Heart Fail Rev (2011) 16:137–145
DOI 10.1007/s10741-010-9184-4

Inflammatory cytokines and nitric oxide in heart failure and potential modulation by vagus nerve stimulation

Weiwei Li · Brian Olshansky

Published online: 25 July 2010

Abstract In heart failure, an inflammatory response may occur. The relationship between inflammatory cytokines, NOS and heart failure progression remains uncertain. Parasympathetic activation can affect heart rate and AV conduction. In heart failure, a relationship between the vagus nerve and the inflammatory response has been proposed. Vagal nerve stimulation can modulate the inflammatory response and affect specific inflammatory mediators including nitric oxide that may be contributory to continued or progressive heart failure. Therefore, vagal nerve stimulation may have beneficial effects that are independent from heart rate or AV conduction in heart failure. Challenges remain regarding the relationship between specific inflammatory markers and heart failure and how to best modulate the cytokines and NOS in patients to achieve beneficial effects. Future studies need to evaluate whether modulating inflammatory cytokines and NOS via vagal nerve stimulation can improve cardiac performance and outcomes in patients with heart failure.

Keywords Inflammatory cytokines · Nitric oxide · Vagus nerve stimulation · Heart failure

Introduction

Cardiac parasympathetic activation affects heart rate and AV conduction, but recent observations in heart failure suggest that vagal nerve stimulation has beneficial effects independent from heart rate or AV conduction [1–6]. In heart failure, an inflammatory response has been observed [7, 8] and may be in part responsible for heart failure and its progression. A relationship between the vagus nerve activation and an inflammatory response seen in heart failure has been proposed [9]. Vagal nerve stimulation can affect the inflammatory response [10, 11] and specific inflammatory mediators, including nitric oxide synthase (NOS), associated with heart failure [1, 4]. Here, we review evidence that vagal nerve activation affects the inflammatory response and alters NOS abnormalities seen in heart failure.

W. Li · B. Olshansky (✉)
Cardiac Electrophysiology, University of Iowa Hospitals, 200 Hawkins Drive, Room 4426a JCP, Iowa City, IA 52242, USA
e-mail: brian-olshansky@uiowa.edu

Inflammatory cytokines and vagal nerve stimulation

Inflammation and heart failure: a relationship exists

Causes for heart failure are manifold and related to hypertension, ischemia, valvular disease, toxins, viruses, diabetes, tachycardia and/or unknown causes. Inflammation may be responsible for or contribute to progression of chronic or acute heart failure due to many of these conditions [12–14]. Inflammatory mediators may be etiology dependent and disease specific [15]. Several reports have shown that heart failure patients have increased serum level of inflammatory cytokines, such as TNF-α, IL-1β, IL-6, IL-18 [16–20], soluble intercellular adhesion molecule (sICAM)-1 [21] and others [22] and that the levels of these constituents are associated with heart failure progression and outcome [16–19]. While these cytokines may be simply markers of disease rather than the cause, it is unlikely that they are innocent bystanders [23]. What remains unclear, but crucial, is how these cytokines affect outcomes and remodeling and whether reduction in the levels of these cytokines has any beneficial effect.

In patients with ischemic heart disease, inflammatory mediators and cells are essential for infarct healing [24].

For some individuals, however, inflammation persists beyond the healing process. While some of the mediators may be beneficial, others may be harmful [25]. Inflammatory cytokines may have a central effect on sympathetic activation [26]. For patients with putative viral cardiomyopathy, an immune inflammatory response, rather than viral damage, may be the cause for worsening outcomes. The inflammatory response, triggered by a virus, may continue and cause progressive cardiomyopathy [27, 28]. Data remain uncertain regarding the effects of hypertension on an inflammatory response that could lead to diastolic dysfunction and subsequent heart failure. Inflammatory pathways are activated during cardiomyocyte hypertrophy and attenuated by peroxisome proliferator-activated receptors PPAR alpha and PPAR delta [29]. Obesity and diabetes have also been associated with activation of an inflammatory response that may cause cardiac injury and ventricular dysfunction [30].

Elevated levels of specific inflammatory cytokines (such as TNF-α) have been shown to relate directly to the deterioration of functional class and left ventricular ejection fraction [16, 17]. Furthermore, circulating inflammatory cytokines were found to be independent predictors of mortality in patients with advanced heart failure [31]. Increased levels of inflammatory cytokines can influence myocardial contractility, induce hypertrophy and promote apoptosis, thereby contributing to the progression of left ventricular dysfunction and remodeling [32].

The inflammatory reflex

In the past decade, regulatory pathways of cytokine production have been elucidated [11, 33]. Cytokine production, regulated, in part, by neural pathways, is termed "the inflammatory reflex" [11, 33, 34]. In the afferent pathway, cytokines activate vagal fibers that travel to the nucleus tractus solitarius [34]. Fibers communicating between the brainstem and hypothalamus stimulate adrenocorticotropic hormone. This leads to increased glucocorticoid secretion that can inhibit proinflammatory cytokines [35]. Ascending vagal sensory fibers can also activate nerve signals to suppress inflammation [36].

Efferent vagal nerve fibers release acetylcholine in the reticuloendothelial system, including the spleen, liver and gastrointestinal tract [34]. Acetylcholine binds to alpha7 nicotinic acetylcholine receptor on peripheral macrophages, and this stimulates Janus kinases (JAK) and signal transducers and activators of transcription (STAT) anti-inflammatory pathways, inhibits NF-kappa B and ultimately by preventing cytokine synthesis and release, inhibits inflammation [34, 37, 38].

In human macrophage cell cultures, acetylcholine inhibits TNF-α, IL-1β, IL-6 and IL-18 release in a dose-dependent manner when exposed to lipopolysaccharides. The inhibitory effect of acetylcholine on the lipopolysaccharide-induced TNF-α response is mediated primarily by α-bungarotoxin-sensitive, nicotinic acetylcholine receptors. The inhibitory effect occurs through post-transcriptional suppression [39].

In animals with intact vagus nerves, efferent vagus nerve activation can inhibit serum TNF-α during endotoxemia [39] without augmenting corticosteroid or IL-10 levels. Serum TNF-α level was higher, and hypotension was worsened with vagotomy [39], indicating that vagus nerve activation has an anti-inflammatory response [39]. These and other data regarding vagal anti-inflammatory reflex may or may not pertain to the heart and may or may not be beneficial.

Immunomodulation and heart failure outcomes

Traditional cardiovascular drugs appear to have little influence on cytokines or the inflammatory reflex seen in heart failure. As several animal and clinical studies suggested that downregulation of inflammation may improve cardiac performance [7, 40], immunomodulatory therapy emerged as a possible new treatment for heart failure [7]. Cytokine-targeted therapy has been attempted to reduce the proinflammatory effects of TNF-α purported to cause worsening ventricular dysfunction. Two trials were performed, the Randomized Etanercept North AmerIcan Strategy to Study AntagoNism of CytokinEs (RENAISSANCE) and the Research into Etanercept CytOkine antagonism in VEntriculaR dysfunction (RECOVER) trials, both of which tested a TNF-α blocker etanercept with difference only in the doses of etanercept used [40]. Etanercept had no effect on clinical status, death or a chronic heart failure hospitalization end point. The anti-TNF Therapy Against Congestive Heart Failure (ATTACH) trial tested infliximab [41]. These trials did not show a beneficial effect of reducing this inflammatory cytokine [42]. The reasons for these results remain uncertain [13].

A novel approach to immune modulation was tested in the Advanced Chronic heart failure Clinical Assessment of Immune Modulation therapy (ACCLAIM) study [43]. In this trial, device-based immunomodulation therapy was applied. Blood samples were exposed ex vivo to controlled oxidative stress which led to apoptosis of leukocytes resulting in reduction of inflammatory cytokine production and upregulation of anti-inflammatory cytokines. In this double-blind, placebo-controlled study, no significant benefit of the therapy was seen. In two prespecified subgroups, including those with no history of previous myocardial infarction and those with NYHA functional class II heart failure ($n = 689$), therapy was associated with a 26% (0.74; 0.57–0.95; $P = 0.02$) and a 39% (0.61; 95% CI

0.46–0.80; $P = 0.0003$) reduction in the risk of primary endpoint events, respectively. The significance of these findings is unclear as precise mechanisms of immune modulation are not completely understood. Other therapies have been tested including immunoglobulin or interferon treatment, immunoabsorption, antioxidants therapies and pentraxins [44].

It remains possible that reduction of cytokines, such as, IL-1, IL-6, IL-18, macrophage inflammatory protein-1 α, monocyte chemoattractant peptide (MCP)-1 and cardiotrophin-1 [16, 17, 19] may improve outcomes. Furthermore, targeting other inflammatory mediators, IL-6, or specific signaling pathways, such as JAK-STAT, Shp2/Ras/ErK or PI3 K/Akt, which can become activated in various cardiovascular diseases, may have beneficial effects.

Potential anti-inflammatory mechanisms of vagus nerve stimulation

Studies have shown that vagus nerve stimulation can modulate the inflammatory response in cell cultures and animal models. Tracey et al. elucidated mechanisms by which vagus nerve stimulation can initiate and modulate such response [10, 11, 34, 45]. Cultured macrophages express nicotinic receptors composed of five α7 subunits. These subunits are required for acetylcholine inhibition of TNF-α release [45]. When exposed to lipopolysaccharides, alpha7 subunit–deficient mice expressed more inflammatory cytokines than wild-type mice. Vagus nerve stimulation inhibited TNF-α synthesis in wild-type mice, but not in alpha7-deficient mice. Therefore, the nicotinic acetylcholine receptor alpha7 subunit is essential for inhibiting cytokine synthesis in the cholinergic anti-inflammatory pathway.

Nicotinic receptor activation can inhibit high-mobility group box 1 (HMGB1) [46, 47], a purported mediator of tissue injury and inflammation released from human macrophages exposed to endotoxin. In experimental model of sepsis, mice were induced for endotoxemia. Treatment with nicotine decreased serum HMGB1 levels, and this inhibition led to a significant decrease in mortality in these animals in a dose-dependent manner [47]. These data suggest that selective nicotinic agonists for the alpha7-nAChR might have therapeutic potential for the treatment of sepsis. Their role to protect against cardiomyopathy is uncertain.

The relationship between cardiac vagal activity and cytokine levels was tested in the human subjects. The Coronary Artery Risk Development In young Adults (CARDIA) study is designed to understand contributors to changes in cardiovascular disease risk factors during transition from adolescence through young adulthood to middle age. In this study, RR interval variability, an index of cardiac vagal modulation, and C-reactive protein (CRP) and IL-6 were measured in 757 subjects. Univariate analysis revealed that all indices of RR interval variability were strongly, and inversely, related to IL-6 and CRP levels. In a multivariate model including gender, race, age, smoking, physical activity, systolic blood pressure, body mass index and disease, there was significant inverse relationship between heart rate variability and inflammatory markers. These findings are consistent with the hypothesis that efferent vagal activity is inversely associated with cytokine and inflammation in humans [48].

The JAK-STAT transcription factors are the signaling pathways for a variety of extracellular signals including cytokines. It is shown that nicotinic receptor activation is anti-inflammatory to macrophages [38]. This effect was dependent on the activation of JAK2 by the alpha7 acetylcholine receptors and subsequent activation of the STAT3. The anti-inflammatory effect of nicotine required phosphorylated STAT3 to bind to its DNA response elements and subsequently activate them. The molecular mechanisms involved the inhibition of nuclear factor NF-kappa B p65 nuclear translocation and activation [38, 49].

This signaling pathway was also tested in animal models. In an animal model of intestinal manipulation, vagus nerve stimulation activated the STAT3 in macrophages and decreased surgery-induced inflammation [49]. When STAT1 and STAT3 were inhibited in rat peritoneal macrophages, which were exposed to lipopolysaccharide, there was a significant decrease in HMGB1 mRNA levels [50]. These data indicate that the molecular mechanism of the cholinergic anti-inflammatory pathway involves activation of JAK2 and STAT3 within macrophages. This ultimately decreases the activation of proinflammatory transcription factor NF-kappa B p65.

Vagus nerve stimulation in congestive heart failure

Despite the results from cytokine targeting therapy, promising data have shown that parasympathetic activation, either pharmacologically or electrically, can induce positive effects in experimental heart failure model (Table 1) [1, 51, 52]. Vagal nerve stimulation can improve survival in a post-ischemic model of heart failure in the rat [53]. The exact mechanisms underlying this observation are not clear. Inhibition of sympathetic activity may partly account for the benefits observed. Another possibility is that vagal stimulation improves survival in severe chronic heart failure through the inhibition of the release of cytokines such as TNF-α, or others. Further studies, including immunological assay, are needed to clarify the mechanisms for beneficial effects of vagal stimulation of the heart when heart failure is present.

Table 1 Effects of vagal nerve stimulation on heart failure and inflammatory cytokines

Hypothesis	Animal model	Findings	Conclusion
Impact of VNS on heart failure development [1]	Canine high-rate ventricular pacing model	LVEDV/LVESV lower LVEF higher Heart rate variability and baroreflex sensitivity improved Plasma NE, Ang-II and CRP levels, markedly attenuated	Chronic VNS improves autonomic control and attenuates systemic inflammation and heart failure progression
Impact of VNS on CHF survival [53]	Rat post-ischemic model of heart failure	Treated rats had significantly lower LVEDP Reduced risk of death (RRR = 73%).	VNS can improve survival in a post-ischemic model of heart failure in the rat
VNS and level of inflammatory cytokines [39]	Rats with lethal endotoxemia	TNF α synthesis and peak serum TNF α level decrease	VNS attenuates the systemic inflammatory response to endotoxin
VNS and level of NOS [86]	Dogs with coronary microembolization–induced chronic heart failure	Improve LV performance Normalization of NOS mRNA	Dysregulation of the nitric pathway can have a direct adverse impact on heart failure state

VNS vagal nerve stimulation, *CHF* congestive heart failure, *NOS* nitric oxide synthase, *LVEDV* left ventricular end-diastolic volume, *LVESV* left ventricle end-systolic volume, *NE* norepinephrine, *Ang-II* angiotensin II, *CRP* C-reactive protein, *TNF* tumor necrosis factor

As discussed elsewhere in this Issue, in a canine model of intracoronary microembolization-induced heart failure, chronic vagal stimulation was shown to exert positive effects on left ventricular function that were additive to those conferred by beta-blockers therapy [2]. The impact of chronic cervical vagus nerve stimulation on heart failure development was also tested in a canine high-rate ventricular pacing model [1]. In this study, the impact of enhancing vagal tone (via chronic cervical vagus nerve stimulation) on heart failure development in a canine high-rate (220 beats/min) ventricular pacing model was tested in 15 dogs randomized to control or vagal nerve stimulation. At 4 and 8 weeks of pacing, both left ventricular end-diastolic and end-systolic volumes were lower, and left ventricular ejection fraction was higher in the group undergoing vagal nerve stimulation. Therapeutic benefit was associated with pronounced anti-inflammatory effects as determined by reduction in CRP levels. Chronic vagal nerve stimulation improved cardiac autonomic control and significantly attenuated heart failure development [1].

A vagus nerve stimulation implantable system can deliver pulses synchronous with heart beats through a multiple contact bipolar cuff electrode. This system has been used in selected heart failure patients. The preliminary efficacy results appear promising (Fig. 1; Table 2) [5, 6]. There was a significant improvement in NYHA functional class, Minnesota Quality–of-Life score and left ventricular end-systolic volume and a favorable trend toward reduction in end-diastolic volume. The exact mechanisms underlying the beneficial effects of vagal nerve stimulation are unknown at this point, but an anti-inflammatory effect is one possible explanation. These findings suggest the opportunity to proceed with a larger multicenter study [3]. Vagal nerve stimulation may ultimately play a contributory role, in the management of heart failure.

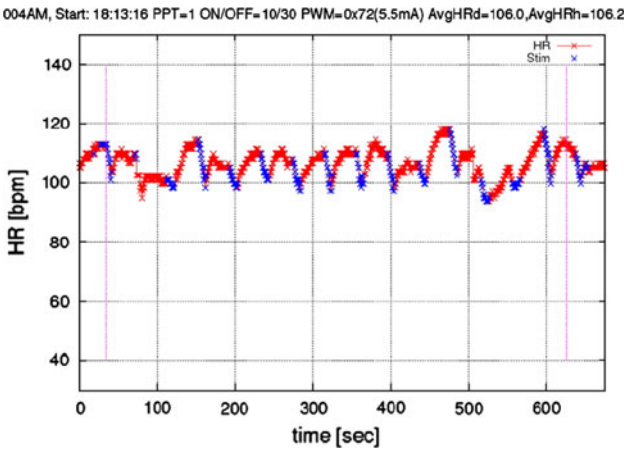

Fig. 1 An example of heart rate reduction during vagal stimulation (5.5 mAmp, 10-s ON time: *red* (*gray*) *crosses*, 30-s OFF time: *blue* (*black*) *crosses*). A reduction of almost 10 beats/min from a baseline heart rate of 110 beats/min occurs during the 10-s train of pulses (from Schwartz et al. [5]). (Color figure online)

Nitric oxide and vagus nerve stimulation

Nitric oxide and nitric oxide synthase

Catalyzed by nitric oxide synthase (NOS), nitric oxide (NO) is generated by the oxidation and conversion of L-arginine to L-citrulline. NO, a highly reactive free radical oxygen species, has multiple roles in cardiovascular

Table 2 Clinical variables after vagal nerve stimulation during follow-up

Variable	Baseline	1 month	3 months	6 months	ANOVA
HR (beats/min)	87 ± 13	78 ± 13	79 ± 13	83 ± 12	0.01
NYHA class (I/II/III/IV)	0/1/7/0	0/7/1/0	0/8/0/0	1/3/4/0	<0.01**
Minnesota QoL	52 ± 14	21 ± 9	25 ± 10	31 ± 18	0.001
6MWT (m)	405 ± 43	462 ± 87	480 ± 95	446 ± 99	0.04
LVEDV (ml)	273 ± S1	242 ± 66	248 ± 73	250 ± 82	0.13
LVESV (ml)	208 ± 71	174 ± 60	184 ± 75	190 ± 83	0.03
LVEF(%)	24 ± 5	29 ± 10	27 ± 12	26 ± 10	0.2
IL-6 (pg/ml)	19.7 ± 12.7	18.2 ± 12.8	14.4 ± 18.5*	26.9 ± 23	0.18

From Schwartz et al. [5]

HR heart rate, *QoL* quality of life, *6MWT* 6-min walk test, *LVEDV* left ventricle end-diastolic volume, *LVESV* left ventricle end-systolic volume, *LVEF* left ventricle ejection fraction, *IL-6* interleukin 6

** Significance was tested by Friedman's test

* ANOVA $P = 0.02$

regulation. It can bind to the heme group of soluble guanylyl cyclase and increase cGMP production. cGMP can activate cyclic nucleotide-gated ion channels and protein kinase G. cGMP-independent effects occur mainly via *S*-nitrosylation, an important protein modification related to cell signaling. NO can also directly activate adenylate cyclase, thus increasing cAMP levels and myocardial contractility. This is mediated through stimulating adenylate cyclase or inhibiting cAMP breakdown by phosphodiesterase 2 or 3 [54]. Similar to inflammatory cytokines, NO elevation can be potentially harmful or helpful in patients with heart failure.

NO has diverse, and often opposing, cardiovascular effects that may be due to the heterogeneity of NOS isoform expression. NOS isoforms exert a variety of location-specific effects [51]. Three isoforms exist: endothelial (eNOS/NOS III), neuronal (nNOS/NOS I) and inducible (inflammatory) NOS (iNOS/NOS II) [52]. All isoforms are present within the heart and are localized subcellularly.

eNOS is expressed in sinoatrial node cells and endothelial cells. In cardiac myocytes, left ventricular epicardial myocytes have more eNOS compared to the endocardial myocytes. eNOS activity is coupled with muscarinic receptor stimulation [55]. eNOS signaling limits the cardiac response to β-adrenergic receptor stimulation by reducing I Ca^{2+} and protects against arrhythmias [51, 56]. In addition to potential antiarrhythmic effects, eNOS signaling can also limit remodeling. Chronic pressure overload in eNOS−/− mice resulted in concentric left ventricular hypertrophy without left ventricular dilation and impaired systolic and diastolic function, suggesting that eNOS limits left ventricular remodeling and dysfunction [57].

nNOS, present in cardiac myocytes, cardiac ganglion cells and nerve fibers innervating the sinoatrial node co-localizes with choline acetyltransferase in intracardiac neurons [58]. In addition to affecting the β-adrenergic receptor pathway [51, 59, 60], nNOS can regulate the force–frequency response in cardiac myocytes. nNOS−/− myocytes have blunted force–frequency response, decreased Ca^{2+} transients and reduced cell shortening amplitudes and prolonged decline of Ca^{2+} currents [61]. nNOS and eNOS are constitutively expressed and are regulated by calcium/calmodulin-dependent as well as calcium-independent mechanisms.

iNOS is expressed in response to inflammation and has been shown to be present during many pathophysiological conditions, such as ischemia–reperfusion injury, sepsis and heart failure. iNOS produces much higher levels of NO compared to the constitutive NOS isoforms [62, 63]. In ischemia–reperfusion injury model, iNOS expression contributes to myocardial dysfunction and extent of infarct [64]. Inhibition of iNOS protects against myocardial dysfunction in sepsis [65]. iNOS elevation in failing hearts correlates with a decreased response to β-adrenergic stimulation [66].

The role of NO in congestive heart failure

Many NO-induced effects are cardioprotective. NO can inhibit ischemia/reperfusion injury, repress inflammation and prevent left ventricular remodeling. However, if NOS becomes uncoupled, reactive oxygen species (ROS) formation with low NO bioavailability predisposes to cardiac damage [67]. Excess NO and co-existence of ROS with NO are injurious [68].

In humans with heart failure and in animals with experimentally induced heart failure, the expression of NOS isoforms is markedly altered [69, 70]. The expression, localization and specific activity of NOS isoforms in the myocardium from patients with dilated cardiomyopathy were different compared with patients who died from

noncardiac causes. Diseased hearts had significant increase in nNOS mRNA and protein expression and activity associated with the translocation of nNOS to the sarcolemma that localize with caveolin-3. Enhanced nNOS activity counteracted a decrease in eNOS expression and activity [69].

In patients with ischemic cardiomyopathy, eNOS activity and expression were reduced compared to those without cardiac decompensation [70]. The role of NOS in decompensated heart failure is controversial. eNOS might be beneficial, and downregulation of eNOS could be a result of decompensation or contribute to decompensation.

In contrast, iNOS activity and expression were significantly higher in failing hearts. eNOS activity and expression is downregulated in cardiac tissue from patients with left ventricular ejection fractions <35% and heart failure. iNOS may represent an alternative mechanism for NO production [70]. Data from a prospective study demonstrate that activation of iNOS in peripheral vessels, associated with proinflammatory cytokines in accordance with the severity of heart failure, is a marker for, or contributes to, adverse events in patients with CHF [71].

Long-term treatment with eNOS enhancer AVE9488 plays beneficial effect on left ventricular dysfunction and remodeling after myocardial infarction. Interventions that increase eNOS-derived NO may be a promising therapeutic approach for the amelioration of post-infarction ventricular remodeling and heart failure [72]. eNOS was also shown to mediate the beneficial effects of cardiovascular drugs commonly used in patients with heart failure [73]. eNOS acts as an "endogenous β-blocker" by restoring the sympathovagal balance and opposing excessive hypertrophy.

NO is also important in regulating excitation–contraction coupling in congestive heart failure as it modulates several key proteins. NO may modulate the ryanodine receptor on the cardiac sarcoplasmic reticulum. eNOS gene deletion can abolish the increase in spontaneous Ca^{+2} spark frequency in cardiomyocytes exposed to sustained stretch, whereas the effect of nNOS-derived NO on RyR2 function remains to be investigated [74]. iNOS expression limits isoproterenol induced increase in contraction. A β-adrenergic hyporesponsiveness in human heart failure is mediated in large part by NO produced via iNOS within cardiac myocytes [66]. Uncoupled cardiac NOS also plays an important role in regulating cardiac diastolic function [75].

NO may modulate the transition from adaptive to maladaptive hypertrophy leading to heart failure, but three NOS isoforms can be neutral, protective or even adverse role in myocardial remodeling depending on the NOS isoforms. Different circuits in NO-signaling pathways in myocardium might be activated, and this principle is key to understand contradictions existing in NO biology in the heart [76].

NO and vagal nerve stimulation

NO may be involved in parasympathetic regulation of myocardial contractility [77]. The role of NO in parasympathetic inhibition of the β-adrenergic contractile response has been evaluated in vivo. Vagal stimulation could attenuate the inotropic response to dobutamine in normal canines. Infusion of a NOS inhibitor reduced the impact of vagal nerve inhibition, whereas infusion of a NOS substrate had the opposite effect [77]. These data suggest that the NO mediates, at least in part, vagal inhibition of the inotropic response to β-adrenergic stimulation and may play a role in normal physiologic regulation of myocardial autonomic responses.

Vagus nerve stimulation causes nNOS activation in the left ventricle and leads to NO release. Isolated innervated rabbit hearts were employed with the use of the NO fluorescent indicator 4, 5-diaminofluorescein diacetate (DAF-2 DA) during stimulation of the cervical vagus nerves [78]. NO-dependent fluorescence was increased in the ventricle in a stimulation frequency-dependent manner during vagus nerve stimulation. This was abolished with a nonspecific NOS inhibitor and an nNOS selective inhibitor [78]. These data suggest that nNOS mediates vagal stimulation-induced NO release. Thus, vagal stimulation may impact HF via a NO-mediated mechanism. Vagal stimulation-induced NO release was not associated with any change in perfusion pressure, suggesting that eNOS is unlikely to contribute significantly to the increase in ventricular NO during vagal stimulation.

Electrical stimulation of the vagus nerve has a strong antiarrhythmic effect in the rabbit ventricle against ventricular fibrillation [79]. Vagal nerve stimulation affects effective refractory period, ventricular fibrillation threshold and electrical restitution. These effects, blocked in the presence of the NOS inhibitor, N(G)-nitro-L-arginine (L-NA), provide indirect evidence that NO is involved. NO can significantly modulate the heart rate response to vagal nerve stimulation without prior adrenergic stimulation [80]. In isolated right atria-vagal nerve preparations from guinea pig, nonspecific NOS and specific nNOS inhibitors can significantly reduce the negative chronotropic response to vagal nerve stimulation [81]. The effect of nNOS on vagal activity was also tested in vivo. nNOS inhibitor can attenuate vagally evoked bradycardia in ferrets and guinea pigs [82]. nNOS−/− mice have a higher basal mean heart rate and lower heart rate variability versus wild-type mice. In nNOS−/− mice, atropine administration led to significant smaller change in mean heart rate and in heart rate variability than in wild-type mice [83]. In an isolated

atria-vagus preparation, nNOS−/− mice had impaired vagal bradycardia compared with wild-type controls [58].

NO may act post-synaptically as a cotransmitter or presynaptically to modulate vagal neurotransmission [58, 83, 84]. In isolated guinea-pig atrial-right vagal nerve preparations, NO donors had no heart rate response effect to acetylcholine [84], but acetylcholine-induced bradycardia was intact in isolated atria from nNOS−/− mice [58]. This pathway may augment the heart rate response to vagal nerve stimulation by increasing presynaptic calcium influx and vesicular release of acetylcholine [58].

Dysregulation of the NO pathway can have a direct adverse impact on heart failure, particularly with respect to left ventricular performance. In dogs with coronary microembolization–induced chronic heart failure, long-term vagus nerve stimulation can improve left ventricular performance, and this is associated with normalization of NOS mRNA [85, 86]. Due to the heterogeneity of the expression and activity of NOS isoforms, NO often has diverse and opposing cardiovascular effects. Future studies need to evaluate whether modulating NOS with vagal nerve stimulation can improve cardiac performance in patients with congestive heart failure.

Conclusion

Levels of inflammatory cytokines and NOS are elevated in heart failure, but the effects of these elevated levels remain uncertain. Vagal nerve stimulation can modulate the inflammatory response and NOS production and, while potentially important, challenges remain with regard to understanding how, and whether, modulation of cytokines and NOS in patients with heart failure results in beneficial effects.

Acknowledgments We gratefully acknowledge Drs. K. J. Tracy, R. Felder and M. Chapleau for helpful discussions and comments on the manuscript.

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