nucleus. In the brainstem, NOS containing neurons are found in the periaqueductal gray, parabrachial nucleus, raphe nuclei, nucleus of the tractus solitarius (NTS), the dorsal motor nucleus and nucleus ambiguus (NA), and caudal and rostral regions of the ventral lateral medulla (VLM). Within these autonomic regions, NOS1 coexists with a variety of neurotransmitters, including glutamate, GABA, glycine, 5-hydroxytryptamine, substance P, somatostatin, and angiotensin among others. There seems to be less co-localization with catecholaminergic neurons. Thus, NOS1 has the potential to modulate sympathetic and parasympathetic function throughout virtually all of the central autonomic pathways known to exist [32].

However, a functional role of NO in the central control of cardiovascular function is fraught with conflicting evidence. Disparate results are most likely driven by the complexity of NO interactions with excitatory and inhibitory neurons at the various levels of the central autonomic network. In general, however, studies performed in conscious animals (mainly rabbits, rats and transgenic mice) support a general consensus that central NO restrains sympathetic outflow and facilitates parasympathetic output, particularly when feedback from the baroreceptor reflex is controlled [15,33]. Consistent with these studies in animals, NOS inhibition in humans increases muscle sympathetic nerve activity when changes in arterial pressure are buffered [34].

Even though NO produces an overall tonic restraint of sympathetic tone, its effects on neuronal excitability throughout the central autonomic circuits are varied. Indeed, at the level of the NTS, NO facilitates excitatory ionotropic receptor activation of second order baroreceptor neurons [35]. These excitatory glutamatergic neurons, however, drive a functional inhibition of sympathetic outflow as they project to intermediate inhibitory GABAergic neurons in the CVLM. This scheme, however, is complicated by recent studies indicating that NO derived from NOS3 in the cerebrovascular endothelium of the NTS drives nearby inhibitory GABAergic projections to inhibit baroreceptor neurons [36]. Thus, theoretically at least, a reciprocal antagonism exists for NO control of baroreceptor traffic through the NTS via opposing effects derived from NOS1 and NOS3.

The complexity of these NO - neuronal interactions is further portrayed at the level of the RVLM, a locus for premotor sympathetic neurons and final central integrating site for activation of preganglionic sympathetic neurons in the spinal cord. NO in this region can be either excitatory or inhibitory to sympathetic activity [32]. Such discrepancy is not surprising given that the region, like the NTS, is endowed with both excitatory glutamatergic and inhibitory GABAergic neurons, and application of NO donors or NOS overexpression increases both glutamate and GABA release [37]. It has been assumed that these effects derive from NOS1 activity. Recent studies, however, have reported the novel finding that NOS2 is expressed in neurons in the RVLM, albeit at very low levels normally [5]. Functional studies using specific NOS inhibitors indicated that NO generated by NOS1 promoted sympatho-excitation via NMDA and nonNMDA receptors; whereas NO derived from NOS2 in the RVLM inhibited sympathetic vasomotor outflow via activation of GABA_A receptors [5]. Both effects were mediated via the guanylyl cyclase/ cGMP cascade. A selective blocker for NOS3 was without discernable effects. However, opposing evidence suggests that inhibition of either NOS1 or NOS3 in the RVLM decreased GABA release and enhanced glutamate levels during reflex sympathetic activation [38,39]. Conversely, NOS2 inhibition enhanced GABA release and attenuated the sympathetic activation [40]. Other studies employing NOS overexpression by adenoviral gene transfer to the RVLM found that NOS3 produced marked reductions in sympathetic vasomotor tone and heart rate [41], whereas NOS2 produced sympathoexcitation via increasing oxidative stress [42]. Thus, it appears that all three isoforms of NOS in the RVLM can have a functional impact on the control of sympathetic output. Whether NO in the RVLM is excitatory or inhibitory may not be governed by the NOS isoform per se, but rather, how the NO interacts with neural inputs to the RVLM affecting the balance of glutamatergic vs GABAergic interactions.

Another important component to the effects of NO on the control of sympathetic outflow lies in the hypothalamus at the level of the PVN. Presympathetic neurons in the PVN provide an additional layer of control of sympathetic outflow beyond the baroreflex by integrating information not only from peripheral sensory receptors, but also from higher regions of the brain influenced by stress and anxiety and by the circumventricular organs that relay signals related to circulating factors. Output from the PVN contributes to sympathetic vasomotor tone, particularly in disease states associated with elevated sympathetic nerve activity such as CHF. Unlike studies on the NTS or RVLM, there is a good experimental agreement that NO in the PVN exerts a tonic inhibitory influence on sympathetic outflow [15]. Presympathetic neurons in the PVN are activated by NMDA receptors driving NO production via NOS1. The increased NO, however, elicits an inhibitory effect on the excitability of these neurons by facilitating local GABAergic inhibitory inputs [43]. Furthermore, NO does not appear to facilitate excitatory NMDA transmission in the PVN [43].

Impaired NO function within these central autonomic areas have been implicated in the sympatho-excitation associated with CHF. NOS1 expression is suppressed in the NTS, RVLM, and PVN in CHF [1,6,15]. The functional impact of this deficit is shown by studies in which gene transfer of NOS1 to either the PVN or RVLM and NOS3 to the NTS reduces sympathetic outflow in CHF rabbits and rats, and improves baroreflex inhibitory control of sympathetic nerve activity [44–46]. Also, downregulation of NO in the PVN contributes to the enhanced sympathetic activation in response to stimulation of the arterial chemoreflex in CHF [47]. Similarly, reduced NO in the RVLM facilitates an enhanced sympathetic activation with stimulation of the cardiac sympathetic afferent reflex in CHF [48].

Recent studies have further documented that the elevated level of sympathetic activation in CHF is achieved not solely by downregulation of NOS function in central and peripheral autonomic loci, but also by an accompanying increase in an excitatory drive exerted by angII. Angiotensin II levels, angiotensin (AT1) receptor protein, and AT1-NADPH oxidase driven

O₂[•] production are enhanced in central (PVN, RVLM) [1,17,50], and peripheral (carotid body) [21] sites where NOS function is downregulated in CHF, and the Ang II- O₂[•] signaling at these sites contributes to the increased sympathetic outflow observed in CHF animals. This suggests that NO and AngII exert mutually inhibitory influences on autonomic neurons to maintain sympathetic balance. In CHF this balance is reset to a higher level of sympathetic activation due to the shift in functionality of these two systems (Figure 1). The reciprocal relationship between expression of the angiotensin AT1 receptor and NOS1 at these loci [1,21,50] suggests that this functional antagonism may be governed at the transcriptional level.

While there is good agreement among various studies that NOS1 is downregulated in central autonomic regions and the resultant loss of NO signaling contributes to sympathetic activation in HF, the possible roles played by central NOS3 and NOS2 have not received attention. NOS3 is suppressed in the endothelium of many vascular beds in CHF, but it is increased in some cases. NOS3 has been shown to be upregulated in the NTS of spontaneously hypertensive rats and contributes to elevated sympathetic vasomotor tone in these animals [51]. It is not clear whether this type of NOS signaling mechanism contributes to sympathetic activation in HF.

A recent study has shown that NOS2 mRNA is increased in the PVN in CHF while NOS1 is decreased [52]. Cytokine blockade prevented these changes, reduced resting sympathetic outflow and increased baroreflex control. These results implicate a role of inflammatory cytokines to signal downregulation of NOS1 in central autonomic areas in CHF. Peroxynitrite levels also were elevated in the PVN in these animals, implicating a role of oxidative stress on impaired NO signaling in the region.

To date there is no information regarding the influence of CHF on NO control of preganglionic vagal activity at the central level. Cardiac vagal preganglionic neurons (CVPNs) are primarily located within the external formation of the NA and in the DMN. A recent electrophysiological study suggests that NO decreases glutamatergic and glycinergic neurotransmission while enhancing GABAergic neurotransmission to CVPNs [53]. Functional interpretation of these effects is limited, but only the inhibition of glycinergic transmission is consistent with the known effect of NO to enhance CVPN activity. Whether disinhibition of glycine receptors in the NA contributes to the attenuation of parasympathetic control of cardiac function found in CHF has not been addressed.

Potential Therapies for CHF by Restoring NOS Function

As summarized above, gene transfer of NOS has proven to be beneficial in restoring NO signaling and lowering sympathetic outflow in CHF animals. Recent studies have shown that the class of HMG-CoA reductase inhibitors widely used in the treatment of hypercholesterolemia, called statins, can upregulate NOS1 protein expression and may be beneficial in lowering sympathetic activity in CHF. An important recent study has shown that selective application of simvastatin to the cerebroventricles over the course of several days led to upregulation of NOS1 in the RVLM of CHF rabbits, reduced sympathetic nerve activity, and improved baroreflex function [54]. The signaling pathway for this effect involves inhibition of HMG-CoA reductase products [54] and possibly activation of Akt/NFkB [55]. Evidence that Akt can phosphorylate and activate NOS1 [56] could implicate an effect of statins to enhance NOS1 activity in addition to upregulation of protein expression.

Exercise conditioning represents another modality of increasing NOS function to reverse sympathetic activation in CHF [57]. Exercise is known to improve endothelial function and improve NOS3 expression in CHF [58]. Exercise also increases expression of NOS1 in the carotid body, the RVLM, NTS and PVN in CHF animals and lowers resting sympathetic nerve activity [59–61]. The signaling mechanisms responsible for normalizing NOS1 expression with exercise in CHF are not clear. It has been proposed that a reduction in angII that occurs with

exercise may lead to decreased $O_2^{\bullet-}$ suppression of NOS function in central autonomic areas [61]. In support of this, maintaining elevated circulating angII levels by chronic infusion during exercise conditioning prevented the exercise-induced normalization of NOS expression and functional restraint of sympathetic outflow in CHF rabbits [61].

Exercise conditioning also has been shown to abrogate changes in afferent and efferent limbs of several autonomic reflexes in CHF, but the role of NO in these effects has not been addressed. However, we have shown that exercise normalizes the exaggerated carotid body chemoreflex activation of sympathetic outflow in CHF [59]. Exercise upregulated NOS3 and NOS1 expression in the carotid body to restore normal NO restraint of chemoafferent activity in CHF animals. Based upon recent evidence that NO signaling in the carotid body is influenced by its blood flow [29], we speculate that periodic increases in blood flow induced during exercise bouts may act to restore NOS signaling mechanisms in the carotid body during CHF by improving endothelial function. The ability of exercise to improve endothelial NOS3 function in CHF may have important implications for central control of sympathetic function as well.

Summary

Studies addressing the role of NO in autonomic control of the cardiovascular system reveal a complex system of excitatory and inhibitory influences of the mediator on peripheral sensory and effector neurons, and central integratory neurons of sympathetic and parasympathetic function. A comprehensive and lucid understanding of the role of NO in the neural control of the heart and circulation even under normal conditions is difficult to reveal because of a great amount of conflicting evidence, and a great number of intriguing but unanswered questions concerning NOS regulation in neurons.

In CHF, the normal functional effects of NO are impaired or downregulated at most autonomic loci studied, leading to a suppressed parasympathetic outflow to the heart and exaggerated sympathetic outflow to the heart and vasculature. A decrease in NOS1 protein and mRNA expression and in bioavailable NO are evident in several autonomic regions in CHF and have lead to the assumption that the suppression of NOS1 activity gives rise to this dysfunctional state. But emerging evidence suggests that a role for NOS3 and NOS2 on autonomic function in CHF should not be ignored. The beneficial effects of statins and exercise in normalizing NOS and autonomic function in CHF open potential new avenues for more effective treatment of the progression of heart failure. Nevertheless, our understanding of regulatory factors governing NOS function and NO influences on autonomic nerves in normal and disease states remains very limited and will require further extensive study.

Abbreviations

NO	nitric oxide
NOS1	neural nitric oxide synthase (nNOS)
NOS2	inducible nitric oxide synthase (iNOS)
NOS3	endothelial nitric oxide synthase (eNOS)
CHF	chronic (or congestive) heart failure
NMDA	N-methyl-D-aspartic acid
GABA	gamma-aminobutyric acid
angII	angiotensin II
$O_2^{\bullet-}$	superoxide anion

NTS	nucleus tractus solitarii
PVN	paraventricular nucleus
RVLM	rostral ventrolateral medulla
NA	nucleus ambiguus

References

• of importance

•• of significant importance

1. Zucker IH, Schultz HD, Li YF, Wang Y, Wang W, Patel KP. The origin of sympathetic outflow in heart failure: the roles of angiotensin II and nitric oxide. *Prog Biophys Mol Biol* 2004;84(2–3):217–232. [PubMed: 14769437]
2. Esler M, Lambert G, Brunner-La Rocca HP, Vaddadi G, Kaye D. Sympathetic nerve activity and neurotransmitter release in humans: translation from pathophysiology into clinical practice. *Acta Physiol Scand* 2003;177(3):275–284. [PubMed: 12608997]
3. Leenen FH. Brain mechanisms contributing to sympathetic hyperactivity and heart failure. *Circ Res* 2007;101(3):221–223. [PubMed: 17673679]
4. Lin LH, Taktakishvili O, Talman WT. Identification and localization of cell types that express endothelial and neuronal nitric oxide synthase in the rat nucleus tractus solitarii. *Brain Res* 2007;1171:42–51. [PubMed: 17761150]
5. Chan SH, Wang LL, Chan JY. Differential engagements of glutamate and GABA receptors in cardiovascular actions of endogenous nNOS or iNOS at rostral ventrolateral medulla of rats. *Br J Pharmacol* 2003;138(4):584–593. [PubMed: 12598412]
6. Patel KP, Zhang K, Zucker IH, Krukoff TL. Decreased gene expression of neuronal nitric oxide synthase in hypothalamus and brainstem of rats in heart failure. *Brain Res* 1996;734(1–2):109–115. [PubMed: 8896816]
7. Bauersachs J, Widder JD. Endothelial dysfunction in heart failure. *Pharmacol Rep* 2008;60(1):119–126. [PubMed: 18276993]
8. Danson EJ, Choate JK, Paterson DJ. Cardiac nitric oxide: emerging role for nNOS in regulating physiological function. *Pharmacol Ther* 2005;106(1):57–74. [PubMed: 15781122]
9. Wang Y, Newton DC, Marsden PA. Neuronal NOS: gene structure, mRNA diversity, and functional relevance. *Crit Rev Neurobiol* 1999;13(1):21–43. [PubMed: 10223522]
10. Kone BC. Protein-protein interactions controlling nitric oxide synthases. *Acta Physiol Scand* 2000;168(1):27–31. [PubMed: 10691776]
11. Garthwaite J. Concepts of neural nitric oxide-mediated transmission. *J Neurosci* 2008;27(11):2783–2802.
12. Schmidt TS, Alp NJ. Mechanisms for the role of tetrahydrobiopterin in endothelial function and vascular disease. *Clin Sci (Lond)* 2007;113(2):47–63. [PubMed: 17555404]
13. Rameau GA, Tukey DS, Garcin-Hosfield ED, Titcombe RF, Misra C, Khatri L, Getzoff ED, Ziff EB. Biphasic coupling of neuronal nitric oxide synthase phosphorylation to the NMDA receptor regulates AMPA receptor trafficking and neuronal cell death. *J Neurosci* 2007;27(13):3445–3455. [PubMed: 17392461]
14. Wiesinger H. Arginine metabolism and the synthesis of nitric oxide in the nervous system. *Prog Neurobiol* 2001;64(4):365–391. [PubMed: 11275358]
15. Patel KP, Li YF, Hirooka Y. Role of nitric oxide in central sympathetic outflow. *Exp Biol Med* (Maywood) 2001;226(9):814–824. [PubMed: 11568303]
16. Bermudez V, Bermudez F, Acosta G, Acosta A, Anez J, Andara C, Leal E, Cano C, Manuel V, Hernandez R, et al. Molecular mechanisms of endothelial dysfunction: from nitric oxide synthesis to ADMA inhibition. *Am J Ther* 2008;15(4):326–333. [PubMed: 18645335]

17. Li YF, Wang W, Mayhan WG, Patel KP. Angiotensin-mediated increase in renal sympathetic nerve discharge within the PVN: role of nitric oxide. *Am J Physiol Regul Integr Comp Physiol* 2006;290(4):R1035–R1043. [PubMed: 16322353]
18. Kung LC, Chan SH, Wu KL, Ou CC, Tai MH, Chan JY. Mitochondrial respiratory enzyme complexes in rostral ventrolateral medulla as cellular targets of nitric oxide and superoxide interaction in the antagonism of antihypertensive action of eNOS transgene. *Mol Pharmacol* 2008;74(5):1319–1332. [PubMed: 18715945] This study reports a novel finding that NOS overexpression in the RVLM can target mitochondrial enzyme complexes to increase superoxide anion production.
19. Wang W, Ma R. Cardiac sympathetic afferent reflexes in heart failure. *Heart Fail Rev* 2000;5(1):57–71. [PubMed: 16228916]
20. Smith SA, Mitchell JH, Garry MG. The mammalian exercise pressor reflex in health and disease. *Exp Physiol* 2006;91(1):89–102. [PubMed: 16282366]
21. Schultz HD, Li YL. Carotid body function in heart failure. *Respir Physiol Neurobiol* 2007;157(1):171–185. [PubMed: 17374517]
22. Brandle M, Wang W, Zucker IH. Ventricular mechanoreflex and chemoreflex alterations in chronic heart failure. *Circ Res* 1994;74(2):262–270. [PubMed: 8293565]
23. Wang W, Chen JS, Zucker IH. Carotid sinus baroreceptor sensitivity in experimental heart failure. *Circulation* 1990;81(6):1959–1966. [PubMed: 2344687]
24. Li Z, Chapleau MW, Bates JN, Bielefeldt K, Lee HC, Abboud FM. Nitric oxide as an autocrine regulator of sodium currents in baroreceptor neurons. *Neuron* 1998;20(5):1039–1049. [PubMed: 9620707]
25. Hohler B, Mayer B, Kummer W. Nitric oxide synthase in the rat carotid body and carotid sinus. *Cell Tissue Res* 1994;276(3):559–564. [PubMed: 7520363]
26. Prabhakar NR. NO and CO as second messengers in oxygen sensing in the carotid body. *Respir Physiol* 1999;115(2):161–168. [PubMed: 10385030]
27. Li YL, Li YF, Liu D, Cornish KG, Patel KP, Zucker IH, Channon KM, Schultz HD. Gene transfer of neuronal nitric oxide synthase to carotid body reverses enhanced chemoreceptor function in heart failure rabbits. *Circ Res* 2005;97(3):260–267. [PubMed: 15994433]
28. Drexler H, Hornig B. Endothelial dysfunction in human disease. *J Mol Cell Cardiol* 1999;31(1):51–60. [PubMed: 10072715]
29. Ding Y, Li Y, Cornish KG, Schultz HD. Reduced Blood Flow in Carotid Arteries is a Trigger Contributing to Peripheral Chemoreflex Hypersensitivity in Chronic Heart Failure Rabbits. *FASEB J* 2007;21:910–913.
30. Esler M, Kaye D. Sympathetic nervous system neuroplasticity. *Hypertension* 2006;47(2):143–144. [PubMed: 16391170]
31. Zhao G, Hintze TH, Kaley G. Neural regulation of coronary vascular resistance: role of nitric oxide in reflex cholinergic coronary vasodilation in normal and pathophysiologic states. *EXS* 1996;76:1–19. [PubMed: 8805785]
32. Krukoff TL. Central actions of nitric oxide in regulation of autonomic functions. *Brain Res Brain Res Rev* 1999;30(1):52–65. [PubMed: 10407125]
33. Zucker IH, Wang W, Pliquett RU, Liu JL, Patel KP. The regulation of sympathetic outflow in heart failure. The roles of angiotensin II, nitric oxide, and exercise training. *Ann N Y Acad Sci* 2001;940:431–443. [PubMed: 11458699]
34. Sartori C, Lepori M, Scherrer U. Interaction between nitric oxide and the cholinergic and sympathetic nervous system in cardiovascular control in humans. *Pharmacol Ther* 2005;106(2):209–220. [PubMed: 15866320]
35. Dias AC, Vitela M, Colombari E, Mifflin SW. Nitric oxide modulation of glutamatergic, baroreflex, and cardiopulmonary transmission in the nucleus of the solitary tract. *Am J Physiol Heart Circ Physiol* 2005;288(1):H256–H262. [PubMed: 15598868]
36. Wang S, Teschemacher AG, Paton JF, Kasparov S. Mechanism of nitric oxide action on inhibitory GABAergic signaling within the nucleus tractus solitarii. *FASEB J* 2006;20(9):1537–1539. [PubMed: 16720728]

37. Kishi T, Hirooka Y, Sakai K, Shigematsu H, Shimokawa H, Takeshita A. Overexpression of eNOS in the RVLM causes hypotension and bradycardia via GABA release. *Hypertension* 2001;38(4):896–901. [PubMed: 11641305]
38. Ishide T, Nauli SM, Maher TJ, Ally A. Cardiovascular responses and neurotransmitter changes following blockade of nNOS within the ventrolateral medulla during static muscle contraction. *Brain Res* 2003;977(1):80–89. [PubMed: 12788516]
39. Ishide T, Preuss CV, Maher TJ, Ally A. Neurochemistry within ventrolateral medulla and cardiovascular effects during static exercise following eNOS antagonism. *Neurosci Res* 2005;52(1):21–30. [PubMed: 15811549]
40. Ally A, Phattanarudee S, Kabadi S, Patel M, Maher TJ. Cardiovascular responses and neurotransmitter changes during static muscle contraction following blockade of inducible nitric oxide synthase (iNOS) within the ventrolateral medulla. *Brain Res* 2006;1090(1):123–133. [PubMed: 16650388]
41. Hirooka Y. Localized gene transfer and its application for the study of central cardiovascular control. *Auton Neurosci* 2006;126–127:120–129.
42. Kimura Y, Hirooka Y, Sagara Y, Ito K, Kishi T, Shimokawa H, Takeshita A, Sunagawa K. Overexpression of inducible nitric oxide synthase in rostral ventrolateral medulla causes hypertension and sympathoexcitation via an increase in oxidative stress. *Circ Res* 2005;96(2):252–260. [PubMed: 15591232]
43. Bains JS, Ferguson AV. Nitric oxide regulates NMDA-driven GABAergic inputs to type I neurones of the rat paraventricular nucleus. *J Physiol* 1997;499(Pt 3):733–746. [PubMed: 9130169]
44. Li YF, Roy SK, Channon KM, Zucker IH, Patel KP. Effect of in vivo gene transfer of nNOS in the PVN on renal nerve discharge in rats. *Am J Physiol Heart Circ Physiol* 2002;282(2):H594–H601. [PubMed: 11788407]
45. Wang Y, Patel KP, Cornish KG, Channon KM, Zucker IH. nNOS gene transfer to RVLM improves baroreflex function in rats with chronic heart failure. *Am J Physiol Heart Circ Physiol* 2003;285(4):H1660–H1667. [PubMed: 12969883]
46. Sakai K, Hirooka Y, Shigematsu H, Kishi T, Ito K, Shimokawa H, Takeshita A, Sunagawa K. Overexpression of eNOS in brain stem reduces enhanced sympathetic drive in mice with myocardial infarction. *Am J Physiol Heart Circ Physiol* 2005;289(5):H2159–H2166. [PubMed: 16006546]
47. Reddy MK, Schultz HD, Zheng H, Patel KP. Altered nitric oxide mechanism within the paraventricular nucleus contributes to the augmented carotid body chemoreflex in heart failure. *Am J Physiol Heart Circ Physiol* 2007;292(1):H149–H157. [PubMed: 16891408]
48. Zhu GQ, Gao XY, Zhang F, Wang W. Reduced nitric oxide in the rostral ventrolateral medulla enhances cardiac sympathetic afferent reflex in rats with chronic heart failure. *Sheng Li Xue Bao* 2004;56(1):47–53. [PubMed: 14985829]
49. Wang WZ, Gao L, Wang HJ, Zucker IH, Wang W. Tonic Glutamatergic Input in the Rostral Ventrolateral Medulla Is Increased in Rats With Chronic Heart Failure. *Hypertension*. 2008
50. Zucker IH, Liu JL. Angiotensin II–nitric oxide interactions in the control of sympathetic outflow in heart failure. *Heart Fail Rev* 2000;5(1):27–43. [PubMed: 16228914]
51. Waki H, Murphy D, Yao ST, Kasparov S, Paton JF. Endothelial NO synthase activity in nucleus tractus solitarii contributes to hypertension in spontaneously hypertensive rats. *Hypertension* 2006;48(4):644–650. [PubMed: 16940227] This study reports a novel finding that NOS3 can influence baroreceptor transmission in the nucleus tractus solitarii.
52. Guggilam A, Patel KP, Haque M, Ebenezer PJ, Kapusta DR, Francis J. Cytokine blockade attenuates sympathoexcitation in heart failure: cross-talk between nNOS, AT-1R and cytokines in the hypothalamic paraventricular nucleus. *Eur J Heart Fail* 2008;10(7):625–634. [PubMed: 18550427] This study reports a novel finding that chronic cytokine blockade in the PVN restores NOS1 expression, decreases sympathetic outflow, and improves survival and cardiac function in CHF rats.
53. Kamendi H, Dergacheva O, Wang X, Huang ZG, Bouairi E, Gorini C, Mendelowitz D. NO differentially regulates neurotransmission to premotor cardiac vagal neurons in the nucleus ambiguus. *Hypertension* 2006;48(6):1137–1142. [PubMed: 17015774] This study reports a novel finding that NO inhibits glycine neurotransmission to cardiac vagal neurons in the nucleus ambiguus.
54. Gao L, Wang W, Zucker IH. Simvastatin inhibits central sympathetic outflow in heart failure by a nitric-oxide synthase mechanism. *J Pharmacol Exp Ther* 2008;326(1):278–285. [PubMed: 18550427]

- 18441251] This study reports a novel finding that chronic central simvastatin treatment restores NOS1 expression in the RVLM and lowers sympathetic outflow in conscious CHF rabbits.
55. Nakata S, Tsutsui M, Shimokawa H, Yamashita T, Tanimoto A, Tasaki H, Ozumi K, Sabanai K, Morishita T, Suda O, et al. Statin treatment upregulates vascular neuronal nitric oxide synthase through Akt/NF-kappaB pathway. *Arterioscler Thromb Vasc Biol* 2007;27(1):92–98. [PubMed: 17082483]
56. Gingerich S, Krukoff TL. Activation of ERbeta increases levels of phosphorylated nNOS and NO production through a Src/PI3K/Akt-dependent pathway in hypothalamic neurons. *Neuropharmacology* 2008;55(5):878–885. [PubMed: 18652836]
57. Zucker IH, Patel KP, Schultz HD, Li YF, Wang W, Pliquet RU. Exercise training and sympathetic regulation in experimental heart failure. *Exerc Sport Sci Rev* 2004;32(3):107–111. [PubMed: 15243206]
58. Moyna NM, Thompson PD. The effect of physical activity on endothelial function in man. *Acta Physiol Scand* 2004;180(2):113–123. [PubMed: 14738470]
59. Li YL, Ding Y, Agnew C, Schultz HD. Exercise training improves peripheral chemoreflex function in heart failure rabbits. *J Appl Physiol* 2008;105(3):782–790. [PubMed: 18583379]
60. Zheng H, Li YF, Cornish KG, Zucker IH, Patel KP. Exercise training improves endogenous nitric oxide mechanisms within the paraventricular nucleus in rats with heart failure. *Am J Physiol Heart Circ Physiol* 2005;288(5):H2332–H2341. [PubMed: 15653768]
61. Mousa TM, Liu D, Cornish KG, Zucker IH. Exercise training enhances baroreflex sensitivity by an angiotensin II-dependent mechanism in chronic heart failure. *J Appl Physiol* 2008;104(3):616–624. [PubMed: 18079268]

Figure 1.

Downregulation of NOS1 and sympatho-inhibitory NO signaling at several autonomic sites contributes to elevated sympathetic activity in CHF. A reciprocal enhancement of sympatho-excitatory angiotensin II - O₂^{•-} signaling at these sites exacerbates the impaired NO restraint. A contribution of NO to the blunted parasympathetic activity seen in CHF is not known. PVN: paraventricular nucleus of the hypothalamus; RVLm: rostral ventrolateral medulla; NTS: nucleus tractus solitarius; NA: nucleus ambiguus/dorsal vagal nucleus; CB: carotid body; NOS1: neural nitric oxide synthase; BH₄: tetrahydrobiopterin; AT1R: angiotensin receptor 1; NOX: NADPH oxidase; O₂^{•-}: superoxide anion

Effects of NO on Neuronal Excitability at Afferent, Central and Efferent Levels of the Cardiovascular Autonomic System.

Table 1

Location	Neuronal type	Condition	NOS isoform implicated	Signaling event	Channel/Receptor Response	Neuronal excitability
AFFERENT						
Carotid sinus	Baroreceptor	Normal CHF	NOS3??	S-nitrosylation??	↓Nav ↑Na/K ⁺ ATPase	↓ [24] ↑ [23]
Carotid body	Glomus cell	Normal CHF	NOS1/NOS3 NOS1↓/NOS3↓	cGMP events S-nitrosylation ↓NO	↑K _V ↓Ca _{V(L)} ↓K _V ↑Ca _V	↓ [21] ↓ [26] ↑ [21]
Heart	Cardiac Sympathetic Afferent	Normal CHF	NOS??	NO / ?? ↑PGs	?? ??	↑ [19] ↑ [19]
Skeletal Muscle	Somatic Afferent	Normal CHF	?? ??	Mechanical/Metabolites??	Various??	↑ [20] ↑ [20]
CENTRAL						
NTS	Barosensitive	Normal CHF	NOS1 NOS3 NOS1↓ NOS3?	cGMP events cGMP events ↓NO	↑(NMDA/nonNMDA) ↑GABA ??	↑ [35] ↓ [36] ↓ *
RVML	Presympathetic	Normal CHF	NOS1 NOS2 NOS3 NOS1↓ NOS2/NOS3??	cGMP events cGMP events cGMP events ↓NO	↑Glutamate ↓GABA ↓Glutamate ↑GABA ↑GABA ↓Glutamate ↓GABA ↑Glutamate ↑Glutamate ↓GABA ↑(NMDA/nonNMDA)	↑ [5] ↓ [38] ↓ [5] ↑ [40] ↓ [39] ↑ [49,50]
PVN	Presympathetic	Normal CHF	NOS NOS1↓ NOS2/NOS3??	cGMP events	↑GABA ↑NMDA ↓GABA	↓ [15,43] ↑ [15]
NA	Cardiac vagal	Normal CHF	NOS NOS??	cGMP events	↓NMDA ↓Glycine ↑GABA ??	↑ [53] ↓ *
EFFERENT						
Heart	Vagal	Normal CHF	NOS1 NOS??	cGMP/↓PD3/↑PKA ??	↑ Ca _{V(N)}	↑ [8] ↓ [8]
	Sympathetic	Normal CHF	NOS1 NOS??	cGMP/↑PD2/↓PKA ??	↓Ca _{V(N)}	↓ [8] ↑ [8]

* Implied from known effects of baroreflex function in CHF

Nav⁺, Kv⁺, Cav⁺ (L-N): voltage gated Na⁺, K⁺, and Ca⁺⁺ channels (L or N type), respectively