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Evidence for impaired vagus nerve activity in heart failure

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Abstract Parasympathetic control of the heart via the vagus nerve is the primary mechanism that regulates beat-to-beat control of heart rate. Additionally, the vagus nerve exerts significant effects at the AV node, as well as effects on both atrial and ventricular myocardium. Vagal control is abnormal in heart failure, occurring at early stages of left ventricular dysfunction, and this reduced vagal function is associated with worse outcomes in patients following myocardial infarction and with heart failure. While central control mechanisms are abnormal, one of the primary sites of attenuated vagal control is at the level of the parasympathetic ganglion. It remains to be seen whether or not preventing or treating abnormal vagal control of the heart improves prognosis.

Keywords Autonomic control · Parasympathetic · Muscarinic receptors · Nicotinic receptors · Intrinsic ganglia · Heart failure

Understanding the complex neurohormonal components and the central role of sympathoexcitation to the progression of heart failure (HF) has resulted in the development of pharmacologic protocols that target the sympathetic nervous system in the clinical treatment of HF. Adding beta blocker therapy alone has resulted in significant improvements in clinical outcomes in HF with a 35% reduction in mortality [1] and has highlighted the importance of the role the autonomic nervous system plays in disease progression [2–4].

Harnessing the parasympathetic limb of the autonomic nervous system as a treatment modality in HF may not be so straight forward, however. Modulating the parasympathetic nervous system will require a fundamental understanding of the abnormalities that are present in HF and the mechanisms that lead to these abnormalities. Dysfunction of the parasympathetic nervous system has been documented early in the development of HF in both animal models and humans [5–7] raising the possibility that stimulation of preganglionic vagal fibers may be ineffective once HF has been diagnosed and parasympathetic dysfunction has ensued. On the other hand, there is evidence that the vagal dysfunction seen in HF may be reversible [8], in which case re-setting autonomic tone to a more physiologic balance may be achievable. In this paper, we will review the current evidence for vagus nerve dysfunction in HF and discuss the significance of the abnormalities that may have relevance to the therapeutic intervention of vagus nerve stimulation.

Vagal pathways to the heart

Parasympathetic control of the heart involves a highly regulated series of mechanisms culminating in profound effects on the heart, including the SA node, the AV node, and to lesser degrees on atrial and ventricular myocardium. In order to understand impairments of vagal nerve activity

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in HF, it is necessary to understand normal anatomy and the physiology of vagal efferent pathways.

Preganglionic parasympathetic neurons arise in the caudal ventrolateral medulla [9] and enter the heart between the right and left superior pulmonary veins and along the superior vena cava, forming a synapse with the postganglionic neuron within the target organ [10, 11]. The postganglionic neuron therefore lies completely within the heart (see Fig. 1), and in the dog and human, this ganglion is located in discrete clusters on the surface of the heart in well-described anatomical locations [12–14]. A cardiotopic organization of parasympathetic control to sinoatrial versus atrioventricular nodal function extends from the central nervous system [15] to the level of the heart. The right atrial ganglionated plexus at the junction of the pulmonary veins and right atrium controls SA nodal function, while the plexus at the junction of the inferior vena cava and left atrium controls AV nodal function [16]. Furthermore, there is now abundant evidence that complex interactions occur in networks of neurons within the heart that are affected by spinal cord afferents as well as preganglionic input from the vagus [17, 18]. At the level of the ganglionic synapse, acetylcholine is released from the preganglionic nerve terminal and binds to nicotinic acetylcholine receptors (nAChR) located on the postganglionic neuron. In response to activation of nAChR, the postganglionic neuron depolarizes and results in the release of acetylcholine at the level of the target organ, predominantly in the SA and AV nodes. Release of acetylcholine in the SA node activates M2 muscarinic receptors, slowing HR by increasing the inward current I(f) at diastolic potentials via changes in cAMP levels and prolonging phase 4 repolarization [19].

The action of acetylcholine at the synapse

The onset and withdrawal of parasympathetic control on heart rate occurs extremely rapidly. Heart reduction in response to vagal stimulation is virtually instantaneous, resulting in a "square wave" response. Moreover, with cessation of stimulation, there is a rapid "off" response due to the effects of acetylcholinesterase that is present in the synaptic cleft and degrades unbound acetylcholine, producing inactive acetate and choline. The relative abundance of acetylcholinesterase, along with its remarkably efficient function to degrade acetylcholine [20], results in these rapid effects of the vagus on the sinus node. This contrasts to sympathetic effects in which the effects of norepinephrine release are longer acting (over seconds), since norepinephrine is dependent primarily upon reuptake mechanisms to be cleared from the synapse and second messengers need to be generated.

While acetylcholine is also released elsewhere in the heart, the contribution of parasympathetic innervation to myocardial function appears to be modest at best, though direct effects of vagal stimulation have been shown to inhibit myocardial contractility. These effects are mediated in part by antagonizing the effects of the sympathetic nervous system ("accentuated antagonism"), but also persist in the presence of beta-blockade, so occur through direct mechanisms as well. These inhibitory effects appear to be greater in atrial rather than ventricular myocardium [21].

Intrinsic cardiac plexus

There is now a substantial body of evidence demonstrating that the intrinsic cardiac nervous system plays an active

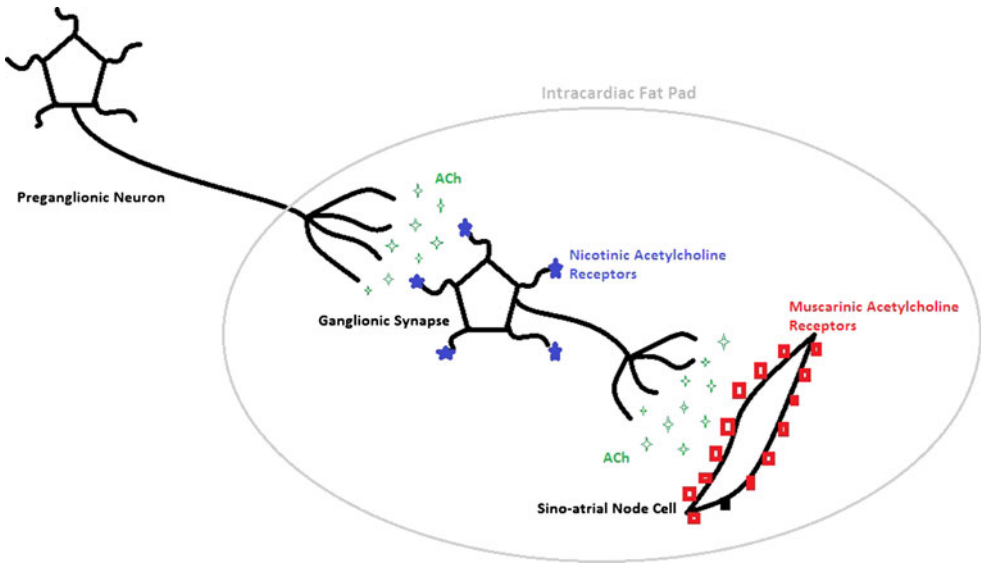

Fig. 1 Nervous pathways involved in vagal control of the heart

role in the regulation of cardiac function. The intrinsic cardiac nervous system contains a heterogeneous population of neurons that include parasympathetic, sympathetic, and afferent cell bodies that are interconnected in a complex network [22]. Specific clusters of intrinsic cardiac ganglia distributed throughout the heart are associated with control of different cardiac regions. For example, the cranial medial ventricular ganglionated plexus predominantly affects ventricular contractility [23], while neural control of dromotropic function is associated with the inferior vena cava–inferior atrial ganglionated plexus [16]. Regulation of atrial function including the sinus node is primarily modulated by the right atrial ganglionated plexus located at the base of the right pulmonary veins [10], but neurons in other intrinsic cardiac ganglia such as the posterior atrial ganglionated plexus may also contribute to the regulation of chronotropic function [24]. Although we have classically ascribed sympathetic and parasympathetic efferent postganglionic interactions to the end-organ cellular level, intrinsic cardiac neurons also seem to play an important role in mediating these interactions. Furukawa et al. [25] reported that a small number of vagal efferent axons that are not contained within the right atrial ganglionated plexus mediate some of the interactive effects between cardiac parasympathetic and sympathetic efferent neurons. Furthermore, there is evidence that the intrinsic cardiac nervous system contributes to the changes seen in heart failure. For example, the dose of nicotine needed to modify intrinsic cardiac neurons was 50 times greater in tachycardia-induced heart failure compared with normal preparations, and neurons demonstrating phasic activity in vitro displayed altered intracellular membrane properties compared with control, including decreased membrane resistance, indicative of reduced excitability [26]. The authors concluded that even early-stage heart failure differentially affects the intrinsic cardiac nervous system's capacity to regulate cardiodynamics.

Abnormal vagal control of the heart

Multiple lines of evidence have shown that parasympathetic control of HR is abnormal in patients with LV dysfunction and HF. Using the phenylephrine bolus technique, Eckberg et al. [27] showed over 40 years ago that arterial baroreflex control of HR was reduced in patients with LV dysfunction. Since that time, numerous groups of investigators have used a variety of techniques to support these findings, including time and frequency domain measures of RR variability, spectral analysis of HR, HR recovery following exercise, HR turbulence, and spontaneous or provoked arterial baroreflex testing [4, 28, 29]. Moreover, altered vagal control of HR is apparent early in the development of LV dysfunction. Using the rapid paced canine model of HF, we previously showed that altered vagal control of HR was apparent within 4 days of initiation of pacing, at a time when cardiac function is impaired minimally [7].

Reduced parasympathetic control portends a poor prognosis in patients both post-MI and in HF. Kleiger et al. [30] studied Holter recordings from 808 patients post-MI and found that HR variability was a significant predictor of mortality, even after adjusting for clinical and demographic features, including ejection fraction. This suggests that altered vagal control of HR is not simply a correlate of severity of LV dysfunction, but that abnormalities of vagal control may directly affect outcomes. In a prospective study following MI in humans, abnormal arterial baroreflex control of HR was predictive of poor outcomes [31]. Moreover, compared to dogs with normal arterial baroreflexes, those with reduced baroreflex control of HR fared more poorly following a subsequent induced MI [32]. Thus, for a given cardiovascular insult, intact baroreflexes appear to be protective against ischemic events and worsening LV dysfunction. Potential mechanisms of this protection include lesser degrees of ischemia, reduced electrical instability, and direct myocardial protective effects, possibly mediated by the release of nitric oxide [33, 34].

Despite the recognition of reduced vagal control in HF and its association with worse outcomes, investigation into the precise anatomical sites and mechanisms has been limited. Potential sites of abnormal vagal control could include a multitude of sites, including the generation of vagal impulses in the CNS, release of ACh by preganglionic fibers, altered signaling at the level of nAChR, release of ACh by postganglionic fibers, alterations in acetylcholinesterase activity, altered density of and/or binding to M2 muscarinic receptors, or intracellular signaling pathways. We have demonstrated previously that acetylcholinesterase is downregulated in HF and therefore not likely a mechanism responsible for diminished parasympathetic activity, since lower levels of acetylcholinesterase would result in more acetylcholine being available to activate nAChR and M2 muscarinic receptors and potentially serve to upregulate vagal activity in HF.

We and others have investigated alterations of M2 muscarinic receptors in HF and found that direct activation of M2 muscarinic receptors leads to enhanced responses in HF versus controls. This was accompanied by the upregulation of muscarinic receptors in the sinus node. These findings suggest that mechanisms at the level of the end-organ receptor and intracellular signaling are not downregulated in HF and therefore do not contribute to reduced vagal control of HR [35, 36].

Since mechanisms downstream to postganglionic fibers do not appear to be responsible for altered vagal control in

HF, we turned our attention to ganglionic mechanisms, including nAChR. nAChR are ligand-gated ion channels that are primarily permeable to cations. Upon binding of agonist (ACh) to receptor, a conformation change results in an influx of ions leading to excitatory postsynaptic currents (EPSCs) that may result in firing of an action potential and propagation of nerve impulses from the brain to the target organ. nAChR can be composed of various combinations of heterologous subunits that impart different ionic and ligand-binding characteristics allowing for diverse physiologic functions [37]. Eleven genes that encode neuronal nAChR have been identified. Based on homology to the muscle nAChR alpha subunit or the ability to substitute for the muscle beta subunit, eight of these have been characterized as alpha (alpha 2–9) and three as beta (beta 2–4). The composition of nAChR responsible for ganglionic transmission in mammalian autonomic neurons has been described by us and others in the last decade [38–40]. Interestingly, the subunits used to assemble a functional receptor are extremely plastic, and exchange of one subunit for another results in significant changes in electrophysiologic characteristics, making the nAChR a strong candidate for abnormal vagal tone in HF.

The parasympathetic ganglion acts as a bottleneck to efferent vagal traffic

In order to identify a specific locus or mechanism responsible for parasympathetic dysfunction in HF, we performed a series of experiments in the paced canine model of HF that included preganglionic as well postganglionic stimulation of parasympathetic nerves to the heart in control versus HF animals [5]. By stimulating preganglionic vagal fibers at the cervical vagus level, we were able to elicit brisk and potent slowing of SA node spontaneous firing resulting in profound bradycardia. At all intensities of electrical vagal stimulation tested, the HF group demonstrated significantly lower responses compared to controls confirming that a functional abnormality was present in the efferent limb. This finding has also been reported in other models of HF [41]. We then stimulated postganglionic neural fibers exclusively by direct electrical stimulation of the ganglion on the surface of the heart in the presence of hexamethonium (to remove all preganglionic influence). In this setting, stimulation of postganglionic fibers resulted in larger responses in the HF group compared to controls. This confirmed a number of concepts. First, an abnormality at the level of the ganglion was responsible for diminished parasympathetic control of the heart in HF. Second, it confirmed that enough acetylcholine could be released from the postganglionic parasympathetic nerve fibers to bind to end-organ muscarinic receptors for an augmented response in HF. This was supported by findings from Vatner et al. [36] who demonstrated that end-organ muscarinic receptors are upregulated in HF in a denervation supersensitivity fashion and that downstream G-protein signaling was intact. Therefore, given upregulated postsynaptic mechanisms, the decreased responses seen with preganglionic vagal stimulation were even more significant and thus amplified the significance of a ganglionic defect. The question therefore remained whether it was presynaptic release of acetylcholine from the preganglionic nerve terminal at the ganglion or ganglionic nAChR that were responsible for decreased vagal function. To test this concept, we developed an isolated atrial preparation perfused via the SA nodal artery. Incremental doses of DMPP, a nAChR agonist that activates all the subtypes of neuronal nAChR, were delivered. At all doses tested, DMPP elicited a decreased response in the HF group despite the presence of upregulated postsynaptic mechanisms. Stimulations in the presence of hexamethonium showed complete block of postganglionic responses. This confirmed that postganglionic responses were mediated via nicotinic mechanisms and that other signaling neurotransmitters and receptors were not responsible for primary synaptic signaling at this ganglion. These experiments therefore confirmed that a defect at the level of the ganglion was acting as a bottleneck to the efferent flow of traffic in vagal pathways to the heart in HF and that this specifically involved nAChR at the ganglion.

Nicotinic acetylcholine receptors demonstrate subunit plasticity leading to altered nAChR function

Although it is known that synaptic transmission at the level of the ganglion utilizes nAChR, the subunit composition of nAChR at this ganglion was not clear. To characterize the receptor subtypes responsible for synaptic transmission in parasympathetic pathways to the heart, Bibevski et al. [38] conducted a series of in vivo experiments using conotoxins specific for different combinations of alpha and beta subunits to block synaptic transmission. They were able to demonstrate that receptors containing alpha 3/beta 2 subunits are the primary species of nAChR responsible for transmission across parasympathetic pathways to the heart in dogs. Subsequent experiments in knockout mice deficient in the alpha3 subunit alone [40] or a combination of beta2 and beta4 subunits [39] demonstrated complete dysautonomia in mice and confirmed a primary functional role for receptors containing alpha3/beta2 subunits. Interestingly, knockout of the beta 2 subunit alone did not result in complete dysautonomia, suggesting that the beta 4 subunit would substitute for beta 2. The primary role of alpha3/beta2 nAChR in peripheral parasympathetic

pathways can probably be extended to other mammalian species since to date, a significant role has not been demonstrated for other subunit combinations in the peripheral autonomic nervous system. The question of whether beta 4 subunits may be substituted for beta 2 in pathologic states such as HF is therefore raised. We have subsequently performed a series of ganglionic blockade experiments with conotoxins in dogs with heart failure in order to assess shifts in subunits. In the HF group, the neurotoxin that was able to block ganglionic transmission in control conditions (alpha-conotoxin MII) was not as effective in HF, while blockade was still afforded by the non-specific nicotinic antagonist hexamethonium (unpublished data). This suggests that while there is a shift in the subunit composition of nAChR in HF, the specific mechanism responsible is still not fully elucidated.

It is likely that abnormalities of parasympathetic control in heart failure are not irreversible, as return of autonomic function to the native sinus node has been described following cardiac transplantation. Whether or not these abnormalities can be prevented from developing is another issue, but evidence suggests that this may be the case, as both ACE inhibition and beta-blockade can augment vagal control of heart rate [42, 43] Further, as opposed to high-affinity receptors that downregulate in the presence of chronic stimulation, nicotinic receptors can be upregulated by chronic exposure to agonist [44, 45]. This approach has been used in the paced canine model of heart failure, in which we showed that exposure with a nicotinic agonist during the development of HF resulted not only in preserved but also in supranormal effects of parasympathetic stimulation on the sinus node [8]. Early studies using chronic vagal stimulation suggest similar protective beneficial effects on autonomic function [46].

Modulators of parasympathetic function

There is an extensive list of neuromodulators that can impact upon parasympathetic function and sympathovagal balance both in vitro and in vivo. These neuromodulators can be intrinsic to the ganglia themselves or can be derived from the myocardium and act upon the intrinsic cardiac nervous system through paracrine effects (see Herring and Patterson, 2008 for review). Furthermore, there is a plethora of neuronal modulators that while having no impact on parasympathetic function directly can alter parasympathetic function over time by changing the expression of neurotransmitters and receptors within neurons. For example, neuronal growth factor (NGF) that is released from cardiac myocytes and parasympathetic neurons can have a dramatic impact upon intrinsic neuronal phenotype, and NGF levels are known to be decreased in HF [47]. Furthermore, other factors such as Neuregulin-1 have been shown to modulate parasympathetic activity [48], and neuregulin expression is altered in various models of HF [49]. This could have a profound effect on autonomic function in HF including changes in nAChR expression as described previously.

Other factors known to be significantly altered in HF that can impact more directly on parasympathetic function include nitric oxide (NO) and angiotensin II. Nitric oxide synthase is localized within intrinsic cardiac vagal neurons as well as stellate sympathetic ganglia. In parasympathetic neurons, NO serves to increase acetylcholine release via stimulation of soluble guanylate cylase [33, 50], and direct modulation of vagal control of cardiac activity has been demonstrated [51] suggesting that NO modulation of parasympathetic activity may be important [34, 52]. As described elsewhere in this issue, NO levels are known to be significantly altered in HF in complex patterns and could represent a significant component to vagal dysfunction in HF [34, 53]. Angiotensin II is elevated in HF and has been demonstrated to modulate autonomic function in both central and peripheral locations. Specifically, it has been demonstrated that administration of angiotensin II by intravenous route inhibited the bradycardia induced by vagal stimulation [54, 55]. Kawada et al. [56] demonstrated that angiotensin II may mediate these effects by decreasing the release of acetylcholine in response to vagal stimulation.

In summary, the parasympathetic nervous system becomes dysfunctional early in the development of HF beyond the obvious physiological withdrawal of spontaneous activity warranted by hypotension and cardiac dysfunction. The entire vagal pathway is under tremendous physiological pressure from circulating factors as well as those acting in autocrine and paracrine ways for altered function. The anatomical level of primary dysfunction seems to lie at the level of the ganglion since postganglionic mechanisms are upregulated and functional. Early evidence suggests that the nAChR subunit at the ganglion may be altered in HF resulting in decreased ganglionic transmission through the efferent pathway. It is yet to be determined at what stage of HF these changes can be reversed or overcome if at all. Chronic stimulation of vagal pathways early during the development of HF stands as a promising approach to reversing vagal dysfunction and augmenting the beneficial effects vagal activity on cardiovascular function in a state of chronic sympathoexcitation.

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