Simvastatin Normalizes Autonomic Neural Control in Experimental Heart Failure

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Background—HMG-CoA reductase inhibitors (statins) have been shown to beneficially affect outcomes in chronic heart failure (CHF). We hypothesized that statins exert effects on autonomic function, as assessed by plasma norepinephrine levels, direct recordings of renal sympathetic nerve activity (RSNA), and baroreflex function.

Methods and Results—Normolipidemic CHF rabbits were treated with simvastatin or vehicle. CHF was induced by continuous ventricular pacing at 320 to 340 bpm for 3 weeks. Two to 3 days after instrumentation of the rabbits with renal nerve electrodes and arterial and venous catheters, blood samples and RSNA recordings were obtained in the conscious state. Baroreflex function was assessed after administration of sodium nitroprusside and phenylephrine. Mean baseline RSNA (±SEM) in normal rabbits was 19.3±3.8%; in CHF rabbits, 39.4±2.9% (P<0.05); in CHF rabbits on low-dose (0.3 mg·kg⁻¹·d⁻¹) simvastatin, 39.8±8.3% (P<0.05); and in CHF rabbits on high-dose simvastatin (3 mg·kg⁻¹·d⁻¹), 21.1±4.5% (P=NS). Similar data were observed for plasma norepinephrine. In CHF rabbits treated with 3 mg·kg⁻¹·d⁻¹ simvastatin, baroreflex regulation of heart rate to transient hypotension with sodium nitroprusside was normalized by 66% compared with CHF controls.

Conclusions—These are the first data showing that non–lipid-lowering statin effects include a normalization of sympathetic outflow and reflex regulation in CHF. The precise neural and cellular pathways involved in these responses need further clarification. This finding may have important implications for the treatment of CHF and progression of the disease process. (Circulation. 2003;107:2493-2498.)

Key Words: heart failure ■ baroreceptors ■ norepinephrine ■ HMG-CoA

HMG-CoA reductase inhibitors such as simvastatin have been shown to beneficially affect the process of atherosclerosis, resulting in fewer deaths caused by myocardial infarction and stroke, lowering the incidence of new-onset heart failure, and improving the outcome among patients with chronic heart failure (CHF). In a canine model of CHF, simvastatin treatment resulted in enhanced coronary vasodilation responses. Pravastatin has been shown to lower arterial pressure in borderline hypertensive, dyslipidemic humans. These favorable statin effects may be a result of both lipid and nonlipid mechanisms affecting endothelial vasoregulation and, as we hypothesize, on sympathovagal balance in disease states. Until now, autonomic function after statin therapy has been given little attention. There is a growing body of evidence that the sympathoexcitation of the CHF state is mediated by changes in central humoral regulation. Overall, the importance of targeting sympathetic tone in CHF is underscored by evidence that an increased sympathetic tone results in more pronounced peripheral vasoconstriction, sodium retention, attenuated cardiovascular reflexes, and higher susceptibility to ventricular arrhythmias and sudden cardiac death. Furthermore, increases in plasma norepinephrine (PNE) have been shown to be a prognostic marker for 5-year survival after the diagnosis of heart failure. Because statins are neuroprotective, in part, by a nitric oxide (NO)–dependent mechanism and because NO is sympathoinhibitory, we hypothesized that chronic administration of a statin to animals with CHF would lower sympathetic outflow.

Statin effects on autonomic neural control in CHF rabbits were investigated in this study by use of both PNE measurements and direct recordings of efferent renal sympathetic nerve activity (RSNA) as sympathetic indices of autonomic outflow. Functional aspects of autonomic control were evaluated by analyzing arterial baroreflex sensitivity. The main goal of this study was to identify the net effect of statin therapy on sympathoexcitation in the CHF state.

Methods

Animals
Experiments were carried out on 54 male New Zealand White rabbits (Harlan, Inc, Indianapolis, Ind) ranging in weight between 3.0 and 3.5 kg. All experiments conformed to the Guidelines for Care and Use of Laboratory Animals of the American Physiological Society.
and the National Institutes of Health. Rabbits were assigned to 1 of 5 groups. Group 1 was a normal control group that underwent similar surgery and had implanted pacing electrodes (n=21). Group 2 was a CHF control (vehicle) group (n=13). Groups 3–5 were CHF groups fed oral simvastatin (via syringe) at a dose of 0.3, 1.5, or 3 mg · kg⁻¹ · day⁻¹ dispersed in 5 mL carrot juice (n=6, 6, and 8, respectively). Control animals were given 5 mL carrot juice every day and otherwise treated in the same manner over the same time period.

**Surgery and the CHF Model**

All rabbits underwent sterile thoracic instrumentation as described previously. Under general anesthesia, a left thoracotomy was performed in the third intercostal space. After the pericardium had been opened, a pair of 5-MHz, 2-mm piezoelectric crystals were sutured to the epicardial surface of the left ventricle across the base of the short axis to chronically record the changes in left ventricular dimensions. A pacing electrode was sutured to the epicardium of the left ventricle in all rabbits. A reference electrode was secured to the left atrium. In some rabbits, an arterial catheter was inserted into the descending thoracic aorta. The chest was closed and evacuated. Rabbits were allowed to recover for 2 weeks before entering into the study. The induction of experimental CHF and the respective treatment occurred concurrently over a period of 3 weeks. CHF was induced by rapid cardiac pacing at 100 beats above the rabbit’s resting heart rate up to a maximum rate of 340 bpm with an external pacing unit. Cardiac dimensions and the first derivative of diameter (dD/dt) were recorded in the conscious state with the pacemaker turned off for 20 minutes. In addition to a left ventricular dimension change of ±2 mm compared with baseline, clinical signs of CHF, such as ascites, pulmonary congestion, and cachexia, were symptoms of this CHF model.

For determination of RSNA, a second surgery for implantation of renal nerve electrodes was performed after the rabbits were in CHF. The renal nerves were identified, and a pair of electrodes (polytetrafluoroethylene-coated, multistranded stainless steel wires) with silicone-cuffed ends were implanted. A ground electrode was sutured to the perirenal fat. The assembly of nerve and cuffed electrodes was covered with a 2-component silicone gel (Wacker Sil-Gel). All electrode wires were tunneled beneath the skin and exited in the area the rabbit was to be trained, and were connected to an intracutaneously implanted silicone-cuffed end. A ground electrode was sutured to the perirenal fat. The assembly of nerve and cuffed electrodes was covered with a 2-component silicone gel (Wacker Sil-Gel). All electrode wires were tunneled beneath the skin and exited in the midscapular area of the back. After 2- to 3-day recovery from this surgery, RSNA was recorded in the conscious state. The RSNA was amplified by a Grass P16 preamplifier and recorded with a Powerlab system. Band-pass filters were set between 30 and 1000 Hz. The raw nerve activity was amplified, rectified, integrated, and displayed as neural spikes per second after a window discriminator had been set above the noise level. Zero nerve activity was assessed by complete suppression of RSNA with administration of either phenylephrine (PE; 20 µg/kg) or hexamethonium (30 mg/kg) at the conclusion of the experiment.

**Blood Sampling**

Blood samples were taken 20 minutes after the pacemaker had been turned off, when the rabbits were calm. Plasma concentrations of norepinephrine were determined with a radioenzymatic assay and obtained in kit form (Amersham Biosciences). Total and HDL cholesterol were assessed with an enzymatic colorimetric test (Roche Diagnostics).

**Evaluation of RSNA**

Resting RSNA was evaluated in 2 ways. First, the percentage of baseline to maximum nerve activity (% max) in response to a reduction in arterial pressure of between 20 and 30 mm Hg was used. However, because the arterial baroreflex may be abnormal in the CHF state, a pressure-independent stimulus, cigarette smoke, was also used to determine maximal RSNA. A puff of smoke (approximately 50 mL) blown smoothly into the nose of the rabbit over a 4- to 5-second period evokes a pronounced sympathetic response that is well described and reproducible. It is comparable to the hypotension-evoked maximal sympathetic response in the normal condition. Resting RSNA can then be expressed as the % max of the smoke response.

**Evaluation of the Arterial Baroreflex**

The arterial baroreflex was evaluated by recording the changes in heart rate and RSNA in response to changes in arterial pressure caused by infusion of PE (20 µg · kg⁻¹ · min⁻¹) and sodium nitroprusside (SNP, 100 µg · kg⁻¹ · min⁻¹) averaged over 3-second intervals. Both drugs were delivered intravenously by infusion pump over a period of 2 minutes. The slope of the linear portion of the relationship between heart rate and mean arterial pressure or between the percent change of RSNA from baseline and mean arterial pressure was taken as a measure of baroreflex sensitivity. This allowed a separate consideration of the slope for the hypertensive and hypotensive responses induced by PE and SNP, respectively. Linear regressions with correlation coefficients of >0.8 were used for analysis.

**Statistical Analysis**

The data for each group were expressed as the mean±SEM. Differences among groups were assessed with a 1-way ANOVA for repeated measures. Post hoc analysis consisted of the Newman-Keuls test. A probability value of P<0.05 was considered significant.

**Results**

**Cholesterol**

Treatment of normolipidemic, CHF rabbits with simvastatin in various doses did not result in any significant changes of total or HDL cholesterol. This finding is consistent with previous studies in rabbits. Total cholesterol in normal animals was 61.6±8.1 mg/dL, whereas in CHF rabbits on 3 mg · kg⁻¹ · day⁻¹ of simvastatin, total cholesterol was 43.5±12.8 mg/dL. HDL cholesterol in normal animals was 10.5±5.7 mg/dL, whereas it was 7.4±5.4 mg/dL in CHF animals on the highest dose of simvastatin.

**Hemodynamics**

Simvastatin did not significantly affect hemodynamics in this model (Table 1). Pacing rabbits exhibited a significant cardiac dilation and a reduction in fractional shortening and dD/dt. The only exception was in the 1.5 mg · kg⁻¹ · day⁻¹ simvastatin group, which showed a smaller dilation response than the other groups. This was because of 1 animal in which the dilation was small but which still showed evidence of CHF, including a decrease in dD/dt and percent fractional shortening. All paced rabbits showed ≥1 clinical signs of CHF, including ascites, pulmonary edema, and cachexia.

**Norepinephrine**

PNE as a marker of sympathoexcitation was clearly elevated in the CHF group (980±191 pg/mL) compared with both normal animals (284±19 pg/mL) and CHF animals on simvastatin 1.5 mg · kg⁻¹ · day⁻¹ (498±148 pg/mL) or 3 mg · kg⁻¹ · day⁻¹ (537±107 pg/mL, Figure 1). The lowest dose of simvastatin (0.3 mg · kg⁻¹ · day⁻¹) did not significantly reduce norepinephrine levels (725±262 pg/mL) compared with the vehicle-treated CHF group.

**Sympathetic Nerve Activity**

Figure 2 shows an original recording from a CHF (vehicle-treated) rabbit (top) and a rabbit treated with 3 mg · kg⁻¹ · day⁻¹ of simvastatin. Although it is difficult to compare baseline values from the raw data, the simvastatin-treated rabbit seems...
to have a substantially lower resting RSNA. Both amplitude and spike frequency seem to be lower in the treated rabbit. As the mean data show in Figures 3 and 4, renal sympathetic nerve recordings confirm the finding of a lower sympathoexcitation in simvastatin-treated animals. The % max of baseline RSNA either to smoke or to SNP-induced peak sympathoactivation was less for simvastatin-treated CHF animals than for vehicle-treated CHF animals. CHF vehicle-treated rabbits exhibited a clear increase in the % max RSNA compared with normal rabbits. Rabbits given the 2 highest doses of simvastatin exhibited a significantly lower % max for both smoke-induced (Figure 3) and SNP-induced (Figure 4) sympathoexcitation compared with the vehicle-treated CHF rabbits.

### Baroreflex Function

Separate slopes were determined for the hypotensive response to SNP and the hypertensive response to PE. Table 2 compares baroreflex slopes for both heart rate and RSNA in normal, CHF, and simvastatin-treated rabbits. Baroreflex slopes were uniformly depressed (heart rate and RSNA) for the SNP responses in CHF vehicle and low-dose-simvastatin–treated rabbits. The PE responses were not altered. The 2 higher doses of simvastatin restored baroreflex slope to the level observed in normal rabbits for the SNP responses; however, there were no effects on the PE responses.

### Discussion

The aim of this study was to determine the effects of chronic statin treatment on autonomic tone in an experimental model of CHF. The pleiotropic effects of statins are such that it is reasonable to hypothesize that they would possess actions on the autonomic nervous system beyond their direct vascular effects. Their ability to enhance NO synthesis in endothelium,4,22 to reduce angiotensin II–induced injury and AT1 receptor expression,23,24 and to reduce ET1 receptor expression25 all point to a potential role for statins in regulating sympathetic and vagal outflow in the central nervous system. Although statins are beneficial in hypertension, after myocardial infarction, and after cerebral ischemia,5,27,28 the present study is the first to show alterations in autonomic tone as a result of chronic statin therapy. We have provided 3 lines of evidence that suggest that simvastatin has sympatholytic effects in the pacing-induced CHF rabbit model. First, PNE concentration was found to be lower in simvastatin-treated CHF rabbits. Second, simvastatin decreased resting RSNA in CHF rabbits. Finally, baroreflex sensitivity was normalized in CHF rabbits treated with simvastatin. All of these effects were observed without changes in plasma total or HDL cholesterol, even though higher dosages of simvastatin were used to reach equipotency to prescribed formulas in humans, as determined previously.21

### Sympathetic Nerve Activity

The evidence that central sympathetic outflow is augmented in heart failure and that this increase in neural activity is detrimental to the downward spiral of ventricular function in heart failure is overwhelming.13,29 Clearly, β-adrenergic blockade has become a mainstay of therapy in heart failure.30 Other pharmacological agents targeting sympathetic function11,12 that were thought to be promising in CHF have been found to have undesirable side effects and thus are unacceptable. PNE concentration is dependent on both release and metabolism that includes activation of various uptake mechanisms. Although measurement of organ-specific and whole-body norepinephrine spillover may be capable of dissecting

| Table 1: Baseline Hemodynamics in Normal, CHF, and Simvastatin-Treated Rabbits |
|---------------------------------|-------|-------|-------|-------|-------|
|                                | Normal (n=21) | CHF (n=13) | CHF 0.3S (n=6) | CHF 1.5S (n=6) | CHF 3S (n=8) |
| Weight, kg                     | 3.1±0.1 | 3.2±0.1 | 3.0±0.1 | 3.1±0.2 | 3.0±0.2 |
| LV/BW, g/kg                    | 1.47±0.08 | 1.53±0.06 | 1.56±0.09 | 1.37±0.07 | 1.57±0.09 |
| Heart Rate, bpm                | 218.3±3.9 | 237.8±5.1 | 231.7±14.2 | 227.2±8.2 | 218.7±8.6 |
| MAP, mm Hg                     | 81.3±2.7 | 74.2±5.4 | 85.6±6.5 | 81.1±1.9 | 70.6±3.2 |
| ΔLVEDD, mm                      | −0.1±0.3 | 2.2±0.6* | 2.3±0.6* | 1.5±0.6 | 3.4±1.1* |
| dD/dt, mm/s                    | 14.3±0.7 | 7.7±0.8† | 8.6±0.9† | 8.8±1.1† | 8.9±1.2† |
| %FS                            | 9.4±0.3 | 3.6±0.7† | 5.0±0.5† | 5.5±0.9† | 5.1±1.2† |

S indicates simvastatin; LV/BW, left ventricular/body wt ratio; MAP, mean arterial pressure; LVEDD, left ventricular end-diastolic diameter; dD/dt, first derivative of diameter; and % FS, fractional shortening.

*P<0.05, †P<0.001 vs normal.
out these mechanisms, the present study confirms that, at least in the case of RSNA, simvastatin treatment decreases central sympathetic outflow and may result in beneficial outcomes in the CHF state. These data support previous evidence that statins may alter both sympathetic and vagal outflow to the heart, as assessed by changes in heart rate variability.

The 3 mechanisms that would be most likely to alter autonomic tone after statin treatment are AT₁ receptor down-regulation, ET-1 downregulation, and upregulation of NO production. Each of these mechanisms has been shown to be altered in the heart failure state and each is associated with changes in sympathetic outflow. In the case of NO, substantial evidence shows that this molecule modulates neurotransmitter function and inhibits sympathetic outflow. Patel et al. showed that the neuronal NO synthase (nNOS) gene is downregulated in the brain stem of rats with coronary artery-induced heart failure. It is intriguing to
speculate that the effects of simvastatin may be mediated by an upregulation of nNOS in the central nervous system.

**Arterial Baroreflex**

We and others have shown that arterial baroreflex sensitivity is reduced in animals and human subjects with CHF.\(^1^\)\(^,\)\(^10\) It is likely that changes in central sympathetic and vagal outflow in the CHF state are responsible for reductions in baroreflex sensitivity. Therefore, restoration of autonomic function should also enhance baroreflex sensitivity. Indeed, the present study revealed an increase in baroreflex sensitivity over the hypertensive range after simvastatin treatment in CHF rabbits. Many of the same factors that are responsible for alterations in resting sympathetic and vagal outflow may operate to change baroreflex sensitivity. For instance, we have shown that central angiotensin II contributes to reduced baroreflex sensitivity.\(^43\) Finally, combination therapy with an NO donor and angiotensin II receptor blockade reduces sympathetic tone in conscious rabbits with CHF.\(^4\) It is not completely clear why there was no effect of statin therapy on the baroreflex response to PE in these experiments. One explanation may be that the CHF vehicle-treated rabbits did not exhibit as great a decrease in the baroreflex response to hypertension as they did in the response to a hypertensive stimulus in these experiments. In a previous study performed in dogs with pacing-induced CHF, we showed that both nitroglycerin and PE baroreflex control of heart rate were blunted after 4 weeks of pacing; however, the nitroglycerin reduction was more pronounced then the PE response.\(^45\)

A possible concern in this study is the dose of simvastatin used compared with the normal therapeutic doses used in humans. Although a dose of 3 mg/kg might seem high, it has been well established that rabbits and smaller species metabolize simvastatin to a greater extent than larger species. The doses we used are similar to those in several other studies in smaller species. These studies were supported in part by National Institutes of Health grants PO-1-HL-62222 and RO-1-HL-38690. Dr Pliquett was supported by a postdoctoral fellowship from the American Heart Association, Heartland Affiliate. The authors would like to acknowledge the expert technical assistance of Johnnie F. Hackley and Pamela Curry. Simvastatin was generously supplied by Merck and Co.

**Heart Failure State**

Left ventricular function in these studies was evaluated primarily by examination of implanted crystal data documenting cardiac dilation and fractional shortening. Although it is possible that subtle differences in regional left ventricular function may be responsible for the lowering of sympathetic nerve activity in the statin-treated groups, we could not find any significant differences in left ventricular function between CHF groups.

In summary, the present study provides the first data showing a potent modulatory effect of statin therapy on sympathetic tone and autonomic function in an animal model of CHF. Because interruption of sympathoexcitation has become a primary therapeutic modality in heart failure, these data are particularly relevant in the current rationale for pharmacotherapy in this state and need to be confirmed in patients. It remains to be seen whether the effects described here are mediated by changes in NOS, AT\(_1\) receptors, ET\(_A\) receptors, or other mechanisms.

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