Mechanisms of altered vagal control in heart failure: influence of muscarinic receptors and acetylcholinesterase activity

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ABNORMAL BAROREFLEX CONTROL of heart rate in patients with cardiac dysfunction has been recognized for over 30 years (10). Abnormalities in this reflex could be located in the afferent limb, centrally, or in efferent control mechanisms. We (7), as well as other investigators (43), have found reduced sensitivity of baroreceptor afferent fibers in a dog model of heart failure (HF). In sinoatrial nodes, Bmax was increased (230 ± 75% of control) and acetylcholinesterase decreased (80 ± 6% of control) in HF. We conclude that muscarinic receptors are upregulated and acetylcholinesterase is reduced in the sinus node in HF. Therefore, reduced vagal control in HF is most likely due to changes of presynaptic function (ganglionic), because postsynaptic mechanisms augment vagal control in HF.

cholinergic; parasympathetic; autonomic

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in acetylcholinesterase activity that serve to increase parasympathetic function in HF.

**METHODS**

**Pacemaker Implantation**

Pacemakers and leads were implanted in seven mongrel dogs weighing 18–22 kg as described previously (8). Briefly, dogs were anesthetized with thiamylol sodium (10 mg/kg) and isoflurane (2–4%). A pacemaker lead was inserted into the right ventricular apex via the jugular vein using fluoroscopic guidance and sterile technique. The lead was tunneled subcutaneously to the interscapular area and connected to a custom-modified Medtronic generator, which was implanted into a pocket and closed in tissue layers. The pacemaker was inhibited, and the dogs were allowed to recover 5–7 days until all wounds showed adequate signs of healing. The pacemaker then was programmed to a rate of 250 beats/min, which was checked weekly by ECG monitoring. The presence of clinical HF was determined by the appearance of ascites, tachypnea, and echocardiographic assessment. Hemodynamic measurements were taken following induction of general anesthesia during the terminal protocol, which occurred within 1 wk of the studies in conscious dogs.

**Studies in Conscious Dogs**

Five control and seven HF dogs were used for the conscious studies. The dogs were acclimated to lie quietly before the study day. On the day of the protocol, the animal was connected to ECG electrodes, a catheter was placed in the brachiocephalic vein, and the dog was allowed to rest for at least 10 min. In the HF dogs, the pacemaker was inhibited before the 10-min rest period. All studies were performed with the same dogs in sinus rhythm. The same dogs were used for different conscious studies, allowing at least 24 h to elapse between successive studies, and the order of the studies was randomized in each animal. Blood pressure or breathing rate was not recorded during any of the conscious studies and was not controlled for during pharmacological challenge. The different studies in conscious dogs are listed below.

**Atropine.** Atropine was administered in cumulative doses of 1, 2, 3, 6, 12, 40, and 100 μg/kg. The low doses (2–3 μg/kg) were used to elicit a vagomimetic effect, and the highest dose was used for complete muscarinic blockade (17, 44). The concentration of each dose was adjusted to administer a volume of 2–6 ml. Baseline R-R interval was recorded for 5 min after administration of vehicle (5 ml saline). Successive doses of atropine were given as intravenous bolus injections followed by 3 ml of saline flush. Two minutes after each dose, the ECG was recorded for 5 min. Mean R-R interval and standard deviation of R-R interval (SD, a measure of resting vagal control (19)) were calculated from each 5-min epoch.

**Bethanechol.** The muscarinic agonist bethanechol [which is neither hydrolyzed by AChE nor crosses the blood-brain barrier (40)] was given in cumulative doses of 60, 120, and 180 μg/kg iv. R-R interval was recorded for 5-min epochs 10 min after each dose of bethanechol. Each dose was administered in a volume of 2–6 ml followed by 3 ml of saline flush. On another day, some of the control dogs were pretreated with the ganglionic blocker hexamethonium (2.5 mg/kg iv), and the same doses of bethanechol were repeated.

**Neostigmine.** The acetylcholinesterase inhibitor neostigmine was given in a single dose of 0.5 mg iv. Ten minutes after each dose of neostigmine, the R-R interval was recorded for 5 min.

**Saline.** A time control study with vehicle alone was performed in the HF dogs in which four doses of saline (5 ml iv) were given instead of the doses of atropine. R-R interval was recorded as in the atropine protocol described above.

**Vagal Stimulation**

The dogs were anesthetized with morphine sulfate (1 mg/kg) and α-chloralose (40–60 mg/kg iv) and placed on mechanical ventilation, and intra-arterial and venous catheters were inserted to monitor arterial blood pressure and allow for intravenous administration of fluids, respectively. Blood pressure was maintained at a constant level during the entire experiment. Both the right and left vagus nerves were isolated, ligated, and sectioned to prevent retrograde activation to identify the region of the sinus node [the crista terminalis, atrial appendage, and the junction of the right atrium and superior vena cava (30)]. The region of the atrioventricular node was dissected (inferior portion of the interatrial septum and floor of the atria close to the septum), and the left atrial appendage and left ventricle also were sampled. Each sample weighed between 0.3 and 0.9 g after blotting. Samples were minced thoroughly with scissors.

**Muscarinic Receptor Assays**

**Tissue preparation.** Five control and seven HF dogs contributed tissue for biochemical studies. The animals were euthanized (under anesthesia) by applying a 9-V charge to the left ventricle to produce ventricular fibrillation, and the heart was excised. The atria were dissected away from the left ventricle and frozen immediately at −70°C. Tissue from one control and one HF dog was processed in parallel. The atria were thawed slowly on ice. Anatomic landmarks were used to identify the region of the sinus node (the crista terminalis, atrial appendage, and the right atrioventricular node was dissected (inferior portion of the interatrial septum and floor of the atria close to the septum), and the left atrial appendage and left ventricle also were sampled. Each sample weighed between 0.3 and 0.9 g after blotting. Samples were minced thoroughly with scissors.

**Membrane preparation.** Cardiac cell membranes were isolated by a modification of previous methods (13). Minced samples each were homogenized by using a polytron (Tekmar Tissuemizer, setting 80 for 2 × 15 s) in 15 ml of ice-cold HEPES-buffered isotonic sucrose (pH brought to 7.4 with Tris base) containing the protease inhibitors (1,10)-phenanthroline (100 μM) and phenylmethylsulfonyl fluoride (50 μM) to inhibit degradation of receptor protein. Homogenates were centrifuged at 1,000 g for 5 min at 4°C to remove nuclei and debris. The pellets (P1) were resuspended in 10 ml of homogenization buffer and centrifuged again at 1,000 g for 5 min. The combined supernatants were centrifuged at 75,000 g for 12 min at 4°C, and the resulting P2 pellet was resuspended in 1.5 ml of 50 mM Tris·HCl buffer (pH 7.7). A 0.5-ml aliquot was then used immediately in the acetylcholinesterase assay (see below). The remaining 1-ml suspension was diluted to 20 ml in Tris·HCl containing 5 mM EDTA. After centrifugation at 75,000 g for 12 min, the resulting membrane pellet was resuspended in Tris·HCl without EDTA, centrifuged again at 75,000 g for 12 min, flash frozen, and stored at −70°C for up to 6 wk.

**H2H}O quinuclidinyl benzilate binding assay.** Radioligand binding assays with [3H]quinuclidinyl benzilate ([3H]QNB) for determination of specific binding to muscarinic cholin-
Acetylcholinesterase Activity

Acetylcholinesterase activity was quantitated as described previously (34). Triton X-100 (1%) was used to disrupt cell membranes (New England Nuclear, Boston, MA), stored at −20°C in ethanol, and diluted in reagent water before assay. Bethanechol was obtained from Merck in sealed ampules and freshly diluted in reagent water for each assay. Other compounds were obtained from Research Biochemicals International (Natick, MA) or Sigma Chemical (St. Louis, MO).

Data Recording and Analysis

All ECG signals were digitized at 500 Hz using an analog-to-digital board (DATAQ Instruments) and stored on a personal computer. The signals were peak detected (CODAS software) and visually inspected to ensure appropriate R wave detection. Mean R-R interval and standard deviation (SD) of R-R interval for each 5-min epoch were calculated. Student’s t-test was used to compare maximum changes in R-R interval and SD of R-R interval to baseline, as well as to compare Bmax and acetylcholinesterase HF and control animals. For the bethanechol and vagal stimulation data, intergroup differences were analyzed by repeated measures ANOVA. A P value < 0.05 was used to indicate statistical significance. Data are presented as means ± SE.

Data from the biochemical studies were obtained as disintegrations per minute and transferred to the Equilibrium Binding Data Analysis program (24) for initial processing, and then 4 to 10 experiments were analyzed simultaneously by using the LIGAND program for nonlinear curve fitting (26). Protein assay data also were analyzed by nonlinear curve fitting.

RESULTS

The mean number of days of rapid ventricular pacing required to induce HF was 32 ± 4 days. Hemodynamic characteristics of the two groups of dogs are presented in Table 1. Pulmonary capillary wedge, right atrial, and mean pulmonary artery pressures were higher and cardiac output was lower in the HF group compared with controls. Mean arterial pressure tended to be lower in the HF group. These hemodynamic parameters are comparable to those obtained by other investigators using this model of HF, which we have reported previously (9).

Studies in Conscious Dogs

Atropine. Figure 1A and B, shows the responses of R-R interval and SD, respectively, to cumulative doses of atropine. Maximal R-R prolongation (Fig. 1A) and SD increase (Fig. 1B) occurred at the 2 µg/kg dose of atropine. Baseline R-R was 595 ± 53 ms and increased to 704 ± 68 ms (P = 0.07) following this dose of atropine. Baseline SD was 127 ± 37 ms and increased to 193 ± 49 ms (P = 0.04) at this dose. After high-dose atropine (100 µg/kg), R-R interval decreased by 308 ms (from 595 ± 53 to 287 ± 15 ms). In the HF group, vagomimetic effects were detectable following 2 µg/kg atropine in both the R-R interval (from 419 ± 25 to 465 ± 30 ms, P = 0.03) and SD (from 18 ± 4 to 34 ± 9 ms, P = 0.04), but both of these were significantly less than in the control group. High-dose atropine decreased R-R interval only by 32 ms (from 419 ± 25 to 387 ± 16 ms) in the HF group, confirming low resting vagal tone.

Bethanechol. The R-R interval responses to the direct muscarinic agonist bethanechol are illustrated in Fig. 2 for both control and HF groups. R-R interval increased in HF suggesting strong sensitivity to muscarinic agonist; however, in controls, R-R interval decreased with bethanechol. Because this decrease in R-R interval in controls probably was mediated by arterial baroreflexes due to reductions in blood pressure, three controls were pretreated with the ganglionic blocker hexamethonium (2.5 mg iv) and again given bethanechol. Under ganglionic blockade to eliminate reflex activation, R-R interval prolongation following bethanechol in the control group increased similar to the HF group, but the response was still more pronounced in the HF group compared with controls.

Neostigmine. In controls animals, the acetylcholinesterase inhibitor neostigmine (0.5 mg iv) increased R-R interval from 578 ± 62 to 717 ± 14 ms but resulted in no change in SD (93 ± 22 to 104 ± 11 ms). Four HF dogs also were given the same dose of neostigmine.

| Table 1. Baseline hemodynamics in control versus heart failure |
|------------------|------------------|------------------|
|                  | Heart Failure    | Control          | P Value |
| CO               | 1.5 ± 0.1        | 2.1 ± 0.2        | 0.02   |
| RAP              | 9.3 ± 1.1        | 0.2 ± 0.9        | 4×10⁻⁶ |
| MPA              | 37.9 ± 2.5       | 16.6 ± 2.7       | 6×10⁻⁶ |
| PCWP             | 25.6 ± 2.0       | 4.0 ± 1.2        | 3×10⁻⁸ |
| MAP              | 107 ± 4.0        | 120 ± 8.7        | 0.13   |

Values are means ± SE. CO, cardiac output (l/min); RAP, right atrial pressure (mmHg); MPA, mean pulmonary artery pressure (mmHg); PCWP, pulmonary capillary wedge pressure (mmHg); MAP, mean arterial pressure (mmHg). P values are for individual t-tests between groups.
Between 6 and 9 min later, three of these four HF dogs had profound bradycardia to the degree that the pacemaker (which was programmed to fire at the lowest possible setting of 30 beats/min) began firing. These animals required urgent administration of cholinergic blocking doses of atropine. Accurate measurement of the SCL was not possible but was >2,000 ms in the HF group (i.e., heart rate <30 beats/min), demonstrating profound sensitivity of postsynaptic cholinergic mechanisms.

Saline. To exclude the influence of time alone causing the vagomimetic effects observed in the HF group, vehicle (saline) was administered. There were no changes in R-R interval or SD over the same period of time in which the vagomimetic effects were noted in response to low-dose atropine (data not shown).

Vagal Stimulation

To demonstrate that a component of parasympathetic dysfunction lies in the peripheral efferent limb, we recorded R-R interval responses to right vagal stimulation (Fig. 3). Prolongation of R-R interval was attenuated in the HF dogs compared with controls, especially at higher stimulus frequencies (1 Hz = 79% of control, 3 Hz = 60% of control, 5 Hz = 35% of control, 7 Hz = 30% of control, and 10 Hz = 48% of control, P < 0.05). These data directly parallel findings in our previous report showing that a physiologically important component of parasympathetic dysfunction lies in the peripheral efferent limb.

Muscarinic Receptor Density and Function

**Binding properties of \[^{3}H\]QNB in canine atria.** The binding of \[^{3}H\]QNB to muscarinic ACh receptors membranes from each region of normal dog heart was specific, displayed high affinity, and was saturable. At a concentration of \[^{3}H\]QNB close to its affinity constant \(K_d\), over 80% of the total binding was specific as defined by inhibition with 4.0 μM atropine. In saturation binding assays, nonspecific binding was a linear function of radioligand concentration. The binding affinity of \[^{3}H\]QNB and the density of muscarinic binding sites \(B_{max}\) were within the range previously reported in other tissues and species.

**Muscarinic receptor density.** The \(B_{max}\) of muscarinic receptor binding sites in the region of the sinus node was increased nearly twofold in dogs with HF compared with controls assayed in parallel \(P < 0.001\) by

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**Fig. 1.** R-R interval (A) and standard deviation (SD) of R-R interval (B) responses in conscious control versus heart failure (HF) dogs. Atropine was administered in cumulative doses. There was a greater increase in R-R interval and SD of heart rate in the control dogs compared with HF at the lower doses.

**Fig. 2.** Change in R-R interval (from baseline) in response to cumulative doses of bethanechol in conscious control and HF dogs. Note the tachycardia in the control group. Because this was due to buffering by arterial baroreflexes, the doses were repeated in three control dogs following hexamethonium (Hex) to eliminate baroreflexes. R-R interval prolongation still was more marked in the HF group.

**Fig. 3.** Change in R-R interval (from baseline) in anesthetized control and HF dogs in response to right vagal stimulation (8 mV, 1.0 ms stimuli). R-R prolongation was attenuated in the HF group, especially at the higher stimulus frequencies.
paired t-test) (Fig. 4). In contrast, the density of muscarinic receptors in the atrioventricular node and left atrial appendage was not altered in HF. The $K_d$ of [3H]QNB was consistent between groups, confirming that the effect of HF on [3H]QNB binding reflected a specific change in receptor density.

High-affinity agonist binding sensitive to guanine nucleotides. In addition to changes in receptor density, changes in postreceptor mechanisms (including coupling to G proteins) may underlie functional alterations in muscarinic ACh transmission in HF. We examined agonist high-affinity binding and its regulation by guanine nucleotide as an index of the interactions between muscarinic receptors and G proteins. The muscarinic agonist bethanechol inhibited [3H]QNB binding to muscarinic receptors in a dose-dependent manner (data for sinus node shown in Fig. 5). For the sinus node samples, the inhibition curve for bethanechol (filled symbols) was best fit by a two-site model, indicating the presence of both high- and low-affinity sites. There were no differences in the values of $pK_i$ for either high- or low-affinity sites (Table 2) between control versus HF groups.

The addition of 0.1 mM GTPγS to irreversibly activate G proteins and prevent their interaction with receptors attenuated bethanechol binding and shifted the bethanechol inhibition curves to the right (open vs. filled symbols). These results confirm the presence of a significant population of high-affinity muscarinic receptors coupled to G protein in both groups. In the sinus node, right atrium, and left ventricle, the HF versus control curves were shifted to the right by comparable amounts, indicating similar proportions of high-versus low-affinity sites (Table 3). In contrast, in the atrioventricular node, there was a significantly higher proportion of high-affinity sites in the HF samples than controls, indicating facilitated coupling between muscarinic receptors and G proteins in the region of the atrioventricular node.

Acetylcholinesterase Activity

Initial rates were measured from the spectrophotometric trace recording and are presented in Fig. 6.
CHOLINERGIC MECHANISMS IN HF

Table 3. Percentage of receptors in the high-affinity state by site before and after addition of GTPγS

<table>
<thead>
<tr>
<th>Site</th>
<th>Heart Failure</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sinus node</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>50.0 ± 7.54</td>
<td>57.7 ± 4.6</td>
</tr>
<tr>
<td>With GTPγS</td>
<td>36.7 ± 11.5</td>
<td>36.9 ± 8.3</td>
</tr>
<tr>
<td>Atrioventricular node</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>69.2 ± 4.33*</td>
<td>46.9 ± 6.7</td>
</tr>
<tr>
<td>With GTPγS</td>
<td>23.0 ± 5.66</td>
<td>28.2 ± 6.4</td>
</tr>
<tr>
<td>Right atrium</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>26.6 ± 2.30</td>
<td>28.2 ± 4.32</td>
</tr>
<tr>
<td>With GTPγS</td>
<td>20.3 ± 6.29</td>
<td>9.1 ± 3.13</td>
</tr>
<tr>
<td>Left ventricle</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>44.9 ± 8.32</td>
<td>26.8 ± 2.20</td>
</tr>
<tr>
<td>With GTPγS</td>
<td>36.8 ± 7.2</td>
<td>14.4 ± 4.07</td>
</tr>
</tbody>
</table>

Values are means ± SE. Inhibition curves for methacholine competition at [3H]quinuclidinyl benzilate binding sites were analyzed by nonlinear curve fitting as described in METHODS. Affinity values obtained in the same analyses are given in Table 2. For inhibition curves in the presence of GTPγS, we assumed that affinity constants were not affected by guanine nucleotide and that an increase in low-affinity sites was responsible for the rightward shift in the inhibition curves. In separate computer modeling analyses, affinity constants were allowed to vary and there was no significant improvement in the residual variance in any group (P > 0.05, F-test).

Results from the order of cholinergic mechanisms contribute to diminished vagal control in HF but that bypass of the ganglion with direct electrical stimulation of postganglionic neurons showed augmented responses in HF (1). To determine whether this augmented response represented upregulation of all postsynaptic mechanisms in a “denervation supersensitivity” type manner, or whether there was a specific compensatory mechanism, we recorded R-R interval responses to the muscarinic agonists bethanecol (direct muscarinic stimulation) and neostigmine (acetylcholinesterase inhibitor). The data with bethanecol showed augmented responses in the HF group, suggesting that not only are muscarinic receptors upregulated, but they are functionally coupled through-out the cellular signaling cascade. Similarly, inhibition of acetylcholinesterase with neostigmine supported the conclusion that postsynaptic mechanisms are upregulated in HF. Although inhibition of acetylcholinesterase elicited a change in R-R interval of 140 ms in the control group, inhibition of the enzyme that degrades ACh at the synapse caused profound bradycardia in HF. Considering that our data on acetylcholinesterase activity showed decreased function of this enzyme in HF (see below), these findings add further support to the concept that postsynaptic mechanisms are upregulated.

To confirm the cellular mechanisms and specific sites responsible for these effects, we performed in vitro studies on myocardial samples from the HF and control groups and examined muscarinic receptor density and acetylcholinesterase activity. We found that muscarinic receptors were increased in number in the sinus node in HF and unchanged at other myocardial sites. There were similar numbers of receptors in the agonist high-affinity state in HF and controls, which reflects coupling of the receptor to G protein and presumably represents the functional receptor population (36). In addition, acetylcholinesterase activity was decreased in this same region of the heart in HF, which would also tend to promote cholinergic signal transduction by reducing degradation of ACh.

Cardiac muscarinic receptors show adaptive responses to changes in the level of receptor stimulation. Chronic treatment with potent agonists causes downregulation of receptors (35, 47), which may be associated with a loss of agonist high-affinity binding sites (23, 47). Conversely, an increase in receptor density has been reported following chronic muscarinic blockade with atropine (47). The increased receptor number and preserved (or augmented) receptor-G protein interactions observed in the present study are consistent with an adaptive response to reduced cholinergic stimulation.

There is evidence for an interaction between sympathetic and parasympathetic systems and their receptors within the heart. For example, chronic treatment with β-adrenergic antagonists downregulates muscarinic receptors while it upregulates β-adrenergic recep-
tors (25). Consistent with this reciprocal relationship, we have found that muscarinic receptors are upregulated in HF, at least within the sinus node, whereas β-adrenergic receptors are known to be downregulated under these same circumstances. It is possible that the chronic sympathoexcitatory state present in HF may contribute to the upregulation of muscarinic receptors found in this study.

In contrast to the present results, a previous study by Vatner and colleagues (42) reported that cardiac muscarinic receptors are decreased in dogs with HF due to pressure overload hypertrophy (42). However, aortic coarctation with accompanying cardiac hypertrophy has been reported to downregulate muscarinic receptors in the absence of HF in the rat (32). Thus the downregulation observed in the prior study (42) may have been due to hypertrophy alone rather than to HF. Alternatively, Vatner et al. (42) used ventricular myocardium for their assays, and the upregulation of muscarinic receptors found in our study was confined to the sinus node.

Site-Specific Differences in Muscarinic Receptor Density and Acetylcholinesterase

The differences we found in Bmax and acetylcholinesterase were confined to the region of the sinus node and were not present in the atrioventricular node or in left atrial appendage. Although this study could not determine the mechanism whereby these changes were confined to the sinus node, we speculate that it may have to do with site-specific changes in efferent vagal control. This selective effect on the sinus node may explain why a study using explanted hearts from patients with HF revealed no changes in muscarinic receptor density (3) because they did not selectively examine sinus node tissue.

Potential Mechanisms of Upregulated Muscarinic Transmission in HF

The HF dogs showed minimal R-R interval changes to muscarinic blocking doses of atropine. This suggests that resting vagal tone was markedly reduced in HF. Smith et al. (38) have shown that dogs with total cardiac denervation demonstrate increased responsiveness to ACh compared with controls, consistent with denervation supersensitivity. In 8-day-old chick hearts, Jo and colleagues (18) demonstrated recently that muscarinic receptor density increased 19% and 46% in the atria 1 and 4 days following vagotomy, respectively. These findings suggest that decreased vagal activity leads to denervation supersensitivity mediated at least in part by increases in muscarinic receptor density. Our results in HF dogs revealed both chronic decreases in vagal tone and muscarinic supersensitivity. We speculate that this supersensitivity may result from chronic decreases in central vagal activity in HF with resulting upregulation of muscarinic cholinergic receptors.

The mechanism by which muscarinic density is increased in HF is not known. Phosphorylation of muscarinic receptors (e.g., by protein kinase C) reduces their number. It has been shown that inhibition of protein kinase C increases muscarinic density in chicken hearts (5). It is possible that the balance of phosphorylation mechanisms may be altered in HF.

In patients with congenitally decreased acetylcholinesterase at the skeletal neuromuscular junction, ACh release at the junction is decreased (11). This suggests that ACh release and expression of acetylcholinesterase may be regulated in parallel. Whereas the mechanism is not known, this is analogous to the current findings in sinus node, where acetylcholinesterase was decreased. Jo et al. (18) were unable to show that vagotomy had any measurable effect on acetylcholinesterase in 8-day-old chicks, but these were studied only 4 days following bilateral vagotomy and may not have had time to compensate for reduced ACh release.

Where Are the Abnormalities in Vagal Control in HF?

These studies show that postsynaptic mechanisms (i.e., hydrolysis of ACh by acetylcholinesterase and density and function of muscarinic receptors) either augment or preserve vagal control in HF. Specifically, there is less breakdown of ACh and greater effect per stimulation of the muscarinic receptor site. Therefore, abnormalities of efferent vagal control must be due to presynaptic mechanisms, including decreased vagal nerve activity and/or ganglionic transmission, or altered synthesis or release of ACh. A previous report by Bibevski and Dunlap (1) has suggested that a component of parasympathetic dysfunction is located at the level of the ganglion. This is likely to involve changes in ACh release at the level of the ganglion or binding of ACh to nAChR or both. Further studies will be needed to identify which of these mechanisms also contribute to altered vagal control in HF. In addition, central processing or central output of vagal activity is likely to be altered in HF. In the early stages of HF, parasympathetic withdrawal is present (20) and likely to persist in the presence of increased sympathetic outflow. Parasympathetic withdrawal may be a contributing factor to abnormalities in the efferent limb over time. For example, it is well documented that both presynaptic and postsynaptic mechanisms contribute to the maintenance and development of nAChR at synapses (22). Further studies are underway by us to address these issues.

Clinical Significance

Patients with HF often have tachycardia. Although compensatory initially, tachycardia may not be well tolerated chronically. We have shown that muscarinic receptors are upregulated in HF. Treatment of the chronic tachycardia in HF by agents designed to stimulate muscarinic receptors may hold some potential as a new therapeutic modality, particularly because low doses of agonist might be expected to exert the largest effect on the sinus node.

Patients who demonstrate reduced vagally mediated sinus arrhythmia are at high risk for sudden cardiac
death following myocardial infarction (21), as are patients with HF. We have shown previously that transcutaneous scopolamine patches increase central vagal outflow in normal volunteers (6), and scopolamine patches also augment vagal control in patients following a myocardial infarction (4). A hypothesis that remains to be tested is whether or not chronically stimulating cholinergic pathways in patients with HF may lead to a lower incidence of sudden cardiac death in these patients. It is also known that vagal mechanisms are impaired in hypertension and aging. It is interesting to speculate that the mechanisms in the current study might contribute to altered autonomic balance in these conditions. If a common mechanism is responsible for the alterations reported here, methods aimed at restoring parasympathetic control could be extended to other clinically important conditions.

Limitations of Study

The experiments in this study were conducted in the pacing model of HF in the dog. Although this model has been shown to generate many of the features of HF in humans, tachycardia as the etiologic cause of HF in humans represents a small proportion of cases. It is possible that the tachycardia might have contributed to some of the changes described in this study, irrespective of the presence of HF. We think that this is unlikely for the following reasons. First, the ventricles, not the atria, were paced at a rate of 250 beats/min. In dogs undergoing ventricular pacing, most do not show intact ventricular-to-atrial conduction, and those that do display a 2:1 ratio (39). Therefore, a dog paced at 250 beats/min would experience atrial stimulation at 125 beats/min, a rate just slightly above normal and therefore unlikely to cause chronic changes in sinus node function. Second, whereas rapid atrial pacing has been shown to cause dramatic effects on sinus node function, no differences were found in sinus node function with rapid ventricular pacing (11). Finally, and perhaps most convincing that the effects seen here are not related to the pacing model per se, are the findings by other investigators that changes in muscarinic receptor function involving G proteins are consistent across pacing-induced HF in dogs (42) and dilated idiopathic cardiomyopathy in humans (15, 16, 23). Therefore, whereas we cannot exclude the effects of tachycardia alone, these results are more consistent with an effect due to the HF state itself.

These studies show that overall vagal efferent control of heart rate is reduced in HF despite postsynaptic compensatory mechanisms that aim to augment muscarinic control in HF. Specifically, muscarinic receptor density is increased, acetylcholinesterase is decreased, and sufficient Ach is released from synaptic terminals to impart synaptic signaling. We therefore conclude that reduced vagal control in HF must be due to abnormal presynaptic mechanisms, most likely involving abnormal function at the level of the ganglion.

REFERENCES


