Prevention of diminished parasympathetic control of the heart in experimental heart failure

Steve Bibevski and Mark E. Dunlap

Department of Physiology and Biophysics, Case Western Reserve University, and Department of Medicine-Cardiology, Louis Stokes Cleveland Veterans Affairs Medical Center, Cleveland, Ohio 44106

Submitted 10 May 2004; accepted in final form 7 June 2004

Bibevski, Steve, and Mark E. Dunlap. Prevention of diminished parasympathetic control of the heart in experimental heart failure. Am J Physiol Heart Circ Physiol 287: H1780–H1785, 2004.—Decreased synaptic transmission in parasympathetic ganglia contributes to abnormal parasympathetic function in heart failure (HF). Because nicotinic ACh receptors (nAChR) mediate synaptic transmission at the ganglion and upregulate in response to chronic exposure to agonist in vitro, we tested the hypothesis that repeated exposures of ganglionic neurons to a nAChR agonist can prevent a loss of parasympathetic control in HF. Two sets of experiments were performed. In set 1, unpaced control dogs and dogs undergoing pacing-induced HF were treated with a repeated intravenous nicotinic agonist during the development of HF. Under conditions of sympathetic blockade, R-R responses to a bolus injection of 200 µg 1,1-dimethyl-4-phenylpyridinium iodide (DMPP; nicotinic agonist) were found to be increased five times over the untreated group after 6 wk. In experimental set 2, dogs treated with weekly DMPP injections and in HF were anesthetized and underwent electrical stimulation of the right vagus nerve, which showed sinus cycle length responses >10 times that of controls (P < 0.05). Complete ganglionic blockade with hexamethonium abolished all responses, confirming that synaptic transmission was mediated entirely by nAChRs in both controls and HF. Despite decreased ganglionic function leading to reduced parasympathetic control of the heart in HF, repeated exposure with a nicotinic agonist during the development of HF results in not only preserved but also supranormal effects of parasympathetic stimulation on the sinus node.

ganglion; nicotinic acetylcholine receptor; vagal; synapse

Heart failure (HF) is characterized by a chronic imbalance of the autonomic nervous system that contributes to the pathophysiology and progression of the disease (20, 33). The role of sympathetic activation in the disease process is well recognized and has been the subject of intense investigation over the past decade, culminating in the use of pharmacological blockade of β-adrenergic mechanisms as a key component in the current therapy of HF (9). Decreased parasympathetic control of the heart in HF also has been recognized since the early 1970s (16); however, the site(s) and mechanism(s) responsible for abnormal vagal function have remained largely unexplored. Under normal conditions, the parasympathetic nervous system exerts effects both directly on the sinus node and myocyte function as well as potent sympatholytic effects mediated via both pre- and postsynaptic mechanisms (29). Augmentation of parasympathetic activity in HF may therefore be beneficial by reducing cardiac norepinephrine (NE) spillover (30), thus reducing the subsequent detrimental effects of high levels of NE on the heart (19). In addition, decreased parasympathetic activity has been closely correlated with risk of sudden death, and previous reports have indicated that patients with left ventricular dysfunction but preserved parasympathetic function have a lower risk of arrhythmia, independent of sympathetic activity (10, 12, 41). Restoration, and perhaps augmentation, of parasympathetic function may therefore provide a more physiological addition to the treatment regimen for patients with HF or at high risk for sudden death. In this regard, attempts have been made in the past to augment parasympathetic innervation to the heart using direct muscarinic stimulation through drugs such as scopolamine (11, 27, 36, 47). These studies were, however, limited by pleiotropic side effects related to nonspecific muscarinic stimulation. There is now mounting evidence that nicotinic ACh receptors (nAChRs) may be a good target for modulation of the autonomic nervous system, and targeting the ganglion to augment parasympathetic activity to the heart may be a more feasible way to achieve increased vagal tone in high-risk patients. nAChRs are known to upregulate rather than downregulate in response to a chronic administration of agonist in cell culture (31, 39, 48). Upregulation of brain nAChRs in smokers and animals treated with nicotine has been described by various groups (6, 8, 38), suggesting that the phenomenon is not limited to cell culture and may therefore provide a novel approach to autonomic dysfunction in HF.

We undertook the present study to investigate the possible role of nicotinic receptor modulation in preventing parasympathetic dysfunction in HF. Specifically, we examined whether or not long-term repeated administration of a nicotinic agonist can prevent nAChR dysfunction in vivo.

Materials and Methods

Induction of HF

The technique used for induction of HF has been discussed by us previously in detail (13). All procedures were carried out according to institutional guidelines for the care and use of laboratory animals. Adult (9–12 mo) male Beagle dogs were anesthetized, and, after the left external jugular vein was exposed under sterile conditions, a Medtronic (Minneapolis, MN) pacing wire was placed in the apex of the right ventricle under fluoroscopic guidance. The lead was then attached to a pacemaker unit placed subcutaneously in the scapular region. Dogs were given Ciprofloxacin (250 mg bid) for 3 days postoperatively and allowed to recover for 7–10 days before the pacemaker was programmed at 250 beats/min. Pacing was continued until the animal showed clinical signs of congestive HF in 6–8 wk. These signs included ascites, tachypnea, rales, decreased appetite, pallor with slowed breathing, ascites, and tachypnea. Dogs were killed by an overdose of pentobarbital sodium after a period of 6 wk of pacing-induced heart failure.

Address for reprint requests and other correspondence: S. Bibevski, VA Medical Center 151W, 10701 East Blvd., Cleveland, OH 44106 (E-mail: steven.bibevski@case.edu).

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capillary refill time in the gums, and dilatation of the ventricles on echocardiographic assessment.

**Induction of nAChR Preservation**

All conscious studies were performed after the pacemaker had been switched off for 30 min. In conscious, unsedated dogs, we administered a total of 300 mg 1,1-dimethyl-4-phenylpiperazinium iodide (DMPP) intravenously in the brachial vein at days 0, 4, and 7 and once weekly thereafter until the development of overt HF. The dose of 300 mg was divided into two separate doses: one at 100 mg and the other at 200 mg. This was done to test the heart rate (HR) response to a low dose and high dose of DMPP as determined from dose-response curves (not shown). The treatment (nicotinic agonist) therefore acted both as the treatment and as the stimulus during developing HF. Previous work has suggested that such an intermittent delivery of nicotinic agonist is sufficient to modify nAChRs chronically because rats exposed to a single dose of nAChR agonist have profound effects on nAChR expression 11 days later (1) and effects of an acute nAChR agonist on Fos protein expression are attenuated after 7 days of initial exposure (40). Two groups of animals received repeated nicotinic agonist: control and HF (n = 5 for both groups). The control group had pacemakers implanted but were not paced. This group acted as a control for assessing the effect of repeated nicotinic agonist exposure without HF and was otherwise treated identically. The HF group was paced at 250 beats/min until the development of HF.

**Assessment of nAChR sensitivity to pharmacological agonist in conscious animals.** To assess the effects of repeated DMPP administration on parasympathetic function in the same dog over time, we measured R-R interval (from lead II) responses to a bolus injection of nicotinic agonist in conscious unsedated dogs (see Fig. 1 for a schematic of the site of action). Blood pressure was monitored continuously and noninvasively using a Finapres blood pressure monitor attached to the tail, which was validated against intra-arterial pressures initially (mean blood pressure from arterial port = 122 ± 3 mmHg vs. mean blood pressure from Finapres = 132 ± 11 mmHg, P > 0.05 by Mann-Whitney rank sum test, n = 13 recordings).

Because sympathetic and parasympathetic ganglia share nAChRs at the ganglionic synapse, DMPP administration would stimulate sympathetic-mediated vasoconstriction and tachycardia, causing reflex changes in parasympathetic activity, and therefore alter the response. To avoid this, we infused Labeletalol HCl slowly (0.5 mg/kg), a combined α- and β-adrenergic antagonist, before administering DMPP to produce adrenergic blockade. This allowed us to record the R-R response to selective parasympathetic cholinergic activation in response to DMPP independently of the underlying sympathetic substrate. To confirm that the entire sinus cycle length (SCL) response was mediated by muscarinic receptors at the sinoatrial (SA) node both at baseline and after the induction of HF, we gave atropine sulfate (2 mg) at the end of some experiments and repeated stimulation with DMPP.

**Assessment of nAChR sensitivity to vagal stimulation.** Animals were induced with thiopental sodium and anesthetized with α-chloralose (100 mg/kg) until the toe pinch reflex was absent. Supplemental chloralose was given via the same means every 30 min. After endotracheal intubation, the dogs were placed on a microprocessor-controlled respirator (Engler). Blood gas and pH were tightly controlled at normal physiological values, and a heating blanket maintained temperature in the physiological range (37–38°C). The femoral artery and vein were cannulated for continuous blood pressure monitoring and drug administration. The vagus nerve trunks were dissected and isolated at the cervical level for stimulation of preganglionic fibers through a single midline incision (see Fig. 1 for site). Each nerve trunk was ligated and then sectioned to prevent retrograde conduction to the brain. A bipolar electrode was inserted into the caudal remnant of the right vagus nerve for stimulation. The right vagus was used for stimulation because it has preferential input to the SA node (23). Stimulation was achieved using pulses delivered at 8 V, 1-ms duration, at 3, 5, and 10 Hz. A right thoracotomy was made at the fourth intercostal space, and a bipolar electrode was placed near the atrial appendage for recording of an atrial electrogram (spontaneous electrical bursts from the SA node) and measurement of SCL (time between successive atrial bursts), which served as a measure of the end-organ response to preganglionic stimulation. Hexamethonium bromide (5 mg) was given at the end of each experiment, and stimulation at 10 Hz was repeated to determine complete perfusion of the ganglion and the nAChR nature of synaptic transmission at the ganglion. Data from control dogs and DMPP-treated HF dogs were compared with data from dogs with HF but no DMPP treatment from previous studies done in our laboratory (3).

**Data Capture and Analysis**

ECG and electrogram signals were captured at 1,000 Hz with an analog-to-digital converter (Ponemah, Gould Instruments). SCL was graphically and numerically plotted online in real time by built-in software macros. Quantitative analysis of R-R responses was made using the peak response during the brief bradycardic period. Quantitative analysis of SCL was made using the specific data points averaged over 30 s during baseline and 15 s during stimulation. Statistical significance was determined by t-test, Mann-Whitney rank sum test, and repeated-measures ANOVA where appropriate using SigmaStat (SPSS; Chicago, IL). Data are presented as means ± SE.

**RESULTS**

Baseline characteristics of control and HF dogs are shown in Table 1.

**Induction of nAChR Preservation**

**Assessment of nAChR sensitivity to pharmacological agonist in conscious animals.** Figure 2 shows a time course of the R-R response to nAChR stimulation with bolus DMPP (200 mg) during the development of HF in conscious dogs. Bolus injec-

Fig. 1. Diagrammatic representation of the anatomic locations of receptors and stimulation (Stim) sites in the parasympathetic efferent pathway. ACh is the neurotransmitter at both ganglionic and end-organ synapses, but nicotinic ACh receptors (nAChRs) mediate signaling at the postganglionic neuron, whereas M2 muscarinic acetylcholine receptors mediate cardiac myocyte signaling. 1,1-Dimethyl-4-phenylpiperazinium iodide (DMPP) was used to stimulate nAChRs on the postganglionic neuron. Hexamethonium was used to block synaptic transmission at the ganglionic synapse (nicotinic), whereas atropine was used to block transmission at the end-organ synapse (muscarinic).
Table 1. Hemodynamic characteristics of control and HF dogs postvagotomy

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>HF</th>
</tr>
</thead>
<tbody>
<tr>
<td>HR, beats/min</td>
<td>109±8.8</td>
<td>145±7.6*</td>
</tr>
<tr>
<td>CO, l/min</td>
<td>1.3±0.29</td>
<td>0.58±0.07*</td>
</tr>
<tr>
<td>MBP, mmHg</td>
<td>122±13</td>
<td>80±5.0*</td>
</tr>
<tr>
<td>PA, mmHg</td>
<td>12.5±2.5</td>
<td>35±3.1*</td>
</tr>
<tr>
<td>PCWP, mmHg</td>
<td>5.5±0.5</td>
<td>24±3.8*</td>
</tr>
<tr>
<td>EF, %</td>
<td>72±9</td>
<td>23±7*</td>
</tr>
<tr>
<td>LVIDd, cm</td>
<td>2.9±0.5</td>
<td>4.3±0.6*</td>
</tr>
<tr>
<td>LVIDs, cm</td>
<td>1.4±0.3</td>
<td>4.3±0.4*</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 8 control and heart failure (HF) dogs. HR, heart rate; CO, cardiac output; MBP, mean blood pressure; PA, pulmonary artery pressure; PCWP, pulmonary capillary wedge pressure; EF, ejection fraction; LVIDd, left ventricular inner diameter in diastole; LVIDs, left ventricular inner diameter in systole. *Statistically significant difference; P < 0.05 (t-test).

tion of DMPP resulted in brief (2–5 beats) periods of bradycardia. The R-R responses were similar to baseline until overt signs of HF appeared at mean of 42 days (arrow), at which point the R-R response showed a trend toward increased responses. Figure 3 shows the group mean response to administration of a 200-μg bolus injection of DMPP at baseline (no treatment, no pacing) compared with 8 wk of treatment in paced (HF) and unpaced (control) dogs (1.5 ± 0.17 s at baseline vs. 2.2 ± 0.34 s after 7 wk of DMPP treatment in unpaced dogs, P > 0.05; and 1.45 ± 0.34 s at baseline vs. 8.46 ± 3.50 s after 8 wk of DMPP treatment in paced dogs, P < 0.05 by Mann-Whitney rank sum test). Instead of observing an overall decreased response to DMPP after pacing as seen in HF without DMPP treatment, we observed an exaggerated response compared with even normal animals. To confirm that the SCL response was completely mediated by cholinergic mechanisms at the neurocardiac synapse, we repeated the stimulation in the presence of atropine. Atropine abolished the response to DMPP (1.88 ± 0.4 s before atropine vs. 0.047 ± 0.018 s after atropine, P < 0.05 in control animals (n = 4); and 7.7 ± 3.3 vs. 0.056 ± 0.004 s in HF dogs, P < 0.05 by Mann-Whitney rank sum test (n = 4)], confirming an exclusive role of cholinergic mechanisms in mediating this response.

Assessment of nAChR sensitivity to vagal stimulation. Figure 4 shows the mean group responses to electrical stimulation of the right cervical vagus in three different groups of dogs: controls (n = 9), untreated HF (n = 5), and HF treated with DMPP (n = 7). The DMPP-treated group exhibited marked increases in response to electrical activation compared with both untreated HF and normal dogs (0.38 ± 0.09, 2.3 ± 1.7, and 6.2 ± 2.6 s in controls vs. 0.42 ± 0.07, 0.81 ± 0.17, and 2.8 ± 1.6 s in untreated HF vs. 1.6 ± 0.5, 12.8 ± 3.6, and 22.5 ± 2. s in DMPP-treated HF, P < 0.05 by repeated-measures ANOVA). Therefore, not only did DMPP treatment protect against decreased nicotinic receptor sensitivity but also it unmasked a supersensitive response in the HF group compared with control. Hexamethonium abolished the SCL response to 10-Hz electrical stimulation (Fig. 5), confirming that ganglionic transmission was mediated exclusively by nAChRs
Parasympathetic dysfunction in HF has been reported since the early 1970s (16), and its impact on morbidity and mortality in the disease has been underscored by trials showing poor clinical outcomes in patients with low resting vagal activity (26, 34). However, the specific mechanisms leading to parasympathetic dysfunction have been less studied, and attempts at overcoming defective vagal control have been met with significant hurdles. Our present study provides data to show that the “bottleneck” in parasympathetic efferent traffic at the ganglion is preventable with repeated exposure of nAChRs to agonist in vivo.

In HF, there is functional upregulation of M2 muscarinic mechanisms at the SA node at multiple anatomic and functional levels (5, 17, 18, 21, 46), which acts to augment parasympathetic function. We have previously shown that muscarinic receptors are increased 230% over control in HF (15) and that upregulated muscarinic mechanisms parlay into functional changes (3). The combination of upregulated postsynaptic mechanisms and the release of ACh from the postganglionic neuron is sufficient to cause augmented sinus node bradycardia in HF when ganglionic nAChRs are bypassed by directly stimulating postganglionic nerve fibers (3). The estimated contribution to actual function from these data is ~280% above control. These results thus support our previous finding that reduced ganglionic transmission is responsible for an attenuated control of HR in HF (3) and extend this work to show that the defect can be prevented in vivo modulation of nAChR function.

**nAChR Stimulation with DMPP**

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**nAChR Modulation by Repeated Exposures to Agonist**

One of the primary aims of this study was to determine whether the in vitro phenomenon of nAChR upregulation in response to nAChR agonist exposure is operative in vivo. Weekly administration of a nicotinic receptor agonist (DMPP) was sufficient to preserve nAChR-mediated ganglionic transmission in HF in our study. Moreover, our data show that such a treatment protocol not only preserves parasympathetic function but also leads to an augmented parasympathetic control of HR in HF in vivo. The explanation for the lack of effect in the control group compared with HF is most likely related to the lack of stimulus for muscarinic upregulation, as is seen in HF. The increased vagal control of HR seen in our experiments is therefore most likely related to preserved nAChR function in combination with increased muscarinic receptor function at the myocyte. The expression of muscarinic receptors and nAChRs has previously been shown to be differentially regulated; therefore, regulation of one class of receptor is not likely to impact on the other (42).

The mechanisms by which repeated DMPP treatment rescues ganglionic function in HF are likely to be multifactorial. Although studies have shown that chronic treatment with nicotinic agonist causes upregulation of receptor density (38, 39, 45, 48), there are important differences between those studies and ours. First, those studies were conducted predominantly in vitro and with continuous application of agonist to the cells expressing the receptor. Indeed, one of the postulated mechanisms by which the agonist induces upregulation involves first desensitizing the receptor or altering its conformation through occupation, which then triggers increased accumulation of the receptor within the cell and on the membrane (2, 37). This mechanism is not likely to explain our findings, because weekly administration of the agonist would not cause long-term desensitization or occupation of the receptors. The immediate effects of DMPP on SCL were not discernable within 30 min after administration in our experiments. Even if DMPP circulated within the system due to a long half-life, the receptors would likely only bind a small proportion of the circulating amount, unlikely to be enough to occupy receptors in the intracardiac ganglion for a week. However, studies have indicated that a single day of exposure to nicotine in rats has profound effects on nAChR expression eleven days later (1) and that effects of nicotine exposure on Fos expression can persist for 7 days (40). Our protocol involved injection of nicotinic agonist every 7 days, well within the time frame to allow for relative upregulation of receptor density.

A potential explanation to globally account for our findings involves reduced central output of parasympathetic drive in HF occurring early, during the development of left ventricular dysfunction (25), resulting in decreased nAChR function with a subsequent upregulation of muscarinic receptors. Repeated activation of nAChRs on the ganglia such as that performed in this study may therefore be sufficient to activate these receptors intermittently, resulting in preserved nAChR function rather than upregulation per se. In combination with increased muscarinic receptors at the SA node, these two mechanisms may yield the augmented parasympathetic function seen in our results. This latter mechanism seems most plausible because we did not see an increase in the SCL response to DMPP injection in the control dogs (no M2 upregulation) that were also treated with nicotinic agonist (Fig. 3). This would support the concept that the augmented response seen in HF is due to preserved nAChR in combination with upregulated muscarinic receptors at the SA node rather than upregulated nAChRs at the ganglion. At the molecular level, previous reports have indicated that presynaptic input and activity are required for
maintenance of nAChR synapse integrity (7, 28). Whether continued firing of action potentials in vagal pathways would lead to preservation of vagal function in HF remains an important question to be answered.

In line with this hypothesis, prevention of ganglionic dysfunction may involve preservation of nicotinic receptors and neuronal viability through effects on nerve growth factor (NGF). Although NGF is not an essential factor for survival of parasympathetic neurons, NGF has been shown to be decreased in the heart in HF (24). Parasympathetic neurons do express NGF (43), and there is preliminary evidence that these neurons express TrkA (the high-affinity receptors for NGF) (14) and may therefore be responsive to NGF. A recent report demonstrated TrkB expression, brain-derived nerve factor (BDNF) sensitivity, and regulation of nAChR expression by BDNF in peripheral parasympathetic ganglia (50). In unpublished observations, we have found that cardiac parasympathetic neurons are decreased in size and demonstrate bidirectional changes in nAChRs consistent with the effects of withdrawal of a factor such as NGF or BDNF. Moreover, nicotine exposure is known to modulate NGF levels (22, 32), and NGF is known to modulate nAChRs (35), suggesting that this entire scheme is a likely mechanism to account for the changes in this ganglion seen in HF.

Regardless of the specific mechanisms, DMPP treatment clearly leads to preservation of parasympathetic ganglionic function in HF. The clinical implications of these findings pose numerous questions. If central vagal drive is reduced in HF, restoring the efferent pathway may be insufficient to increase resting parasympathetic tone to the heart. On the other hand, reclaiming efferent parasympathetic function by preserving nAChR function may pave the way for means to augment resting parasympathetic function. Further studies are needed to determine whether augmented ganglionic transmission parleys into augmented parasympathetic control of the heart.

It is intriguing to speculate as to whether this modality might afford beneficial effects in patients with HF and whether the benefits of chronic exposure to nicotinic agonist might outweigh any negative effects. Particularly provoking is a recent report showing that patients with nonischemic HF who had smoked previously and quit for at least 2 yr had a slightly lower relative risk for HF hospitalization than those who had never smoked (44). A possible explanation may involve higher baseline levels of nAChR leading to somewhat preserved ganglionic function later in HF. Although such studies do not indicate that smoking is beneficial, they suggest that effects of nicotine may be beneficial in the autonomic neurons to the heart much like the effects reported for Alzheimer’s disease, addiction, memory, and schizophrenia in the central nervous system (49). Work is currently under way to determine the subunit composition of nAChRs in various anatomic and functional sites, which will aid in targeting pharmacological modalities more specifically with fewer side effects than currently available (4).

Finally, we did not address the issue of the effects of repeated nicotinic agonist on other organ systems. We did not observe any overt detrimental effects in our studies other than a marked prolongation of SCL leading to >20-s sinus arrest in some of the HF dogs. In view of the importance of parasympathetic control of the heart in protection from arrhythmia, and attenuation of sympathetic drive in HF, our findings of a role for nAChRs in parasympathetic dysfunction provide a pertinent and therapeutically viable approach to modulating parasympathetic function in vivo.

ACKNOWLEDGMENTS

We thank Herrick Finkelstein for the help in preparing this manuscript.

GRANTS

This study was supported by the Department of Veterans Affairs and by National Heart, Lung, and Blood Institute Grant HL-50669.

REFERENCES


