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) induced by rapid pacing. Muscarinic blockade reduced the R-R interval by 308 ms in controls but only by 32 ms in HF, indicating low levels of resting vagal tone. Vagomimetic doses of atropine sulfate prolonged the R-R interval by 109 ms in controls and increased standard deviation of the R-R interval by 66 ms but only by 46 and 16 ms, respectively, in HF. Bradycardia elicited by electrical stimulation of the vagus nerve was also attenuated in the HF group. Conversely, muscarinic receptor activation by betahanechol, and indirectly by neostigmine, elicited exaggerated R-R interval responses in HF. To investigate possible mechanisms, we measured muscarinic receptor density (Bmax) and acetylcholinesterase activity in different areas of the heart. 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 thiopental 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 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) 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.[The right vagus nerve contributes the majority of parasympathetic fibers to the sinus node (48), the right cervical vagus was stimulated electrically using 8 V, 1-ms duration, and frequencies of 0.5, 1, 3, 5, 7, and 10 Hz presented in random order. This was done to obtain a “dose-response” curve of vagal stimulation to identify any differences at low versus high levels of vagal activity. Baseline R-R was recorded for 10 s, and the mean R-R interval during the final 10 s of a 20-s stimulation was compared with the control period.

**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 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.

**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 mMEDTA. 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.

**[3H]quinuclidinyl benzilate binding assay.** Radioligand binding assays with [3H]quinuclidinyl benzilate ([3H]QNB) for determination of specific binding to muscarinic cholin-
Cholinergic receptors were performed by a modification of methods described previously (12). Membranes were slowly thawed and resuspended in Tris·HCl buffer at a concentration of 0.1–0.2 mg protein/ml. Assays were conducted in a total volume of 250 μl in polystyrene 96-well plates (Beckman Macrowell), and each well contained 125 μl membrane suspension, 25 μl radioligand, and 100 μl drug or vehicle. Incubations were initiated by the addition of membrane and were carried out for 60 min at 25°C. Nonspecific binding was defined in the presence of 4.0 μM atropine. Incubations were terminated by vacuum filtration over Reves-Angel glass fiber filters using a cell harvester (Brandel). The filters were washed three times with 4 ml ice-cold Tris·HCl, placed in scintillation vials, covered with 4 ml scintillation cocktail (BioSafe II, Research Products International), and counted at 50% efficiency (Beckman LS5801). Protein was assayed by a modified Lowry method (29).

Materials. [3H]QNB (60 Ci/mmol) was obtained from 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).

Acetylcholinesterase Activity

Acetylcholinesterase activity was quantitated as described previously (34). Triton X-100 (1%) was used to avoid partial losses of activity (46). A 30% adjustment to approximate standard pH-stat assay conditions (33) was calculated assuming an apparent binding constant \(K_{\text{app}}\) of 100 μM and an active site pKₐ of 6.3. From the absorbance coefficient \(ΔA_{412\text{nm}}\) = 14.15 mM⁻¹ cm⁻¹ for thiolate dianion produced in the assay (31), one activity unit (1 μM of acetylcholine hydrolyzed per minute) was estimated to correspond to 3.67 \(ΔA_{412\text{nm}}/\text{min}\).

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 \(B_{\text{max}}\) 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 1, A 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**

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<tr>
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<th>Heart Failure</th>
<th>Control</th>
<th>P Value</th>
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<tbody>
<tr>
<td>CO</td>
<td>1.5 ± 0.1</td>
<td>2.1 ± 0.2</td>
<td>0.02</td>
</tr>
<tr>
<td>RAP</td>
<td>9.3 ± 1.1</td>
<td>0.2 ± 0.9</td>
<td>4 × 10⁻⁶</td>
</tr>
<tr>
<td>MPA</td>
<td>37.9 ± 2.5</td>
<td>16.6 ± 2.7</td>
<td>6 × 10⁻⁶</td>
</tr>
<tr>
<td>PCWP</td>
<td>25.6 ± 2.0</td>
<td>4.0 ± 1.2</td>
<td>3 × 10⁻⁶</td>
</tr>
<tr>
<td>MAP</td>
<td>107 ± 4.0</td>
<td>120 ± 8.7</td>
<td>0.13</td>
</tr>
</tbody>
</table>

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 [3H]QNB in canine atria. The binding of [3H]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 [3H]QNB close to its affinity constant (Kd), 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 [3H]QNB and the density of muscarinic binding sites (Bmax) were within the range previously reported in other tissues and species.

Muscarinic receptor density. The Bmax 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
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 $[\text{3H}]$QNB was consistent between groups, confirming that the effect of HF on $[\text{3H}]$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 $[\text{3H}]$-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 pKi 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.

Acetylcholinesterase was reduced significantly in the sinus node in HF but was unchanged in the atrioventricular node and left atrial appendage.

**DISCUSSION**

This study demonstrates that vagal control of heart rate is reduced, despite muscarinic receptor upregulation.
were not affected by guanine nucleotide and that an increase in by nonlinear curve responses in the HF dogs. This last compared with the controls. Third, right vagal stimulation ond, vagomimetic effects of low-dose atropine, al-

First, R-R interval responses to complete muscarinic decreases in acetylcholinesterase function are found in the sinoatrial node, the primary target of parasympathetic innervation of the heart.

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The percentage of receptors in the high-affinity state in HF and controls, which 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 down-regulation 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 innervation of the heart. Three different components of the current investigation examined overall efferent vagal control in HF. First, R-R interval responses to complete muscarinic receptor blockade with atropine (100 μg/kg) showed markedly less resting vagal tone in HF animals. Second, vagomimetic effects of low-dose atropine, although present, were markedly diminished in HF compared with the controls. Third, right vagal stimulation in anesthetized dogs revealed attenuated R-R interval responses in the HF dogs. This last finding is similar to a study by White (45) and by Bibevski and Dunlap (1), which demonstrated attenuated heart rate responses to right vagal stimulation in dogs with HF. These three findings show that overall vagal control of R-R interval is diminished in HF. We have shown previously that ganglionic 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 bethanechol (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.

<table>
<thead>
<tr>
<th>Site</th>
<th>Heart Failure</th>
<th>Control</th>
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<tbody>
<tr>
<td>Sinus node</td>
<td>50.0 ± 7.54</td>
<td>57.7 ± 4.6</td>
</tr>
<tr>
<td>Control</td>
<td>36.7 ± 11.5</td>
<td>36.9 ± 8.3</td>
</tr>
<tr>
<td>With GTPγS</td>
<td>69.2 ± 4.33x</td>
<td>46.9 ± 6.7</td>
</tr>
<tr>
<td>Atrioventricular node</td>
<td>23.0 ± 5.66</td>
<td>28.2 ± 6.4</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>Right atrium</td>
<td>44.9 ± 8.32</td>
<td>26.8 ± 2.20</td>
</tr>
<tr>
<td>Control</td>
<td>36.8 ± 7.2</td>
<td>14.4 ± 4.07</td>
</tr>
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Values are means ± SE. Affinity 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).

Fig. 6. Acetylcholinesterase activity (y-axis) at different sites in control and HF dogs. Acetylcholinesterase was reduced significantly in SAN and unchanged at other myocardial sites.
Carinic receptors found in our study was con
Alternatively, Vatner et al. (42) used ventricular myo-
downregulation observed in the prior study (42) may
trophy has been reported to downregulate muscarinic
aortic coarctation with accompanying cardiac hyper-
receptors are decreased in dogs with HF
by Vatner and colleagues (42) reported that cardiac
-upregulation of muscarinic receptors
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 hyper-
trophy 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 myo-
cardium for their assays, and the upregulation of musca-
rinic 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 acetylcholine-
sterase 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 deter-
mine the mechanism whereby these changes were con-
fined to the sinus node, we speculate that it may have
to do with site-specific changes in efferent vagal con-
trol. 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 respon-
siveness 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 super-
sensitivity. We speculate that this supersensitivity
may result from chronic decreases in central vagal
activity in HF with resulting upregulation of musca-
rinic cholinergic receptors.
The mechanism by which muscarinic density is in-
creased in HF is not known. Phosphorylation of musca-
rinic 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 acetylcholi-
nerase at the skeletal neuromuscular junction,
ACh release at the junction is decreased (11). This
suggests that ACh release and expression of acetylcho-
linesterase may be regulated in parallel. Whereas the
mechanism is not known, this is analogous to the
current findings in sinus node, where acetylcholinest-
erase was decreased. Jo et al. (18) were unable to show
that vagotomy had any measurable effect on acetylcho-
linesterase 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
diagnosinaptic mechanisms, including decreased vagal
nerve activity and/or ganglionic transmission, or al-
tered synthesis or release of ACh. A previous report by
Bibevsri and Dunlap (1) has suggested that a compo-
nent of parasympathetic dysfunction is located at the
level of the ganglon. This is likely to involve changes
in ACh release at the level of the ganglon 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, parasym-
pathetic withdrawal is present (20) and likely to per-
sist 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 presyn-
aptic 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 stim-
ulate 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

H1638 CHOLINERGIC MECHANISMS IN HF
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


