Selegiline improves cardiac sympathetic terminal function and β-adrenergic responsiveness in heart failure

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Selegiline (l-deprenyl) is a noncompetitive monoamine oxidase type B inhibitor used to treat Parkinson’s disease (18). The drug is known to cause central sympathetic inhibition secondary to stimulation of medullary α2-adrenergic receptors (4, 47). In addition, selegiline has been shown to produce neuroprotective and neuronal rescue effects (28, 38). Studies have shown that selegiline may restore the sympathetic reinnervation after chemical sympathectomy by 6-hydroxydopamine (45). Thus we carried out the present study to determine whether selegiline treatment would reduce the sympathetic nervous system overactivity and prevent the cardiac sympathetic neurodegenerative changes that occur in CHF. Furthermore, to assess the functional significance of changes in the noradrenergic nerve terminals, we measured myocardial systolic function, myocardial β-adrenergic receptor density, and baroreflex sensitivity in the selegiline-treated animals. Our present investigation is the first one to determine whether selegiline improves cardiac noradrenergic nerve function in heart failure.

METHODS

Animal model. Adult New Zealand White rabbits (3.14 ± 0.04 kg, 4–6 mo old) were chosen for the study. CHF was produced by rapid ventricular pacing using a modified technique of Spinale et al. (36). The study was approved by the University of Rochester Committee on Animal Resources and conformed to the guiding principles approved by the Council of the American Physiological Society and the National Institutes of Health Guide for the Care and Use of Laboratory Animals [DHHS Publication No. (NIH) 85-23, Revised 1985, Office of Science and Health Reports]. Under general isoflurane anesthesia and an aseptic subxiphoid thoracotomy and pericardiotomy, a shielded pacing lead (TPW50, Ethicon, Somerville, NJ) was sutured onto the apical left ventricular free wall. A second pacing lead was sutured onto the left pectoral muscle. Both leads were routed subcutaneously and exteriorized to the interscapular region. One week after surgery, rabbits were randomly assigned to receive pacing at a rate of 360 beats/min with an...
implantable model 8086 Prevail VHRP programmable pacemaker (Medtronic, Minneapolis, MN). Sham animals underwent identical surgery for wire placement but received no cardiac pacing.

Experimental protocol. Commencing with cardiac pacing or 1 wk after thoracotomy in sham animals, animals were started on either oral selegiline (1 mg/day) or placebo for 8 wk. Animals were cared for clinically by veterinary staff. Heart failure was assessed by chest auscultation, weekly measurements of body weight, and echocardiographic determinations of left ventricular dimension and function. After 8 wk of treatment, cardiac pacing was discontinued, and the animals were prepared for hemodynamic and blood NE measurements.

Finally, animals were killed with a lethal dose (>100 mg/kg) of intravenous pentobarbital sodium. The heart was removed and rinsed in ice-cold oxygenated normal saline. The amount of fluid in the chest and abdominal cavity was measured. The left ventricular weight includes both the septum and the left ventricular free wall. Fresh muscle blocks were taken from left ventricular free wall for measurement of NE uptake activity and noradrenergic nerve terminal profiles by NE histofluorescence and tyrosine hydroxylase immunocytochemistry. Remaining left ventricular tissue was stored in liquid nitrogen and was prepared later for crude muscle membrane fractions by homogenization for measurement of myocardial NE uptake-1 carrier sites and β-adrenoceptor density. Tissue protein was determined using a bichromatic acid protein assay reagent (Pierce, Rockford, IL) with bovine serum albumin as a standard.

Echocardiography and hemodynamic measurements. Two-dimensional and M-mode echocardiographic studies were performed using a Toshiba sonographic Scanning System Heart model (Toshiba America Medical Systems, Tustin, CA). A 5-MHz transducer was used to measure the maximal left ventricular end-diastolic dimension (EDD) and end-systolic dimension (ESD). Left ventricular fractional shortening (%) was measured as [(EDD − ESD) × 100]/EDD.

For the hemodynamic studies, animals were anesthetized with ketamine (35 mg/kg) and midazolam (0.8 mg/kg). A 20-gauge fluid-filled catheter (Insyte; Deseret Medical, Becton-Dickinson, Sandy, UT) was introduced into the left carotid artery, and a 2-Fr micromanometer-tipped catheter (Millar Instruments, Houston, TX) was introduced into the left ventricle via the right carotid artery. A Brush multichannel recorder (model 480; Gould, Cleveland, OH) was used to record an electrocardiogram for heart rate, arterial and left ventricular pressures, and the first derivative of left ventricular pressure (dP/dt) using an electronic differentiator. Resting hemodynamic measurements were obtained in triplicate over a 15- to 20-min steady-state period at least 1 h after introduction of the Miller catheter. The averages of triplicate measurements were used for statistical analysis. An arterial blood sample was taken for measurement of plasma NE (34) before the hemodynamic measurements were completed.

After resting hemodynamic measurements were taken, isoproterenol (0.8 μg/kg) was administered intravenously to measure the peak β-adrenergic heart rate and left ventricular dP/dt responses. After the animals recovered, phenylephrine (10–20 μg/kg) was injected intravenously to increase the systolic pressure by 15–50 mmHg. Electrocardiograms and arterial blood pressures were recorded continuously. We plotted the R-R interval against the systolic blood pressure of the preceding beat; only those regression lines with correlation coefficients >0.95 were accepted. The slope of the regression line was taken as an index of baroreflex sensitivity (13).

Myocardial NE uptake activity. Myocardial NE uptake activity was measured in quadruplicate by incubating fresh tissue slices at 37°C for 15 min in 50 nmol/l 1-[3H(N)]NE (13.8 Ci/mmol; NEN, Boston, MA). Specific 3H-uptake activity, defined as the difference in radioactivity between tissue slices incubated in an [3H]NE-containing solution at 37°C and those at 4°C, is considered to represent NE uptake activity (25).

Cardiac sympathetic nerve profiles. Cardiac sympathetic nerve integrity was assessed by histofluorescence for catecholamines and immunocytochemistry for tyrosine hydroxylase as previously described (12). Briefly, histofluorescence specific for catecholamines was performed using sucrose-potassium phosphate-glyoxylic acid. Sections for NE histofluorescence were photographed at ×30 magnification onto 35-mm slides. The number of stained catecholamine profiles was counted in a 0.221-mm2 (0.003536 mm3) field. For immunocytochemical visualization of tyrosine hydroxylase, a sheep anti-tyrosine hydroxylase primary antibody was used. Slides for tyrosine hydroxylase immunocytochemistry were photographed at ×20 magnification onto 35-mm slides, and the number of tyrosine hydroxylase immunostained profiles was counted in a 0.00855-mm2 field. All the data were expressed as the average of six fields.

Myocardial NE uptake-1 carrier site density. Myocardial NE uptake-1 carrier site density was measured using a radioligand assay with specific binding of [3H]nisoxetine (NEN) (43). Approximately 80 μg of membrane protein were incubated in 250 μl of a Tris buffer (50 mM Tris, 300 mM NaCl, and 5 mM KCl, pH 7.4) containing 3 nM [3H]nisoxetine and eight concentrations of cold nisoxetine (0.3125–10 μM). Incubation was performed in triplicate at room temperature for 90 min. For determination of nonspecific binding, 10 μM cold nisoxetine was used. The reaction was terminated by addition of an ice-cold Tris buffer. The membranes were rapidly washed three times with Tris buffer and immediately filtered through Whatman GF/B filters on a Brandel cell harvester (Biomedical Research and Development Laboratories, Gaithersburg, MD). The filters were dried and counted for 125I radioactivity by liquid scintillation spectrometry (Tri-Carb 460 CD; Packard Instruments, Downers Grove, IL). The difference between binding in the absence and presence of 10 μM nisoxetine was considered as specific binding. The number of receptor binding sites and dissociation constant were calculated using the EBDA computer software program (Elsevier Science, Cambridge, UK) (32).

Myocardial β-adrenoceptor density. Myocardial β-adrenoceptor density was measured by specific binding of [125I]iodocyanopindolol (ICYP; 2,200 Ci/mmol; NEN) (19). Approximately 20 μg of membrane protein, suspended in 50 mM Tris-HCl buffer (pH 7.4) containing 120 mM NaCl and 5 mM KCl, were incubated with eight concentrations of [125I]ICYP (10–150 pM) in the presence of either 20 μM propranolol or vehicle at 37°C for 60 min in a final volume of 0.25 ml. The reaction was terminated by addition of an ice-cold Tris buffer and counted for radioactivity by the same method described for the NE-uptake-1 carrier site density. The difference between tissue binding of the radioligand in the presence and absence of propranolol was considered the specific binding to the β-adrenoceptors. The number of receptor-binding sites was calculated using the same computer program as that used for NE uptake-1 carrier site assay.

Statistical analysis. Results are expressed as means ± SE. Two-way factorial analysis of variance (ANOVA) followed by post hoc contrast comparison tests was used to determine the significance of differences between treatment (selegiline vs. placebo), intervention (CHF vs. sham), and interaction be-
between treatment and intervention. For the serial echocardiographic data, ANOVA for repeated measures was used to determine the effect of treatment (selegiline vs. placebo) in CHF and sham groups. A probability value of <0.05 was considered significant.

RESULTS

Animals were divided into four experimental groups according to pacing assignment and drug treatment: 1) sham animals treated with placebo (n = 13), 2) sham animals treated with selegiline (n = 10), 3) CHF animals treated with placebo (n = 17), and 4) CHF animals treated with selegiline (n = 10).

Echocardiographic indexes. Figure 1 shows the serial changes of left ventricular EDD and fractional shortening during the 8 wk of pacing. Neither parameter changed significantly in sham animals. In contrast, rapid cardiac pacing caused progressive dilation of the left ventricle and a concomitant decline of left ventricular fractional shortening. Selegiline treatment had no effects in sham animals but attenuated the decline of fractional shortening in the CHF animals (F = 4.685, P = 0.046).

Table 1. Resting hemodynamics and body fluids

<table>
<thead>
<tr>
<th></th>
<th>Sham</th>
<th>Selegiline</th>
<th>Placebo</th>
<th>Selegiline</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of animals</td>
<td>13</td>
<td>10</td>
<td>17</td>
<td>10</td>
</tr>
<tr>
<td>Body weight, kg</td>
<td>3.13 ± 0.06</td>
<td>3.10 ± 0.08</td>
<td>3.17 ± 0.08</td>
<td>3.15 ± 0.06</td>
</tr>
<tr>
<td>Heart weight, g</td>
<td>6.1 ± 0.2</td>
<td>6.2 ± 0.1</td>
<td>6.9 ± 0.3*</td>
<td>7.7 ± 0.3*</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>262 ± 10</td>
<td>278 ± 13</td>
<td>253 ± 6</td>
<td>241 ± 9</td>
</tr>
<tr>
<td>RA pressure, mmHg</td>
<td>2.1 ± 0.5</td>
<td>1.6 ± 0.4</td>
<td>5.0 ± 0.9*</td>
<td>4.3 ± 0.8</td>
</tr>
<tr>
<td>Mean aortic pressure, mmHg</td>
<td>98 ± 3</td>
<td>102 ± 4</td>
<td>89 ± 4</td>
<td>98 ± 5</td>
</tr>
<tr>
<td>LV EDP, mmHg</td>
<td>7.5 ± 0.8</td>
<td>6.7 ± 1.0</td>
<td>20.8 ± 2.4*</td>
<td>18.2 ± 2.9*</td>
</tr>
<tr>
<td>LV dp/dt, mmHg/s</td>
<td>4,348 ± 229</td>
<td>3,962 ± 1882</td>
<td>773 ± 87*</td>
<td>3,044 ± 229*</td>
</tr>
<tr>
<td>Plasma [NE], ng/ml</td>
<td>0.09 ± 0.01</td>
<td>0.08 ± 0.02</td>
<td>0.46 ± 0.09*</td>
<td>0.24 ± 0.06†</td>
</tr>
<tr>
<td>Pleural effusion, ml</td>
<td>0</td>
<td>0</td>
<td>14 ± 8</td>
<td>4 ± 2</td>
</tr>
<tr>
<td>Ascites, ml</td>
<td>0</td>
<td>0</td>
<td>46 ± 30</td>
<td>16 ± 9</td>
</tr>
</tbody>
</table>

Values are means ± SE. CHF, congestive heart failure; RA, right atrial; LV, left ventricular; EDP, end-diastolic pressure; [NE], nor-epinephrine concentration. *P < 0.05 compared with sham. †P < 0.05 compared with CHF placebo.

Resting hemodynamics and plasma NE. Table 1 shows body weight, heart weight, resting hemodynamics, plasma NE, and amounts of pleural effusions and ascites in the four experimental groups. There were no differences in body weight among the groups. Rapid pacing increased heart weight (F = 21.95, P < 0.001), right atrial pressure (F = 13.22, P = 0.001), and left ventricular end-diastolic pressure (F = 32.75, P < 0.001) and decreased left ventricular dp/dt (F = 44.22, P < 0.001). These changes were associated with an increase of plasma NE but no alterations in heart rate or mean aortic pressure. Selegiline treatment produced no significant effects on resting hemodynamics in either sham or CHF animals but reduced the increase in plasma NE that was seen in untreated CHF animals. Pleural effusions and ascites were present only in CHF animals. Selegiline treatment reduced pleural effusions and ascites in CHF animals, but because the amount of fluid was highly variable, there were no significant differences between the placebo- and selegiline-treated CHF animals.

Myocardial β-adrenergic sensitivity and β-adrenoceptor density. Isoproterenol increased heart rate and left ventricular dp/dt in all animals with and without heart failure. Two-way ANOVA indicates that the increases of heart rate and dp/dt in response to isoproterenol were reduced in CHF compared with sham animals (Fig. 2). There were also statistically significant interactions between the pacing modality and drug treatment. Although selegiline had no effects in sham animals, selegiline treatment reduced the impairment of isoproterenol-induced heart rate and dp/dt responses in CHF animals.

Figure 3 shows that myocardial β-adrenoceptor density was reduced in CHF animals. Selegiline treatment had no effect in sham animals but returned the myocardial β-adrenoceptor density toward normal values in CHF animals.

Baroreflex sensitivity. Baroreflex sensitivity, as represented by heart rate reduction in response to the blood pressure increase produced by phenylephrine, was significantly reduced by pacing (F = 8.90, P =
In addition, there was a significant interaction between pacing modality and drug treatment. Selegiline reduced baroreflex sensitivity in sham animals (P < 0.002) but increased it in CHF animals (P < 0.018).

Myocardial NE-uptake activity, NE uptake-1 site density and cardiac sympathetic nerve profiles. CHF was associated with reductions of myocardial NE uptake activity and uptake-1 carrier site density as well as decreased adrenergic neuronal profiles as identified by catecholaminergic histofluorescence and tyrosine hydroxylase immunocytochemical staining. Selegiline treatment produced no effects on any of the parameters in sham animals but attenuated the reductions of myocardial NE uptake activity and NE uptake-1 carrier site density in CHF animals (Fig. 5). Selegiline treatment almost completely prevented the reduction of adrenergic neuronal profiles in CHF animals (Figs. 6 and 7).

**DISCUSSION**

Our present study is the first one to investigate the effects of selegiline on the sympathetic nerve ending function in CHF. We found that selegiline treatment prevented downregulation of myocardial β-adrenoceptors and reductions of the chronotropic and inotropic responses to isoproterenol in CHF. This was associated with reduction of plasma NE and preservation of the integrity of noradrenergic nerve terminals as assessed by myocardial NE uptake activity, NE uptake-1 carrier site density, catecholaminergic histofluorescence, and immunostained tyrosine hydroxylase profiles.

The mechanisms by which selegiline exerts beneficial effects in CHF have not been fully established. However, because selegiline reduced plasma NE in CHF, we speculate that selegiline may exert its effect, at least in part, by its central inhibition of the sympathetic nervous system (4, 47). Clonidine and moxonidine are two other agents that have been shown to...
stimulate the central $\alpha_2$-adrenoceptors and imidazoline receptors and cause peripheral sympathoinhibition (9, 37). Acute administration of clonidine and moxonidine has been shown to reduce plasma NE and produce hemodynamically beneficial effects in patients with CHF (1, 6). Short-term studies also have reported these agents to cause significant amelioration of heart failure symptoms, an increase in left ventricular ejection fraction, and reduction of malignant arrhythmias in patients with heart failure (29, 51). Clonidine treatment also improves heart rate variability by increasing the parasympathetic tone (10) and increases blood flow and muscle efficiency in exercising muscle in patients with heart failure (20). However, published data are lacking on the effects of centrally acting sympathoinhibitors on cardiac noradrenergic nerve terminal function or on the long-term effects in patients with heart failure.

Selegiline treatment prevented the decreases of catecholaminergic histofluorescence and immunostained tyrosine hydroxylase profiles, suggesting preservation of the adrenergic nerve terminals in CHF. This is consistent with the neuroprotective effect of selegiline on both the central nervous system and peripheral sympathetic nerves (28, 45). Administration of selegiline also has been shown to reduce axotomy-induced spinal motor neuronal death in neonatal rats (14) and increase the number of surviving motoneurons after facial nerve axotomy in immature rats (46). The findings suggest that selegiline can rescue neurons by partially compensating for the loss of target-derived trophic support. This neuroprotective effect may be related to the increased gene expression of nerve growth factor (35). In addition, selegiline inhibits apoptosis of PC12 and human melanoma cells (27, 28). This anti-apoptotic effect of selegiline probably is independent of monoamine oxidase type B inhibition, because it occurs at concentrations too low to inhibit monoamine oxidase type B (41, 42, 50) and because it is not shared by the $d$-isomer of selegiline (28, 41), an active monoamine oxidase type B inhibitor. Moreover, the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-induced neurocytotoxicity can be prevented by selegiline in a hybrid clone.
dopaminergic cell line that does not contain monooxidase type B enzyme (23).

Recent work from Tatton et al. (39, 40) indicates that selegiline alters expression of a number of genes and the synthesis of a number of proteins in nerve and glial cells and that these changes are accompanied by a decrease in DNA fragmentation and changes in Bcl-2, Bax, and superoxide dismutase proteins. Xu et al. (50) showed that selegiline blocked apoptosis induced by hypoxia in cultured retinal neurons by regulating the expression of apoptosis-related genes (c-jun and hsp70). Selegiline administered to rats and mice increases cytosolic copper-zinc and mitochondrial manganese superoxide dismutase activity (44). It also has been shown to increase the expression of superoxide dismutase and catalase (2, 3, 17) and reduce oxygen free radical formation (49). It prevents a reduction of mitochondrial membrane potential and increases in intramitochondrial calcium ion and cytoplasmic peroxyl radical levels in preapoptotic neurons (39, 48). Selegiline is also capable of potentiating the effect of nerve growth factor on gene expression of superoxide dismutase (24). These findings led Tatton et al. (40) to propose that selegiline acts on transcription factors to maintain mitochondrial function, decrease cytoplasmic oxidative radicals, and reduce apoptosis.

The dose of selegiline employed in the present study was chosen on the basis of pilot studies. The dose employed was sufficient to produce the desired effects on the cardiac sympathetic nerve endings. A larger dose of selegiline (5 mg/day) produced less neuroprotective effects. This finding is consistent with the observation that there is an optimal dose for selegiline in exerting its antioxidant and antiapoptotic effects (17, 23). The doses of selegiline required to produce long-term optimal antioxidant effects such as superoxide dismutase and catalase in selective brain regions and survival benefit in animals have been shown to be in the range of 0.25–0.5 mg/kg 3 days per week (16–18). The dose we employed in the present study (0.3 mg·kg$^{-1}$·day$^{-1}$) was within this range.

Our present study shows that the effects of selegiline on the sympathetic nervous system in heart failure are functionally important, as demonstrated by the improvements in myocardial β-adrenergic density and baroreflex sensitivity. We speculate that the preserved sympathetic nerve endings can effectively remove NE and keep interstitial NE at a relatively low level, thus preventing the agonist-induced myocardial and keep interstitial NE at a relatively low level, thus sympathoexcitation. The actions of selegiline are salutary in animals, as demonstrated by the improvements of myocardial β-adrenoceptor density and β-adrenergic responsiveness as well as the baroreflex function in the CHF animals. Further investigations of the effects of selegiline or other similar agents in heart failure are warranted.

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