J Physiol Sci (2010) 60:67–74
DOI 10.1007/s12576-009-0071-5

ORIGINAL PAPER

Effect of the cholinesterase inhibitor donepezil on cardiac remodeling and autonomic balance in rats with heart failure

Yoshihisa Okazaki · Can Zheng · Meihua Li · Masaru Sugimachi

Received: 28 July 2009 / Accepted: 4 November 2009 / Published online: 1 December 2009

Abstract In an earlier study we demonstrated the beneficial effect of direct vagal electrical stimulation on cardiac remodeling and survival. In the study reported here, we attempted to reproduce the effect of vagal enhancement through the administration of an acetylcholinesterase inhibitor, donepezil. A rat model of heart failure following extensive healed myocardial infarction was used. Compared to their nontreated counterparts, rats given donepezil (5 mg/kg/day) in their drinking water had a smaller biventricular weight (3.40 ± 0.13 vs. 3.02 ± 0.21 g/kg body weight, $P < 0.05$), and maximal rate of rise (3256 ± 955 vs. 3822 ± 389 mmHg/s, $P < 0.05$) and the end-diastolic value (30.1 ± 5.6 vs. 23.2 ± 5.7 mmHg, $P < 0.05$) of left ventricular pressure were improved. Neurohumoral factors were suppressed in donepezil-treated rats (norepinephrine 1885 ± 1423 vs. 316 ± 248 pg/ml, $P < 0.01$; brain natriuretic peptide 457 ± 68 vs. 362 ± 80 ng/ml, $P < 0.05$), and the high-frequency component of heart rate variability showed a nocturnal increase. These findings indicated that donepezil reproduced the anti-remodeling effect of electrical vagal stimulation. Further studies are warranted to evaluate the clinical usefulness of donepezil in heart failure.

Y. Okazaki · M. Sugimachi
Department of Artificial Organ Medicine, Division of Surgical Medicine, Osaka University Graduate School of Medicine, 2-2 Yamadaoka, Suita, Osaka 565-0871, Japan

Y. Okazaki · C. Zheng · M. Li · M. Sugimachi (✉)
Department of Cardiovascular Dynamics, Advanced Medical Engineering Center, National Cardiovascular Center Research Institute, 5-7-1 Fujishirodai, Suita, Osaka 565-8565, Japan
e-mail: su91mach@ri.ncvc.go.jp

Keywords Heart rate variability · Myocardial infarction · Neurohumoral activation · Vagal stimulation

Introduction

Profound imbalances in the autonomic nervous system, such as overactive sympathetic activity as well as diminished vagal activity, are considered to be important factors that aggravate heart failure [1, 2]. Various therapeutic agents, including beta-blockers [3, 4], angiotensin converting enzyme inhibitors [5, 6], and angiotensin receptor antagonists [7, 8] have proven to be useful pharmacotherapy, not a little by correcting the abnormally augmented sympathetic activity. However, few attempts have been made to date to actively remedy the reduced vagal activity as a treatment for heart failure. As a first attempt to testing this therapeutic strategy, our group has shown that in rats with aggravating chronic heart failure after experimentally induced healed myocardial infarction, electrical stimulation of the vagus nerve markedly improved survival by preventing cardiac remodeling [9].

Since the efferent vagal nerve activity is transmitted by acetylcholine, drugs that increase acetylcholine concentration at the neuro-effector junction are expected to have an effect similar to that of electrical stimulation. In support of this hypothesis, clinical trials in which patients with chronic heart failure were treated with the acetylcholinesterase inhibitor pyridostigmine reported decreased ventricular arrhythmia, enhanced heart rate variability at rest, increased heart rate reserve and oxygen pulse during exercise, and improved heart rate recovery after exercise [10, 11]. However, these studies examined the effect of short-term administration (1–2 days), and to date the long-term effect of pyridostigmine has not been investigated.

Clinical trials have also been conducted on scopolamine, which stimulates vagus nerve centrally at low doses [12, 13]. Transdermal administration of a small dose of scopolamine in patients with heart failure following myocardial infarction was found to increase heart rate variability and enhance baroreflex sensitivity. These studies have not shown, however, an anti-remodeling effect as more direct evidence against the progression of heart failure.

We hypothesized that donepezil, a novel acetylcholinesterase inhibitor, would show various clinically relevant beneficial effects through its preferential effects on neural true cholinesterase (rather than hepatic pseudocholinesterase) [14]. Therefore, in the study reported here, we investigated the effect of donepezil on hemodynamics, neurohumoral activation, and cardiac remodeling in rats with chronic heart failure. We also analyzed the high-frequency (HF) component of the heart rate variability to assess changes in vagal tone [15, 16]. Our results suggest that donepezil reproduces the anti-remodeling effect of electrical stimulation of the vagus nerve and increases vagal tone.

Materials and methods

The protocol of this study was performed in accordance with the Guiding Principles for the Care and Use of Animals in the Field of Physiological Sciences and was approved by the Experimental Animal Committee of the National Cardiovascular Center.

Chronic heart failure model

Male Sprague–Dawley rats (8 weeks of age) were used. A thoracotomy was performed under halothane anesthesia, and the main branch of the left coronary artery was ligated with nylon to produce myocardial infarction. The ligation resulted in myocardial infarction of 45–55%. The rats recovered from this extensive myocardial infarction and progressed to the chronic state of heart failure (see Results). The ventricular fibrillation that occurred within 1 h of ligation was treated actively by defibrillation and cardiac massage in order to salvage as many as possible rats with extensive myocardial infarction.

Experimental protocol

One week after the induction of myocardial infarction, the surviving rats underwent a second operation under halothane anesthesia in which an electrocardiogram (ECG) telemetry device was implanted in each rat to continuously monitor the electrical activity of the heart and heart rate (Fig. 1a).

Rats that survived the second week were divided into a nontreated group and a donepezil group. The donepezil group was administered the acetylcholinesterase inhibitor

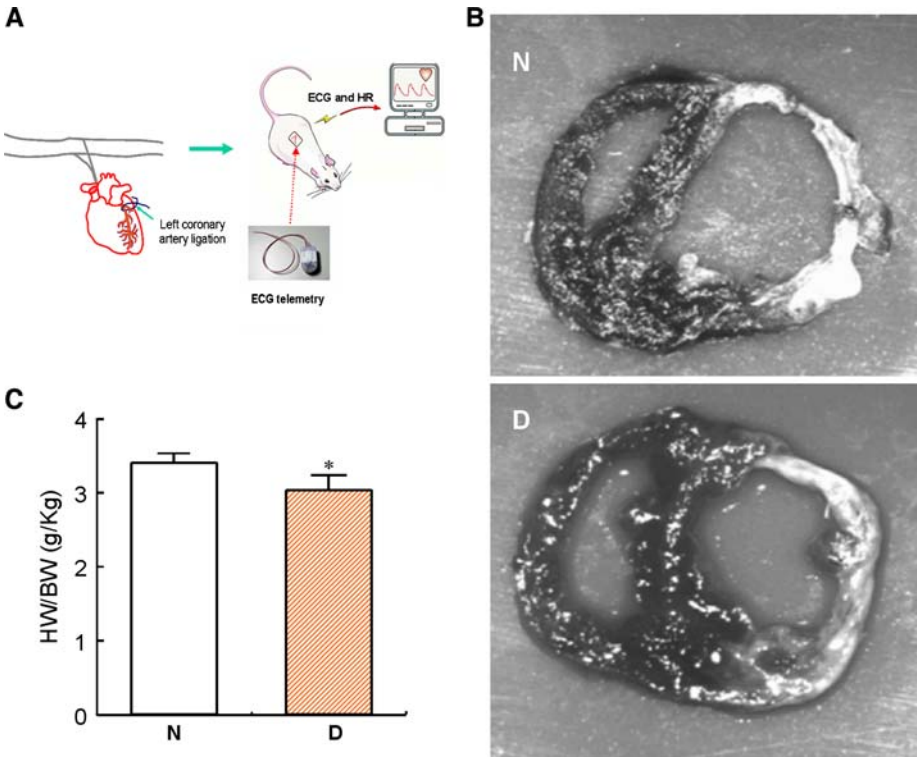

Fig. 1 **a** Schematic representation of the experimental design. Electrocardiogram (*ECG*) was recorded continuously using a telemetric system. **b** Ventricular sections of representative animals at week 6 of treatment (8 weeks post-infarction). No significant difference in the size of the myocardial infarction was observed between the donepezil group and the nontreated group. Compared with the nontreated heart (*N*), the donepezil-treated heart (*D*) had a thicker scar in the infarct area with more spared myocardium in the border area. **c** Combined weight of left and right ventricles per body weight (*HW/BW*) at week 6 of treatment. Ventricular weight was significantly lower ($*P < 0.05$) in the donepezil group (*shaded bar*, *D*) than in the nontreated group (*open bar*, *N*)

donepezil (Aricept; Eisai, Tokyo, Japan) dissolved in drinking water at a concentration of 50 mg/dl. The dose estimated from the volume of water consumed was 5 mg/kg/day on average. The selection of donepezil rested on the fact that, in comparison to other drugs, its inhibition action is directed much more towards the (true) acetylcholinesterase at synapses and effectors and less towards pseudocholinesterase (butyrylcholinesterase) in the liver [14].

At week 6 post-treatment (week 8 after infarction was induced), 13 rats in the nontreated group and 14 rats in the donepezil group were subjected to a hemodynamic study under halothane anesthesia. Following this study and blood collection, the rats were euthanized by an overdose of halothane, and a histological examination was conducted.

In 11 other rats with a similar healed myocardial infarction, the heart rate variability was calculated from the continuous ECG recordings between weeks 12 and 20 post-myocardial infarction induction. Five of these 11 rats served as the nontreated group (weeks 12–20 post-infarction), and six received the donepezil treatment (weeks 17–19 post-infarction). Preliminary analysis indicated no differences in heart rate variability at 8 weeks post-infarction.

Hemodynamic measurement

The hemodynamic study was conducted in rats under halothane anesthesia at week 6 of the treatment period. A Millar catheter (SPC-320; Millar Instruments, Houston, TX) was inserted from the carotid artery into the left ventricle to measure left ventricular pressure (LVP) with a high-fidelity catheter. Based on the LVPs, we calculated the maximal first derivative of left ventricular pressure over time (dP/dt_{max}) and the left ventricular end-diastolic pressure (LVEDP). The right atrial pressure (RAP) was measured by an external transducer via a catheter filled with physiological saline.

Neurohumoral factor measurements

Blood samples (3 ml) were collected and the neurohumoral factors in the blood assayed. As indices of sympathetic activity, norepinephrine (NE) and epinephrine (Epi) were measured by high-performance liquid chromatography with electrochemical detection. The plasma level of brain (or B-type) natriuretic peptide (BNP) was measured by an enzyme-linked immunosorbent (ELISA) assay (BNP-32 Enzyme Immunoassay kit, Peninsula Lab, San Carlos, CA). We included BNP in the assay due to its importance as a strong predictor of prognosis [17, 18]. BNP has been useful in detecting new patients with heart failure and in predicting mortality and cardiac events in both patients and asymptomatic subjects. BNP may also be a useful predictor of heart failure with preserved systolic function.

Heart tissue examination

The left and right ventricles were excised and the total weight measured. Both ventricles were then sectioned into 3-mm-thick slices, starting from the apex towards the base of the heart. Myocardial infarction size was assessed from the proportion of the length of the infarct to the left ventricular perimeter measured on each section.

Power spectral analysis of heart rate variability

The ECG telemetric data were processed as follows. Signals from the transmitter (model TA11CTA-F40; Data Sciences Int, St. Paul, MN) were recorded on a recording software (HEM; Notocord, Newark, NJ). An analysis software program (HRT10a1; Notocord) was used to extract the RR intervals from the data of the continuous recording (1-kHz sampling). All of the RR intervals were extracted from 24-h continuous recording data for the nontreated and the donepezil groups. The text data of 2-h intervals were stored in files to be analyzed later using the heart rate variability analysis software that we developed. Due to the frequent occurrence of extrasystoles in chronic heart failure, it was necessary to develop an original algorithm to process the data, as explained below.

Heart rate variability analysis software

The following procedures were conducted.

1. Data preparation. The 2-h data were combined to obtain 24-h data. The time of R-wave detection and the RR interval were saved as combined data.
2. Removal of extrasystole. A 20-point median filter was applied to all of the RR interval data to produce a sequence. Heart beats with RR intervals differing from the median value by 15 ms (threshold) or above were recognized and recorded as extrasystole or post-extrasystole. These data were excluded from analysis.
3. Resampling of valid interval data. The 24-h data were divided into 6-min data (with 50% overlap). After excluding the RR intervals associated with extrasystole, the valid RR interval data were resampled at intervals of 1/10 s using linear interpolation.
4. Power spectral analysis. In the power spectral analysis, 1024 points of 1/10-s data were grouped into a segment (segment length = 102.4 s) for fast Fourier transformation (FFT). The power spectra obtained from six segments were ensemble-averaged. Prior to FFT, the linear trend was removed from each segment
5. Data selection. Even though extrasystoles are removed, segments with many deleted data cannot be expected to yield reliable power spectral analysis results. Therefore,

data with ≥40 extrasystoles within 6 min were excluded from analysis.

6. Definition of HF component. In this study, the effect of bigeminy that occurs in heart failure was observed in the higher frequency range. Therefore, we excluded frequency range >1.5 Hz, and HF was defined as the power from 0.5 to 1.5 Hz. The power of the HF component was determined during daytime (0600–1800 hours) and nighttime (1800–0600 hours).

Statistical analysis

All data are presented as mean ± standard deviation (SD). Continuous variables were compared using the unpaired *t* test between two groups. The differences were considered significant when $P < 0.05$.

Results

Hemodynamics

Figure 2 shows the measurements of the hemodynamic parameters in rats under anesthesia 6 weeks after the onset of donepezil administration. A LVP waveform and its first derivative (d*P*/d*t*) in a nontreated rat are shown in Fig. 2a. Figure 2b shows that the dP/dt_{max} of the nontreated rat was significantly lower than that of the donepezil group (3,256 ± 955 vs. 3,822 ± 389 mmHg/s, $P < 0.05$). The LVEDP and RAP was significantly lowered by donepezil administration compared to the nontreated rat [23.2 ± 5.7 vs. 30.1 ± 5.6 mmHg, $P < 0.05$ (Fig. 2c) and 4.1 ± 2.9 vs. 7.0 ± 4.0 mmHg, $P < 0.05$ (Fig. 2d), respectively]. The contractility index dP/dt_{max} is known as a heart rate-

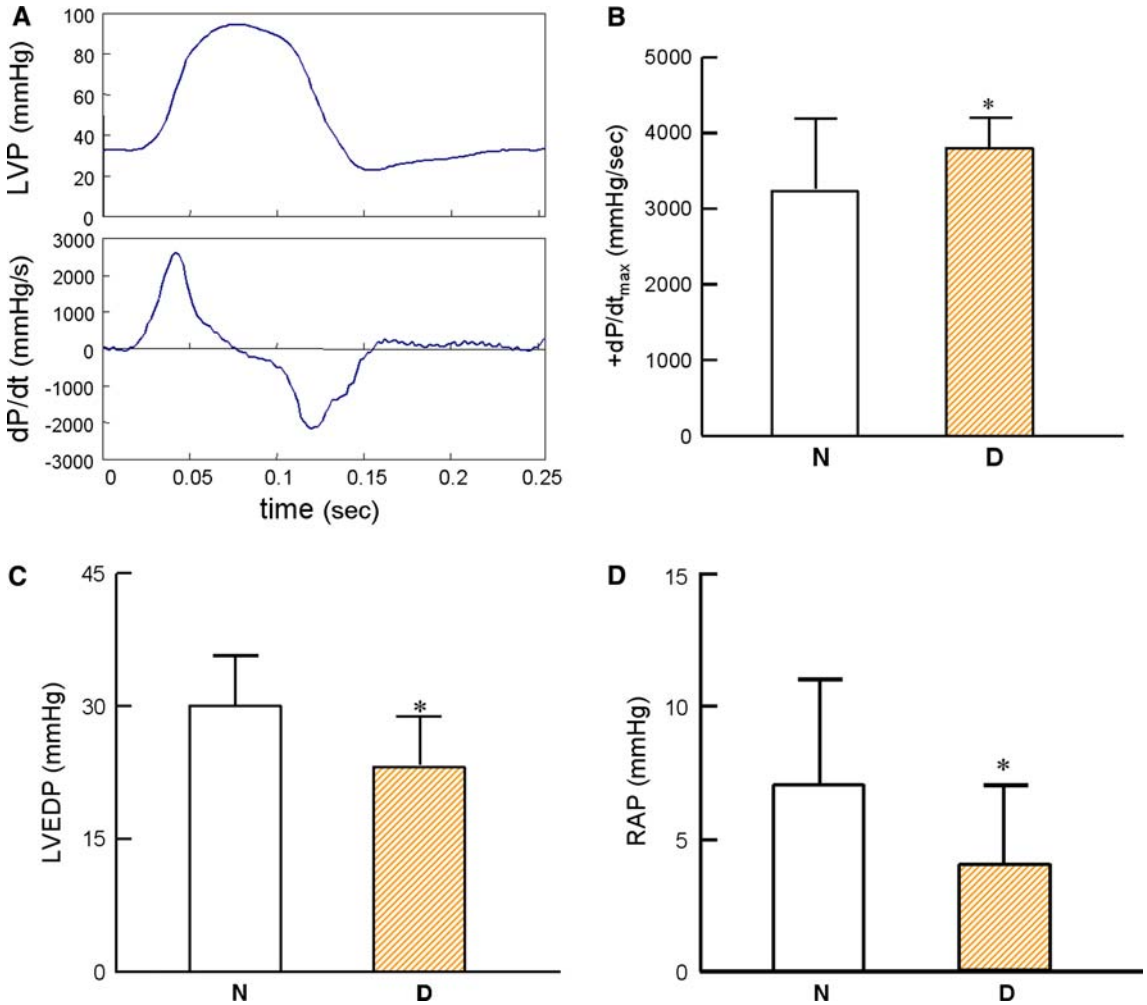

Fig. 2 **a** A representative example of the left ventricular pressure (*LVP*) waveform and its derivative in a nontreated rat. **b** Maximal first derivative of left ventricular pressure (dP/dt_{max}) at week 6 of treatment. The dP/dt_{max} was significantly (*$P < 0.05$) higher in the donepezil group (*shaded bar*, *D*) than in the nontreated group (*open bar*, *N*). **c** Left ventricular end-diastolic pressure (*LVEDP*) at week 6 of treatment. The LVEDP was significantly lower in the donepezil group (*shaded bar*, *D*) than in the nontreated control group (*open bar*, *N*). **d** Right atrial pressure (*RAP*) at week 6 of treatment. The RAP was significantly (*$P < 0.05$) lower in the donepezil group (*shaded bar*, *D*) than in the nontreated control group (*open bar*, *N*)

and preload-dependent index. Because heart rate was higher in the nontreated group than in the donepezil group (354 ± 37 vs. 324 ± 23 bpm, difference of approx. 9%) and LVEDP was higher in the nontreated group than in the donepezil group, the difference in heart rate and preload would have underestimated the true difference in contractility. Moreover, decreased LVEDP with decreased RAP in the donepezil group suggested that body fluid retention was suppressed.

Neurohumoral factors

Figure 3 shows the blood concentrations of norepinephrine, epinephrine, and BNP measured 6 weeks after donepezil administration was started. Compared to the nontreated group, donepezil administration resulted in significant decreases in the concentrations of norepinephrine (316 ± 248 vs. 1,885 ± 1423 pg/ml, $P < 0.01$), epinephrine (347 ± 153 vs. 1,694 ± 1,355 pg/ml, $P < 0.05$), and BNP (362 ± 80 vs. 457 ± 68 ng/ml, $P < 0.05$) in the blood. These results indicated that donepezil effectively suppressed the overactive sympathetic nervous system, which is a hallmark pathophysiology of heart failure.

Infarct size and heart weight

Figure 1b shows representative ventricular sections in the nontreated and the donepezil groups. The myocardial infarction resulted from obliteration of the left coronary artery was 48 ± 6% of the left ventricular perimeter in the nontreated group and 53 ± 3% in the donepezil group, with no significant difference in infarct size between two groups. Therefore, donepezil administration started 2 weeks after myocardial infarction did not reduce the size of the infarct, suggesting that infarct size did not account for the differences in hemodynamics and neurohumoral factors described above.

Figure 1c compares the ventricular weight per body weight between the nontreated and the donepezil groups. The combined weight of the left and right ventricles was significantly lower in the donepezil group than in the nontreated group (3.02 ± 0.21 vs. 3.40 ± 0.13 g/kg body weight, $P < 0.05$). This result indicated that donepezil reduced cardiac remodeling after myocardial infarction was completed.

Power spectral analysis of heart rate variability

The left panel of Fig. 4a shows a representative change in RR intervals with respect to time in a rat from the donepezil group. The RR intervals connected with dotted lines were assessed to be extrasystoles or post-extrasystoles and were removed before spectral analysis. The right panel shows the result of spectral analysis from the same data. The solid area was calculated as the HF component. The HF components during the daytime (0600–1800 hours, Fig. 4b) and nighttime (1800–0600 hours, Fig. 4c) were calculated for the donepezil group ($n = 6$) and the nontreated group ($n = 5$). The log-transformed HF components [log(HF)] of the two groups were analyzed statistically.

During the night, log(HF) significantly increased in the donepezil group compared to the untreated group. On the other hand, there was no significant difference in log(HF) during the day between the two groups. These results indicated that heart rate variability at night was enhanced by donepezil administration in rats.

Discussion

Imbalances in the autonomic nervous system, particularly overactive sympathetic activity together with reduced

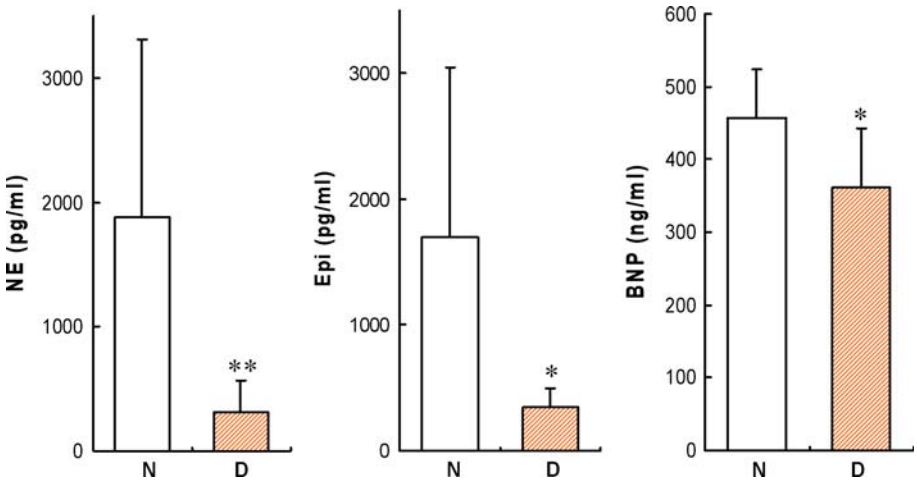

Fig. 3 Blood concentrations of norepinephrine (*NE*), epinephrine (*Epi*), and brain natriuretic peptide (*BNP*) at week 6 of treatment. Significant decreases (*$P < 0.05$, **$P < 0.01$) in blood NE, Epi, and BNP concentrations were observed in the donepezil group (*shaded bar*, *D*) compared to the nontreated group (*open bar*, *N*)

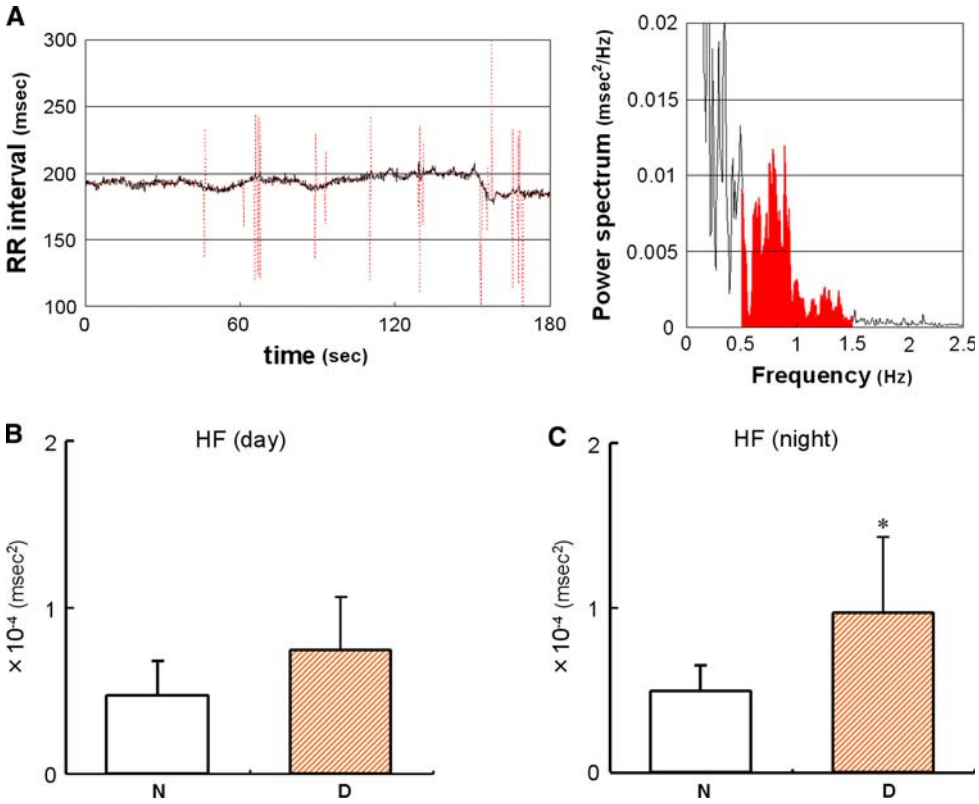

Fig. 4 **a** A representative example of time series of RR interval (*left*) and its power spectrum (*right*) in a donepezil-treated rat. RR intervals shown with *dotted lines* were assessed to be extrasystoles or post-extrasystoles and were removed before the power spectrum was calculated. *Solid area* indicates the high-frequency (HF) component. **b** HF of heart rate variability during the day. No significant difference in daytime HF value was observed between the donepezil group (*shaded bar*, *D*) and the nontreated group (*open bar*, *N*). **c** HF component of heart rate variability during the night. The nocturnal HF value of the donepezil group (*shaded bar*, *D*) was significantly higher than that of the nontreated group (*open bar*, *N*). $*P < 0.05$ by *t* test using log(HF) values

vagal activity, have been considered to be major factors aggravating heart failure. In an earlier study, we demonstrated that upstream treatment using electrical stimulation of the vagal nerve improves the survival rate in rats with heart failure after a healed extensive myocardial infarction. Although pharmacological reproduction of the vagotonic treatment of heart failure would be of benefit clinically, no vagotonic drugs have successfully shown anti-remodeling, which is the most direct evidence of a lack of progression of heart failure.

The results presented here clearly demonstrate that, in our rat model system, donepezil treatment improved hemodynamics, ameliorated cardiac remodeling, and prevented neurohumoral activation. Because donepezil exerted no significant effects on infarct size and was administered after the infarction had been established, these effects cannot be attributed to the reduction in ischemic insult. Although we have not shown the benefits on survival in this study, the similar hemodynamic, anti-remodeling, and neurohumoral effects as electrical vagal stimulation may also be translated into survival. Further studies on survival are needed to test the clinical application of donepezil.

We did not prepare sham-operated rats that would serve as a true control. To compensate for this limitation in study design, we used historical control values for hemodynamic measurements (dP/dt_{max} 11,237 ± 1,389 mmHg/s, LVEDP 6.5 ± 2.3 mmHg; RAP 1.9 ± 1.3 mmHg), neurohumoral factor measurements (NE 392 ± 205 pg/ml, Epi 164 ± 46 pg/ml, BNP 62 ± 7 pg/ml), and biventricular weight (2.22 ± 0.11 g/kg) obtained from the same strain and similar age of rats. These control values indicate that hemodynamic deterioration, neurohumoral activation, and cardiac remodeling were only partially reversed, with the exception of NE. Notwithstanding, the results with the electrical stimulation of vagal nerves indicate that these small benefits may accompany a larger improvement in survival.

We selected donepezil, a novel cholinesterase inhibitor, in order to be able to maximize inhibitor action on neuronal acetylcholinesterase but not on hepatic butyrylchoinesterase inhibitor [14]. We intentionally used donepezil, a drug acting both peripherally and centrally, to simulate electrical stimulation of the vagus nerve. Electrical stimulation affects both the afferent and efferent pathways of the vagus nerve, although detailed knowledge of the therapeutic mechanisms, including which of the two pathways plays a greater role in the therapeutic effect, is not yet available. However, a drug with dual central and peripheral action is certainly inappropriate for deepening mechanistic insights.

A mechanistic study would be important as donepezil itself may not be clinically applicable. The dose we chose in our study was aimed at decreasing the heart rate in the rats by 10%; it is 50-fold larger than the dose used for

treating Alzheimer's disease. Although the objective of our study was not to elucidate how large the contribution of each effect of donepezil is on the peripheral vagus nerve, ganglion, and central nervous system, we would like to add discuss some mechanistic aspects in terms of designing future studies.

Regarding the mechanism downstream of the neuro-effector junction, the neurotransmitter acetylcholine per se may provide some protective effect for cardiomyocytes. Based on their results from acute studies, Sato et al. have obtained several lines of evidence supporting this hypothesis. First, acetylcholine promotes the phosphorylation of connexin 43, a gap junction molecule located between cardiomyocytes, which in turn normalizes the intercellular ion flow and prevents the occurrence of fatal arrhythmia [19]. Second, acetylcholine directly enhances the phosphorylation of Akt via PI3K in the cardiomyocytes and activates the PI3/Akt pathway to enhance the expression of hypoxia-inducible factor-1α (HIF-1α), which may protect the cardiomyocytes from the hypoxic state induced by ischemia [20]. As shown by these findings, the acetylcholine concentration increases in the neuro-effector junction by vagal efferent activation; this acetylcholine possesses various functions that support the survival of cardiomyocytes. Further studies are required to study the contribution of acetylcholine in cardiomyocytes at the molecular level. Vagal enhancement at the effector site may potentiate its anti-inflammation effects [21] and may ameliorate progression of heart failure through alpha 7-nicotinic receptors.

On the other hand, experiments using rat and canine models of heart failure suggest the presence of abnormalities in the ganglia of the vagus nerve. For example, a comparison of control rats to those with heart failure following myocardial infarction revealed that the bradycardiac response to pre-ganglionic vagus stimulation in the rats with infarction was attenuated, while the bradycardiac response to acetylcholine was unchanged [22]. In dogs with heart failure induced by tachypacing, pre-ganglionic vagus stimulation showed lower heart rate responses, while postganglionic stimulation at the fat pad showed no difference in heart rate response compared to control dogs [23]. Taken together the above observations, in our model system, donepezil may act on the ganglia of the vagus nerve.

As donepezil passes the blood–brain barrier, the drug can act on the central nervous system. To gain an insight into the central effect, we conducted an analysis of heart rate variability. Heart rate variability, especially its HF component (at respiratory frequency) reflects background vagal tone and has been shown to be a strong prognostic determinant [15, 16]. Our results revealed that donepezil increased the HF of heart rate variability during the night, indicating enhanced vagal activity. On the other hand, the HF of the heart rate variability tended to increase, although not significantly, during the day. These finding may suggest a central effect of donepezil, but again a secondary effect of improved hemodynamics cannot be ruled out. Regardless of the detailed mechanism, increased HF may be associated to a better outcome in these rats, as shown in the ATRAMI study [24, 25]. These issues require further investigations.

In summary, the results of the study reported here suggest that donepezil treatment, similar to electrical stimulation of the vagus nerve, confers beneficial effects in terms of the prevention of cardiac remodeling in rats with heart failure following myocardial infarction. Future studies should examine if survival would be improved by the administration of donepezil in rats with healed myocardial infarction.

Acknowledgments This study was supported by Health and Labor Sciences Research Grants (H19-nano-Ippan-009, H20-katsudo-Shitei-007) from the Ministry of Health, Labor and Welfare of Japan.

References

1. Packer M (1992) The neurohormonal hypothesis: a theory to explain the mechanism of disease progression in heart failure. J Am Coll Cardiol 20:248–254
2. Floras JS (1993) Clinical aspects of sympathetic activation and parasympathetic withdrawal in heart failure. J Am Coll Cardiol 22:72A–84A
3. Packer M, Bristow MR, Cohn JN, Colucci WS, Fowler MB, Gilbert EM, Shusterman NH, U.S. Carvedilol Heart Failure Study Group (1996) The effect of carvedilol on morbidity and mortality in patients with chronic heart failure. N Engl J Med 334:1349–1355
4. Bristow MR, Gilbert EM, Abraham WT, Adams KF, Fowler MB, Hershberger RE, Kubo SH, Narahara KA, Ingersoll H, Krueger S, Young S, Shusterman N, MOCHA Investigators (1996) Carvedilol produces dose-related improvements in left ventricular function and survival in subjects with chronic heart failure. Circulation 94:2807–2816
5. The CONSENSUS Trial Study Group (1987) Effects of enalapril on mortality in severe congestive heart failure. Results of the Cooperative North Scandinavian Enalapril Survival Study (CONSENSUS). N Engl J Med 316:1429–1435
6. The SOLVD Investigators (1991) Effect of enalapril on survival in patients with reduced left ventricular ejection fractions and congestive heart failure. N Engl J Med 325:293–302
7. Cohn JN, Tognoni G, Valsartan Heart Failure Trial Investigators (2001) A randomized trial of the angiotensin-receptor blocker valsartan in chronic heart failure. N Engl J Med 345:1667–1675
8. McMurray JJ, Ostergren J, Swedberg K, Granger CB, Held P, Michelson EL, Olofsson B, Yusuf S, Pfeffer MA, CHARM Investigators and Committees (2003) Effects of candesartan in patients with chronic heart failure and reduced left-ventricular systolic function taking angiotensin-converting-enzyme inhibitors: the CHARM-added trial. Lancet 362:767–771
9. Li M, Zheng C, Sato T, Kawada T, Sugimachi M, Sunagawa K (2004) Vagal nerve stimulation markedly improves long-term survival after chronic heart failure in rats. Circulation 109:120–124

10. Serra SM, Costa RV, Teixeira De Castro RR, Xavier SS, Lucas Da Nóbrega AC (2009) Cholinergic stimulation improves autonomic and hemodynamic profile during dynamic exercise in patients with heart failure. J Card Fail 15:124–129
11. Behling A, Moraes RS, Rohde LE, Ferlin EL, Nóbrega AC, Ribeiro JP (2003) Cholinergic stimulation with pyridostigmine reduces ventricular arrhythmia and enhances heart rate variability in heart failure. Am Heart J 146:494–500
12. Casadei B, Conway J, Forfar C, Sleight P (1996) Effect of low doses of scopolamine on RR interval variability, baroreflex sensitivity, and exercise performance in patients with chronic heart failure. Heart 75:274–280
13. Venkatesh G, Fallen EL, Kamath MV, Connolly S, Yusuf S (1996) Double blind placebo controlled trial of short term transdermal scopolamine on heart rate variability in patients with chronic heart failure. Heart 76:137–143
14. Liston DR, Nielsen JA, Villalobos A, Chapin D, Jones SB, Hubbard ST, Shalaby IA, Ramirez A, Nason D, White WF (2004) Pharmacology of selective acetylcholinesterase inhibitors: implications for use in Alzheimer's disease. Eur J Pharmacol 486:9–17
15. Kleiger RE, Miller JP, Bigger JT Jr, Moss AJ (1987) Decreased heart rate variability and its association with increased mortality after acute myocardial infarction. Am J Cardiol 59:256–262
16. Schwartz PJ, La Rovere MT (1998) ATRAMI: a mark in the quest for the prognostic value of autonomic markers. Autonomic tone and reflexes after myocardial infarction. Eur Heart J 19:1593–1595
17. Anand IS, Fisher LD, Chiang YT, Latini R, Masson S, Maggioni AP, Glazer RD, Tognoni G, Cohn JN, Val-HeFT Investigators (2003) Changes in brain natriuretic peptide and norepinephrine over time and mortality and morbidity in the Valsartan heart failure trial (Val-HeFT). Circulation 107:1278–1283
18. Doust JA, Pietrzak E, Dobson A, Glasziou P (2005) How well does B-type natriuretic peptide predict death and cardiac events in patients with heart failure: systematic review. Br Med J 330:625
19. Ando M, Katare RG, Kakinuma Y, Zhang D, Yamasaki F, Muramoto K, Sato T (2005) Efferent vagal nerve stimulation protects heart against ischemia-induced arrhythmias by preserving connexin 43 protein. Circulation 112:164–170
20. Kakinuma Y, Ando M, Kuwabara M, Katare RG, Okudela K, Kobayashi M, Sato T (2005) Acetylcholine from vagal stimulation protects cardiomyocytes against ischemia and hypoxia involving additive non-hypoxic induction of HIF-1alpha. FEBS Lett 579:2111–2118
21. Wang H, Yu M, Ochani M, Amella CA, Tanovic M, Susarla S, Li JH, Wang H, Yang H, Ulloa L, Al-Abed Y, Czura CJ, Tracey KJ (2003) Nicotinic acetylcholine receptor alpha7 subunit is an essential regulator of inflammation. Nature 421:384–388
22. Du XJ, Cox HS, Dart AM, Esler MD (1998) Depression of efferent parasympathetic control of heart rate in rats with myocardial infarction: effect of losartan. J Cardiovasc Pharmacol 31:937–944
23. Bibevski S, Dunlap ME (1999) Ganglionic mechanisms contribute to diminished vagal control in heart failure. Circulation 99:2958–2963
24. La Rovere MT, Bigger JT Jr, Marcus FI, Mortara A, Schwartz PJ (1998) Baroreflex sensitivity and heart-rate variability in prediction of total cardiac mortality after myocardial infarction ATRAMI (autonomic tone and reflexes after myocardial infarction) Investigators. Lancet 351:478–484
25. La Rovere MT, Pinna GD, Hohnloser SH, Marcus FI, Mortara A, Nohara R, Bigger JT Jr, Camm AJ, Schwartz PJ, ATRAMI Investigators (2001) Baroreflex sensitivity and heart rate variability in the identification of patients at risk for life-threatening arrhythmias: implications for clinical trials. Circulation 103:2072–2077