Parasympathetic Control of Cardiac Sympathetic Activity
Normal Ventricular Function Versus Congestive Heart Failure
Eduardo R. Azevedo, MD; John D. Parker, MD

Background—Muscarinic receptors on adrenergic nerve terminals attenuate norepinephrine release. The role of these receptors in the modulation of cardiac norepinephrine release in humans remains uncertain.

Methods and Results—Twelve patients with normal left ventricular (LV) function and 18 with congestive heart failure (CHF) were studied. A radiotracer technique was used to measure cardiac norepinephrine spillover (CANESP) in response to intracoronary acetylcholine (ACh, 5×10^{-5} Mol), and in response to intracoronary atropine (12 μg/min). ACh did not affect CANESP in the group of subjects with normal LV function, but it caused a significant reduction in those with CHF [197 (150 to 302) versus 168 (87 to 288) pmol/min, \(P<0.05\)]. Atropine caused a significant increase in CANESP in those with normal LV function [47 (27 to 51) versus 64 (38 to 139) pmol/min, \(P<0.05\)], but no change was observed in the CHF group.

Conclusions—Therefore, in the setting of heart failure and sympathetic activation, muscarinic receptor stimulation decreases CANESP, an effect not observed in patients with preserved LV function. Blockade of muscarinic receptors with atropine increased CANESP in patients with normal LV function, suggesting that cardiac parasympathetic tone has inhibitory effects on cardiac sympathetic activity. This basal inhibition was not observed in CHF patients in response to atropine. The lack of basal parasympathetic inhibition of cardiac sympathetic activity may play a role in the pathogenesis of cardiac sympathetic activation in heart failure. (Circulation. 1999;100:274-279.)

Key Words: nervous system, autonomic ■ acetylcholine ■ norepinephrine ■ receptors

Congestive heart failure (CHF) is characterized by elevated cardiac sympathetic activity, an abnormality that is felt to have important effects on the prognosis of this disease.1,2 Therapy aimed at counteracting this increase in adrenergic drive to the heart has been shown to be beneficial.3,4 The mechanisms responsible for cardiac sympathetic nervous system activity in patients with CHF are poorly understood. Although the process is likely multifactorial, one potential mechanism may involve abnormalities in parasympathetic neuronal outflow to the heart, which appears to be reduced in the setting of chronic heart failure.5,6

In isolated organ preparations and in animal models, local muscarinic receptors, when stimulated, inhibit norepinephrine release from adrenergic nerve terminals.7–10 Human in vitro data, although limited, confirms the presence and functional significance of muscarinic modulation of norepinephrine release from adrenergic nerve terminals.11,12 Indeed, in human papillary muscle, muscarinic receptor stimulation decreases norepinephrine release, whereas a muscarinic antagonist has the opposite effect.12 The exact location of these muscarinic receptors remains unclear. Although they are present on sympathetic nerve endings in a prejunctional distribution, it is now recognized that they play a role in neurotransmission within the intrinsic cardiac sympathetic nervous system. To date, no human in vivo data are available concerning the importance of this receptor pathway in the modulation of norepinephrine release.

The present study was designed to investigate the functional importance of local muscarinic receptors in the control of cardiac norepinephrine release in patients with normal ventricular function and heart failure. We hypothesize that muscarinic receptor stimulation with acetylcholine inhibits cardiac sympathetic efferent neuronal activity and that this effect is more prominent in the setting of CHF, a state of cardiac sympathetic activation.13 We also examine the effects of muscarinic receptor blockade on cardiac sympathetic neuronal spillover. In those with normal ventricular function, we hypothesize that atropine will cause an increase in cardiac sympathetic activity. In contrast, in patients with CHF we anticipate that atropine will have no effect on this index because heart failure also represents a state of parasympathetic withdrawal.5,6

Methods

Study Population
The study population consisted of 30 patients referred for angiography. Among the group with normal left ventricular (LV) function (n=12 patients; 6 males and 6 females), there were 8 patients with
normal coronary anatomy and 4 patients with either single or 2-vessel coronary disease. All patients had normal LV function. Six patients were hypertensive and 1 was diabetic. Medical therapy consisted of calcium channel blockers (n = 7), nitrates (n = 4), β-blockers (n = 3), diuretics (n = 2), and an angiotensin converting-enzyme inhibitor (n = 2).

Eighteen patients with a diagnosis of chronic heart failure secondary to a dilated cardiomyopathy were studied. All subjects had normal coronary arteries and an ejection fraction measured by radionuclide angiography of ≥ 35%. The etiology of the cardiomyopathy was idiopathic in 15 patients and ethanol-related in 3. Concomitant diseases included hypertension (n = 3) and diabetes (n = 4). Medical therapy consisted of diuretics (n = 18), angiotensin converting-enzyme inhibitors (n = 17), digoxin (n = 13), β-blockers (n = 4), amiodarone (n = 3), nitrates (n = 3), and hydralazine (n = 1). In both groups, medical therapy was held on the morning of the study.

This protocol was approved by the Ethical Review Committee for Human Experimentation of the University of Toronto. Written informed consent was obtained from all patients.

**Hemodynamic, Catecholamines, and Coronary Flow Measurements**

A diagnostic right and left heart catheterization was performed using a femoral approach without sedation. The pulmonary artery catheter was left in place after completion of the diagnostic procedure. A 7F coronary sinus thermodilution flow catheter (type CCS-7U-90B, Webster Laboratories) was inserted from an antecubital vein and positioned in the coronary sinus for flow measurements and blood sampling. A 7F left Judkins catheter (Cordis Laboratories) was advanced to the ostium of the left main coronary artery for intracoronary drug infusions. Systemic arterial pressure was monitored from an 8F sidearm sheath (Cordis Laboratories). The ECG, pulmonary artery pressure, and systemic arterial pressure were recorded on a strip-chart recorder. For each variable, the results were expressed as an average of 15 cardiac cycles. Arterial and coronary sinus blood samples were obtained for analysis of catecholamines at the end of each drug infusion. Coronary sinus blood flow measurements were performed in duplicate at each measurement point according to the method of Ganz et al.14

**Norepinephrine Spillover Measurements**

Sympathetic outflow was estimated by the measurement of cardiac and total body norepinephrine spillover, using techniques that are well established in our laboratory.15,16 For these measurements, tritiated norepinephrine (1.6 μCi/min with a 16 μCi priming bolus of L-[2,5,6-3H]norepinephrine; New England Nuclear) was infused into the femoral vein via a Harvard pump (model ‘11’, Harvard Apparatus Inc) to steady-state concentration in plasma. Cardiac norepinephrine spillover (CANESP) was calculated as follows:14

\[
\text{CANESP (pmol/min)} = \left( \text{NE}_{\text{cs}} - \text{NE}_{\text{art}} + (\text{NE}_{\text{art}} \times \text{NE}_{\text{cs}}) \right) \times \text{CSPF}
\]

where \(\text{NE}_{\text{cs}}\) and \(\text{NE}_{\text{art}}\) indicate coronary sinus and arterial plasma norepinephrine concentrations, respectively; \(\text{NE}_{\text{cs}}\), transcadiac fractional extraction of tritium-labeled norepinephrine; and CSPF, coronary sinus plasma flow.

**Analysis of Plasma Catecholamines**

Plasma catecholamines were analyzed using high-performance liquid chromatography with electrochemical detection, as previously described.15,17 The biochemical analysis was performed by personnel unaware of the patient status or the purpose of this experiment.

**Drug Infusion Protocols**

After the diagnostic heart catheterization and insertion of catheters for hemodynamic monitoring, patients were left undisturbed for a minimum of 20 minutes for tritium-labeled norepinephrine to reach steady state concentration in plasma. Two intracoronary drug infusion protocols (A and B) were performed. In protocol A, the effect of intracoronary acetylcholine was examined using the following sequence of drug infusions: 1) D2W, the vehicle for acetylcholine, was infused into the left main coronary artery at a rate of 1.25 mL/min, 2) acetylcholine was infused at a rate of 1.25 mL/min to achieve estimated intracoronary concentrations of 10⁻⁶ and 10⁻⁵ mol/L, 3) acetylcholine was discontinued and D2W was reinitiated at the same rate of 1.25 mL/min. Protocol B was designed to test the effects of atropine. The sequence of infusions was as follows: 1) D2W was infused intracoronary at the rate of 1.25 mL/min, 2) atropine was infused at a rate of 12 μg/min.18 We did not attempt to recontrol for the results of atropine because this drug has a half-life >2 hours.19 All drugs were infused into the left main coronary artery via a 7F Judkins catheter using a Harvard infusion pump. All measurements, including hemodynamics, coronary sinus blood flow, arterial, and coronary sinus blood sampling were performed after 10 minutes of each drug infusion. At the end of each infusion protocol, the position of the catheter was confirmed by injection of radiocontrast.

Twelve subjects with normal ventricular function were studied. Eight patients received acetylcholine (Protocol A) and 10 patients received atropine (Protocol B). Six patients participated in both protocols. Eighteen patients with CHF were studied. All patients participated in protocol A and 8 patients were also submitted to protocol B.

**Statistical Analysis**

Most variables were not normally distributed. Therefore, nonparametric tests were used for statistical analyses. Within-group comparisons of hemodynamics and norepinephrine kinetics were made by the Friedman repeated measures ANOVA on ranks with application of the Dunnett’s method for correction for multiple comparisons. Between-group comparisons of baseline characteristics were performed with a Mann-Whitney rank sum test. Between-group comparisons of observed changes were made using ANCOVA with the appropriate baseline as the covariate. Exact probability value were calculated using SigmaStat for Windows, version 2.0 (Jandel Scientific). \(P<0.05\) was required for statistical significance.

**Results**

**Baseline Characteristics**

The group of normal LV function subjects had essentially normal hemodynamics, except for mildly elevated blood pressure. Heart rate, pulmonary artery pressures, and cardiac norepinephrine spillover were significantly elevated in the CHF group compared with the group with normal ventricular function (Table 1).

**Hemodynamic Responses**

There were no significant hemodynamic changes in response to the intracoronary infusion of acetylcholine or atropine in either group. There was, however, a significant increase in

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**TABLE 1. Baseline Characteristics**

<table>
<thead>
<tr>
<th></th>
<th>Normal LV</th>
<th>CHF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>55 (46–63)</td>
<td>58 (49–63)</td>
</tr>
<tr>
<td>LVEF, %</td>
<td>59 (50–60)</td>
<td>18 (12–22)*</td>
</tr>
<tr>
<td>HR, bpm</td>
<td>66 (61–74)</td>
<td>87 (75–102)*</td>
</tr>
<tr>
<td>PAmax, mm Hg</td>
<td>18 (15–21)</td>
<td>31 (26–33)*</td>
</tr>
<tr>
<td>MAP, mm Hg</td>
<td>105 (92–113)</td>
<td>98 (80–104)</td>
</tr>
<tr>
<td>CANESP, pmol/min</td>
<td>50 (39–111)</td>
<td>197 (150–302)*</td>
</tr>
</tbody>
</table>

LVEF indicates left ventricular ejection fraction; HR, heart rate; PAmax, pulmonary artery mean pressure; MAP, mean arterial pressure; and CANESP, cardiac norepinephrine spillover. Data presented as median with 25th–75th percentiles. *P<0.01 vs normals.
TABLE 2. Responses to Acetylcholine in the Group With Normal LV Function

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>ACh</th>
<th>Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>HR, bpm</td>
<td>66 (62–75)</td>
<td>72 (66–78)</td>
<td>69 (60–74)</td>
</tr>
<tr>
<td>PAmax, mm Hg</td>
<td>18 (16–21)</td>
<td>20 (17–21)</td>
<td>17 (14–19)</td>
</tr>
<tr>
<td>MAP, mm Hg</td>
<td>111 (98–117)</td>
<td>111 (98–123)</td>
<td>110 (100–120)</td>
</tr>
<tr>
<td>CSPF, mL/min</td>
<td>60 (51–67)</td>
<td>81 (72–94)*</td>
<td>48 (42–58)</td>
</tr>
<tr>
<td>NEart, nmol/L</td>
<td>0.8 (0.6–2.1)</td>
<td>1.1 (0.9–2.3)*</td>
<td>1.1 (0.9–2.7)*</td>
</tr>
<tr>
<td>NEcs, nmol/L</td>
<td>1.0 (0.8–2.2)</td>
<td>0.9 (0.8–2.5)</td>
<td>1.1 (0.8–2.7)</td>
</tr>
<tr>
<td>CANESP, pmol/min</td>
<td>54 (43–111)</td>
<td>41 (20–157)</td>
<td>47 (31–98)</td>
</tr>
<tr>
<td>TBNESP, nmol/min</td>
<td>1.7 (1.1–4.5)</td>
<td>1.9 (1.4–4.4)</td>
<td>2.2 (1.2–5.2)</td>
</tr>
</tbody>
</table>

*P < 0.05 vs control.

TABLE 3. Responses to Acetylcholine in Patients With CHF

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>ACh</th>
<th>Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>HR, bpm</td>
<td>87 (75–102)</td>
<td>92 (73–103)</td>
<td>88 (73–105)</td>
</tr>
<tr>
<td>PAmax, mm Hg</td>
<td>31 (26–33)</td>
<td>31 (26–34)</td>
<td>32 (25–37)</td>
</tr>
<tr>
<td>MAP, mm Hg</td>
<td>98 (80–104)</td>
<td>99 (85–103)</td>
<td>99 (87–108)</td>
</tr>
<tr>
<td>CSPF, mL/min</td>
<td>96 (73–141)</td>
<td>116 (91–147)*</td>
<td>93 (85–138)</td>
</tr>
<tr>
<td>NEart, nmol/L</td>
<td>1.2 (0.8–3.2)</td>
<td>1.8 (1.0–3.1)</td>
<td>1.8 (1.0–3.2)</td>
</tr>
<tr>
<td>NEcs, nmol/L</td>
<td>2.8 (2.0–4.2)</td>
<td>2.0 (1.2–3.2)*</td>
<td>3.2 (2.1–4.3)</td>
</tr>
<tr>
<td>CANESP, pmol/min</td>
<td>197 (150–302)</td>
<td>168 (87–288)*</td>
<td>200 (135–367)</td>
</tr>
<tr>
<td>TBNESP, nmol/min</td>
<td>2.5 (1.8–4.4)</td>
<td>3.0 (2.0–4.6)</td>
<td>3.3 (2.1–6.3)</td>
</tr>
</tbody>
</table>

Data presented as median with 25th–75th percentiles. *P < 0.05 vs control.

TABLE 4. Responses to Atropine in the Group With Normal LV Function

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Atropine</th>
</tr>
</thead>
<tbody>
<tr>
<td>HR, bpm</td>
<td>67 (60–75)</td>
<td>76 (60–97)</td>
</tr>
<tr>
<td>PAmax, mm Hg</td>
<td>18 (14–20)</td>
<td>17 (12–19)</td>
</tr>
<tr>
<td>MAP, mm Hg</td>
<td>107 (95–116)</td>
<td>106 (97–119)</td>
</tr>
<tr>
<td>CSPF, mL/min</td>
<td>58 (45–68)</td>
<td>69 (57–82)</td>
</tr>
<tr>
<td>NEart, nmol/L</td>
<td>1.1 (0.7–1.2)</td>
<td>0.9 (0.7–1.1)</td>
</tr>
<tr>
<td>NEcs, nmol/L</td>
<td>0.9 (0.7–1.4)</td>
<td>1.1 (0.9–1.5)*</td>
</tr>
<tr>
<td>CANESP, pmol/min</td>
<td>47 (27–51)</td>
<td>64 (38–139)*</td>
</tr>
<tr>
<td>TBNESP, nmol/min</td>
<td>1.8 (1.0–2.4)</td>
<td>1.8 (1.1–2.3)</td>
</tr>
</tbody>
</table>

*P < 0.05 vs control.

Discussion

The results of this study demonstrate that myocardial muscarinic receptor stimulation reduces cardiac norepinephrine spillover in patients with heart failure. In those individuals with normal ventricular function, the local release of norepinephrine from cardiac sympathetic neurons did not change in response to the infusion of acetylcholine. Atropine did not affect the local modulation of cardiac sympathetic activity in the setting of CHF but did cause a significant increase in cardiac norepinephrine spillover in those with normal ventricular function.

This is the first human in vivo study to examine the effect of muscarinic receptor activation and blockade on a measure of norepinephrine release. In animal models, the functional importance of this receptor pathway has been demonstrated in several organ beds, including the heart. In isolated rabbit heart submitted to electrical stimulation, Löffelholz and Muscholl confirmed that acetylcholine causes inhibition of norepinephrine release. More recently, Casado et al demonstrated that the noradrenergic nerve endings in guinea pig carotid arteries possess M2 inhibitory muscarinic receptors that modulate norepinephrine release. To date, observations made in human tissue have been limited to in vitro studies using field stimulation. Different muscarinic agonists have been tested in human iris-ciliary body and papillary muscle, demonstrating an inhibitory effect on norepinephrine release. The present study demonstrates that muscarinic receptor stimulation has inhibitory effects on
norepinephrine release from cardiac sympathetic nerves, presumably through its effects on cardiac neuronal muscarinic receptors. This suggests that the prejunctional muscarinic receptor system has functionally important effects on norepinephrine release from adrenergic nerve terminals in the human heart.

Traditionally, it has been assumed that the local modulation of norepinephrine release by muscarinic receptors occurred at a prejunctional level. Importantly, local cardiac ganglia have recently been described, which have important effects on cardiac sympathetic responses.20 Indeed, Armour and colleagues have demonstrated that muscarinic receptors are involved in the local neurotransmission.21,22 In either case, the specific muscarinic receptor subtypes that are responsible for the modulation of sympathetic responses we described remain uncertain.11,12,23,24

Muscarinic receptor stimulation had no effect on cardiac norepinephrine spillover in those subjects with normal ventricular function. The most likely explanation for this observation has to do with differences in the level of baseline cardiac sympathetic activity in both groups. It is well known that the inhibitory effects of muscarinic receptor stimulation are greater as sympathetic activity is increased, a phenomenon referred to as accentuated antagonism.25–28 Indeed, previous observations concerning the inhibitory prejunctional effects of muscarinic receptor stimulation on norepinephrine release from adrenergic nerve terminals have been made in the setting of some form of sympathetic nerve stimulation.7,8,10–12 Therefore, the fact that baseline sympathetic efferent neuronal input to the heart was significantly greater in the CHF group [197 (150–302) versus 50 (39–111) pmol/min; P<0.01], may explain the differences in the observed response to acetylcholine. There are other potential explanations for the neutral effect of acetylcholine observed in those with normal ventricular function. In this group, intracoronary atropine caused a significant increase in cardiac norepinephrine spillover. This would suggest that basal parasympathetic activity in this population exerts tonic inhibitory effects on cardiac norepinephrine release. As such, the administration of exogenous acetylcholine may not have additional effects, particularly in a setting where sympathetic nerve activity is relatively low. The fact that acetylcholine caused a significant increase in coronary blood flow may also be involved, because an increase in flow may have masked an inhibitory effect of acetylcholine on norepinephrine release as measured by the spillover technique.

Limited information is available concerning the effects of atropine on the local modulation of norepinephrine release from adrenergic nerve terminals. Some in vitro studies suggest that this muscarinic antagonist has no effect on norepinephrine overflow.10 Importantly, one study using human cardiac tissue demonstrated that atropine caused an increase in norepinephrine release from papillary muscles.12 This is consistent with our observation in subjects with normal ventricular function in whom the intracoronary administration of atropine caused a significant increase in heart rate. Atropine did not cause a significant increase in heart rate despite the observed increase in cardiac sympathetic activity. This is not unexpected, because cardiac norepinephrine spillover reflects...
changes in efferent sympathetic nerve activity primarily within ventricular myocardium and does not necessarily account for changes at the level of the sinus node. Furthermore, because atropine was administered into the left coronary artery in the majority of patients, it would not reach significant concentrations in the sinus node.

Previous human in vivo studies examining the effect of systemic atropine on sympathetic activity have reported decreases in plasma norepinephrine levels and muscle sympathetic nerve activity. These results reflect systemic administration of atropine in doses that are known to increase cardiac output and systemic blood pressure, both of which could lead to reflexive decreases in central sympathetic outflow. Although low doses of systemic atropine have been reported to have parasympathomimetic effects in humans, we found no evidence of these effects when atropine was administered locally (total dose of 120 μg).

Observations related to the effect of atropine on the local modulation of norepinephrine release have important implications concerning the role of the parasympathetic nervous system in the control of cardiac sympathetic activity. As mentioned above, the fact that atropine administration caused a significant increase in cardiac norepinephrine spillover in those with normal ventricular function suggests that the parasympathetic nervous system exerts tonic inhibitory effects on cardiac sympathetic activity. The absence of an atropine effect on cardiac sympathetic activity in subjects with CHF is consistent with previous observations that have demonstrated that the norepinephrine spillover technique allows the calculation of extraction and organ clearance of norepinephrine, it does not provide direct measurements of norepinephrine release. Nevertheless, previous work has shown that the norepinephrine spillover technique provides a reliable estimate of the neuronal release of norepinephrine. Patients in this study were taking a variety of cardioactive medications, some of which may have had an impact on baseline cardiac sympathetic activity. This is particularly true in the CHF group where the majority of patients were taking angiotensin converting-enzyme inhibitors and digitalis glycosides. Despite these medications, this group had significantly elevated cardiac sympathetic activity, as compared with those with normal ventricular function, and muscarinic receptor stimulation had potent inhibitory effects on this variable. Acetylcholine and atropine are nonselective in their effects on muscarinic receptors. Therefore, the present study cannot address the question as to which muscarinic receptor subtypes are involved in the control of cardiac sympathetic response. It might be suggested that atropine did not increase cardiac norepinephrine spillover in patients with CHF because neuronal activity is already maximally stimulated in this disease state. This does not appear to be the case, because previous studies in patients with CHF have demonstrated further increases in cardiac norepinephrine spillover in response to short-term β-blockade and exercise. Finally, this represents an acute study and it remains to be determined whether chronic muscarinic receptor stimulation would be associated with continued inhibitory effects on cardiac sympathetic activity.

Therefore, we have demonstrated that prejunctional muscarinic receptors play an important role in the local modulation of cardiac sympathetic activity in both normal and heart failure patients. To the best of our knowledge, this is the first study to demonstrate the importance of this receptor system in the modulation of sympathetic activity in a human organ bed in vivo. The fact that muscarinic receptor stimulation has a very important effect on cardiac sympathetic activity in the setting of CHF has implications concerning the genesis of cardiac sympathetic activation in this disorder. This is of particular interest if we consider the potential benefits of increasing parasympathetic tone in patients with heart failure and the results of preliminary clinical investigations which have been performed in this area.

**Acknowledgments**

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**References**


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