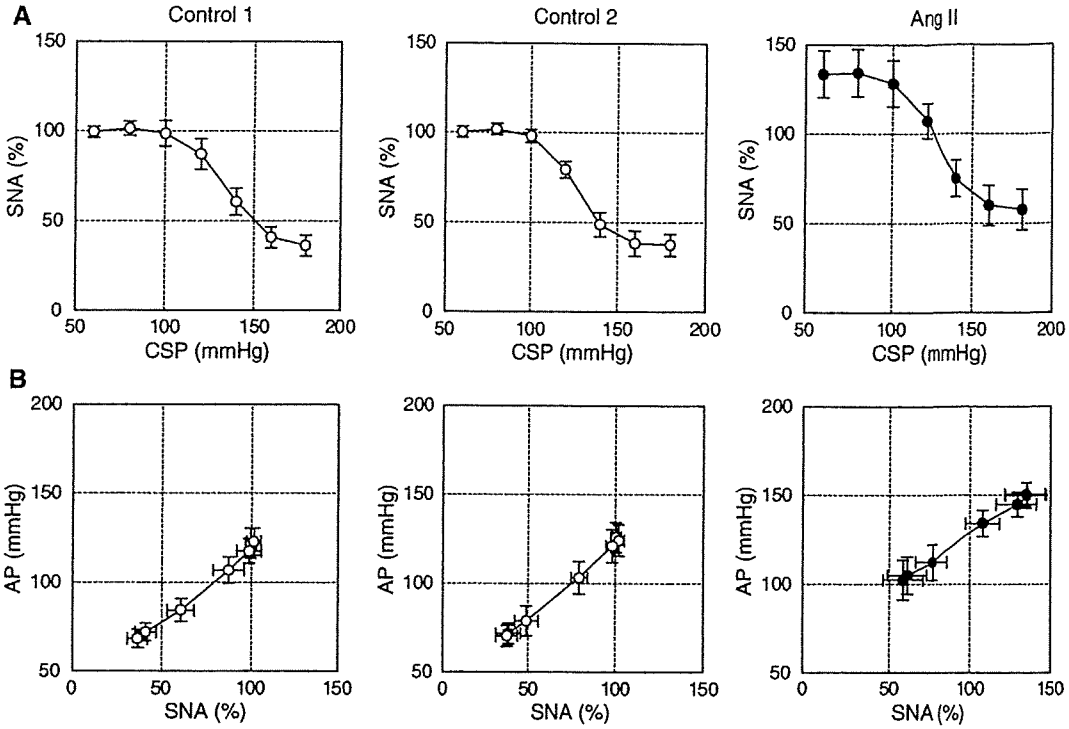

Fig. 3 **a** Averaged input–output relation of the baroreflex neural arc or the arterial baroreflex control of SNA. SNA decreased in response to an increase in the CSP. ANG II increased SNA, while the range of the SNA response was preserved. **b** Averaged input–output relation of the baroreflex peripheral arc. AP increased in response to an increase in SNA. ANG II increased the AP, an effect that was greater for lower SNA

HR by ANG II was not observed in the present study because the vagal nerves were sectioned.

Limitations

First, we performed the experiments in anesthetized animals, and comparisons with results obtained in conscious animals should be made carefully. Circulating levels of ANG II may vary under anesthesia, which could have affected the present results. For instance, reported plasma ANG II concentration in pithed rats is approximately 400 pg/ml [16], which exceeds the plasma ANG II concentration reported in rats with heart failure [34]. Second, although the dose of ANG II used in the present study was within or below those used in previous studies in rats [12, 16, 17], Brown et al. demonstrated that intravenous ANG II at 20 and 270 ng kg^{-1} min^{-1} increased the plasma ANG II concentration from approximately 80 pg/ml to 140 and 2,000 pg/ml, respectively [35]. Based on those data, the plasma ANG II concentration might have been increased beyond a physiologically relevant range to approximately 1,200 pg/ml in the present study. Therefore, the observed effect of ANG II on the arterial baroreflex should be interpreted as pharmacologic. Effects of circulating ANG II can be different when examined in different doses. Third, there was large variation in HR values among the animals (Fig. 2b). Increasing the number of animals would reduce this variation. Nevertheless, data from the eight rats was sufficient to perform statistical analyses and draw reasonable conclusions. Fourth, we occluded the common carotid arteries to isolate the carotid sinuses. Although the vertebral arteries were kept intact and the effects of ANG II were examined using the same preparation, the possibility cannot be ruled out that the carotid occlusion affected the present results. Finally, we cut the vagal nerves to obtain the open-loop condition for the carotid sinus baroreflex. Further studies are needed to clarify the effects of ANG II on the baroreflex control of the cardiovascular system through the vagal system.

Conclusion

The present study indicates that high circulating levels of ANG II significantly increased splanchnic SNA but did not acutely attenuate the range of arterial baroreflex control of SNA. The ranges of the total baroreflex response and the baroreflex control of HR were also preserved during ANG

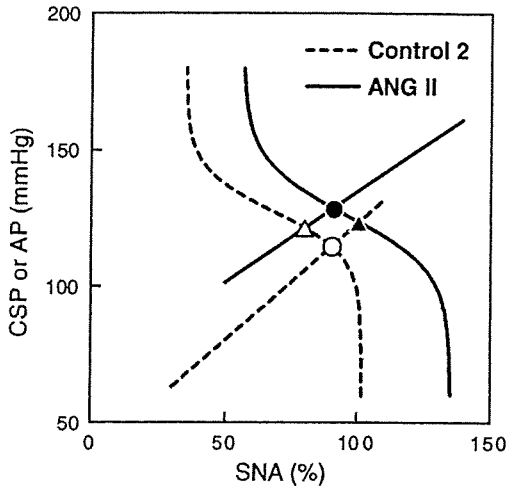

Fig. 4 Equilibrium diagrams between the arterial baroreflex neural and peripheral arcs. The *dashed* and *solid curves* represent the open-loop characteristics of the baroreflex neural arc under the control and ANG II-treated conditions, respectively. The *dashed* and *solid lines* represent the open-loop characteristics of the baroreflex peripheral arc under the control and ANG II-treated conditions, respectively. The *open circle* indicates the closed-loop operating point under the control condition. ANG II causes an upward shift in the peripheral arc. If ANG II does not affect the neural arc, the closed-loop operating point would be at the point depicted by the *open triangle*. In this case, the estimation of baroreflex control of SNA based on the closed-loop operating points (the *open circle* and *open triangle*) approximates the slope of the baroreflex neural arc (*dashed curve*). ANG II, however, causes a rightward shift in the neural arc. Thus, the estimation of the baroreflex control of SNA based on closed-loop operating points (the *open* and *filled circles*) does not match the slope of the neural arc under either the control (*dashed curve*) or ANG II-treated condition (*solid curve*)

II administration. ANG II does modify the arterial baroreflex in that it increases SNA at a given baroreceptor pressure level but does not appear to attenuate the range of arterial baroreflex control of SNA, HR or AP.

Acknowledgments This study was supported by Health and Labour Sciences Research Grants (H18-nano-Ippan-003, H19-nano-Ippan-009, H20-katsudo-Shitei-007, and H21-nano-Ippan-005) from the Ministry of Health, Labour and Welfare of Japan; by a Grant-in-Aid for Scientific Research (No. 20390462) from the Ministry of Education, Culture, Sports, Science and Technology of Japan; and by the Industrial Technology Research Grant Program from the New Energy and Industrial Technology Development Organization (NEDO) of Japan.

References

1. Ikeda Y, Kawada T, Sugimachi M, Kawaguchi O, Shishido T, Sato T, Miyano H, Matsuura W, Alexander J Jr, Sunagawa K (1996) Neural arc of baroreflex optimizes dynamic pressure regulation in achieving both stability and quickness. Am J Physiol 271:H882–H890
2. Kawada T, Yamamoto K, Kamiya A, Ariumi H, Michikami D, Shishido T, Sunagawa K, Sugimachi M (2005) Dynamic characteristics of carotid sinus pressure-nerve activity transduction in rabbits. Jpn J Physiol 55:157–163
3. Sato T, Kawada T, Shishido T, Miyano H, Inagaki M, Miyashita H, Sugimachi M, Kneupfer MM, Sunagawa K (1998) Dynamic transduction properties of in situ baroreceptors of rabbit aortic depressor nerve. Am J Physiol Heart Circ Physiol 274:H358–H365
4. Chapleau MW, Abboud FM (1987) Contrasting effects of static and pulsatile pressure on carotid baroreceptor activity in dogs. Circ Res 61:648–658
5. Kawada T, Fujiki N, Hosomi H (1992) Systems analysis of the carotid sinus baroreflex system using a sum-of-sinusoidal input. Jpn J Physiol 42:15–34
6. Kawada T, Yanagiya Y, Uemura K, Miyamoto T, Zheng C, Li M, Sugimachi M, Sunagawa K (2002) Input-size dependence of the baroreflex neural arc transfer characteristics. Am J Physiol Heart Circ Physiol 284:H404–H415
7. Kawada T, Zheng C, Yanagiya Y, Uemura K, Miyamoto T, Inagaki M, Shishido T, Sugimachi M, Sunagawa K (2002) High-cut characteristics of the baroreflex neural arc preserve baroreflex gain against pulsatile pressure. Am J Physiol Heart Circ Physiol 282:H1149–H1156
8. Reid IA (1992) Interactions between ANG II, sympathetic nervous system, and baroreceptor reflexes in regulation of blood pressure. Am J Physiol Endocrinol Metab 262:E763–E778
9. Sanderford MG, Bishop VS (2000) Angiotensin II acutely attenuates range of arterial baroreflex control of renal sympathetic nerve activity. Am J Physiol Heart Circ Physiol 279:H1804–H1812
10. McMullan S, Goodchild AK, Pilowsky PM (2007) Circulating angiotensin II attenuates the sympathetic baroreflex by reducing the barosensitivity of medullary cardiovascular neurons in the rat. J Physiol 582:711–722
11. Kumagai K, Reid IA (1994) Angiotensin II exerts differential actions on renal nerve activity and heart rate. Hypertension 24:451–456
12. Tan PS, Killinger S, Horiuchi J, Dampney RA (2007) Baroreceptor reflex modulation by circulating angiotensin II is mediated by AT_1 receptors in the nucleus tractus solitarius. Am J Physiol Regul Integr Comp Physiol 293:R2267–R2278
13. Zucker IH (2006) Novel mechanisms of sympathetic regulation in chronic heart failure. Hypertension 48:1005–1011
14. Shoukas AA, Callahan CA, Lash JM, Haase EB (1991) New technique to completely isolate carotid sinus baroreceptor regions in rats. Am J Physiol Heart Circ Physiol 260:H300–H303
15. Sato T, Kawada T, Miyano H, Shishido T, Inagaki M, Yoshimura R, Tatewaki T, Sugimachi M, Alexander J Jr, Sunagawa K (1999) New simple methods for isolating baroreceptor regions of carotid sinus and aortic depressor nerves in rats. Am J Physiol Heart Circ Physiol 276:H326–H332
16. Grant TL, McGrath JC (1988) Interactions between angiotensin II, sympathetic nerve-mediated pressor response and cyclo-oxygenase products in the pithed rat. Br J Pharmacol 95:1220–1228
17. Haywood JR, Fink GD, Buggy J, Phillips MI, Brody MJ (1980) The area postrema plays no role in the pressor action of angiotensin in the rat. Am J Physiol Heart Circ Physiol 239:H108–H113
18. Kent BB, Drane JW, Blumenstein B, Manning JW (1972) A mathematical model to assess changes in the baroreceptor reflex. Cardiology 57:295–310
19. Glantz SA (2002) Primer of biostatistics, 5th edn. McGraw-Hill, New York
20. Mohrman DE, Heller LJ (2006) Cardiovascular physiology, 6th edn. McGraw Hill, New York, pp 172–177
21. Sato T, Kawada T, Inagaki M, Shishido T, Takaki H, Sugimachi M, Sunagawa K (1999) New analytic framework for

understanding sympathetic baroreflex control of arterial pressure. Am J Physiol Heart Circ Physiol 276:H2251–H2261
22. Kawada T, Shishido T, Inagaki M, Zheng C, Yanagiya Y, Uemura K, Sugimachi M, Sunagawa K (2002) Estimation of baroreflex gain using a baroreflex equilibrium diagram. Jpn J Physiol 52:21–29
23. Kashihara K, Takahashi Y, Chatani K, Kawada T, Zheng C, Li M, Sugimachi M, Sunagawa K (2003) Intravenous angiotensin II does not affect dynamic baroreflex characteristics of the neural or peripheral arc. Jpn J Physiol 53:135–143
24. Sanderford MG, Bishop VS (2002) Central mechanisms of acute ANG II modulation of arterial baroreflex control of renal sympathetic nerve activity. Am J Physiol Heart Circ Physiol 282:H1592–H1602
25. Fukiyama K (1972) Central action of angiotensin and hypertension—increased central vasomotor outflow by angiotensin. Jpn Circ J 36:599–602
26. Guo GB, Abboud FM (1984) Angiotensin II attenuates baroreflex control of heart rate and sympathetic activity. Am J Physiol Heart Circ Physiol 246:H80–H89
27. Kamiya A, Kawada T, Yamamoto K, Michikami D, Ariumi H, Uemura K, Zheng C, Shimizu S, Aiba T, Miyamoto T, Sugimachi M, Sunagawa K (2005) Resetting of the arterial baroreflex increases orthostatic sympathetic activation and prevents postural hypotension in rabbits. J Physiol 566:237–246
28. Yamamoto K, Kawada T, Kamiya A, Takaki H, Miyamoto T, Sugimachi M, Sunagawa K (2004) Muscle mechanoreflex induces the pressor response by resetting the arterial baroreflex neural arc. Am J Physiol Heart Circ Physiol 286:H1382–H1388
29. Lumbers ER, McCloskey DI, Potter EK (1979) Inhibition by angiotensin II of baroreceptor-evoked activity in cardiac vagal efferent nerves in the dog. J Physiol 294:69–80
30. Hughes J, Roth RH (1971) Evidence that angiotensin enhances transmitter release during sympathetic nerve stimulation. Br J Phrmacol 41:239–255
31. Zimmerman BG, Gomer SK, Liao JC (1972) Action of angiotensin on vascular adrenergic nerve endings: facilitation of norepinephrine release. Federation Proc 31:1344–1350
32. Reid IA, Chou L (1990) Analysis of the action of angiotensin II on the baroreflex control of heart rate in conscious rabbits. Endocrinology 126:2749–2756
33. Brooks VL (1995) Chronic infusion of angiotensin II resets baroreflex control of heart rate by an arterial pressure-independent mechanism. Hypertension 26:420–424
34. Schunkert H, Tang SS, Litwin SE, Diamant D, Riegger G, Dzau VJ, Ingelfinger JR (1993) Regulation of intrarenal and circulating renin–angiotensin systems in severe heart failure in the rat. Cardiovasc Res 27:731–735
35. Brown AJ, Casals-Stenzel J, Gofford S, Lever AF, Morton JJ (1981) Comparison of fast and slow pressor effects of angiotensin II in the conscious rat. Am J Physiol Heart Circ Physiol 241:H381–H388

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

Authors: Yoshihisa Okazaki [1,2], Can Zheng [2], Meihua Li [2], Masaru Sugimachi [1,2]

Institutions: [1] Department of Artificial Organ Medicine, Division of Surgical Medicine, Osaka University Graduate School of Medicine, Suita 565-0871, Japan

[2] Department of Cardiovascular Dynamics, Advanced Medical Engineering Center, National Cardiovascular Center Research Institute, Suita 565-8565, Japan

Correspondence: Masaru Sugimachi, MD, PhD

Department of Cardiovascular Dynamics, Advanced Medical Engineering Center, National Cardiovascular Center Research Institute, Suita 5658565, Japan

TEL: +81-6-6833-5012 x2509

FAX: +81-6-6835-5403

e-mail: su91mach@ri.ncvc.go.jp

Abstract

We have previously shown the beneficial effect of direct vagal electrical stimulation on cardiac remodeling and survival. In this study, we tried to reproduce the effect of vagal enhancement by administration of an acetylcholinesterase inhibitor, donepezil. A rat model of heart failure following extensive healed myocardial infarction was used. In rats given donepezil (5 mg/kg/day) in drinking water, biventricular weight was smaller (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 enddiastolic value (30.1±5.6 vs. 23.2±5.7 mmHg, $p<0.05$) of left ventricular pressure were improved. Neurohumoral factors were suppressed (norepinephrine, 1885±1423 vs. 316±248 pg/ml, $p<0.01$; BNP, 457±68 vs. 362±80 ng/ml, $p<0.05$). 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.

(149 words)

Keywords

Myocardial infarction, Vagal stimulation, Heart rate variability, Neurohumoral activation

Introduction

Profound imbalance of the autonomic nervous system has been considered to be an important factor that aggravates heart failure [1]. The imbalance includes not only overactive sympathetic activity but also diminished vagal activity [2]. Various therapeutic agents including beta-blockers [3, 4], angiotensin converting enzyme inhibitors [5, 6], and angiotensin receptor antagonists [7, 8] have proven useful at least partly by correcting the abnormally augmented sympathetic activity. However, few endeavors have been made to actively remedy the reduced vagal activity as a treatment for heart failure. As the first attempt of 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 through prevention of cardiac remodeling [9].

Since the efferent vagal nerve activity is transmitted by acetylcholine, drugs that increase the acetylcholine concentration at the neuro-effector junction are expected to have the similar effect as electrical stimulation. In fact, clinical trials of the acetylcholinesterase inhibitor pyridostigmine have been conducted in patients with chronic heart failure [10, 11], resulting in decreased ventricular arrhythmia, enhanced heart rate variability at rest, increased heart rate reserve and oxygen pulse during exercise, as well as improved heart rate recovery after exercise. However, these studies examined the effect of short-term administration (one to two days), and the long-term effect of pyridostigmine has not been investigated. Clinical trials have also been conducted on scopolamine that 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 increased heart rate variability and enhanced baroreflex sensitivity. These studies have not shown, however, anti-remodeling effect, 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 present study, we investigated the effect of donepezil on hemodynamics, neurohumoral activation, and cardiac remodeling in rats with chronic heart failure. In addition, we analyzed high-frequency component of the heart rate variability to assess changes in vagal tone [15, 16]. The results indicated that donepezil reproduced anti-remodeling effect of electrical stimulation of the vagus nerve, and increased 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. Under halothane anesthesia, a thoracotomy was performed 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 to 55%. The rats recovered from extensive myocardial infarction and progressed to the chronic state of heart failure (see Results). For ventricular fibrillation that occurred within one hour of ligation, defibrillation was conducted actively by cardiac massage in order to salvage as far as possible the rats with extensive myocardial infarction.

Experimental protocol

One week after induction of myocardial infarction, the surviving rats underwent another operation under halothane anesthesia. An electrocardiogram (ECG) telemetry device was implanted in each rat to monitor ECG and heart rate continuously (Figure 1A).

Rats that survived another week were divided into a nontreated group and a donepezil group. The donepezil group was administered the acetylcholinesterase inhibitor 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 it more inhibits the (true) acetylcholinesterase at synapses and effectors but less inhibits pseudocholinesterase (butyrylcholinesterase) in liver than other drugs [14].

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

In other 11 rats with similar healed myocardial infarction, heart rate variability was calculated from the continuous ECG recordings between weeks 12 to 20 after myocardial infarction, In 11 rats, 5 served as the nontreated group (weeks 12-20 after infarction) and 6 received the donepezil treatment (weeks 17 to 19 after infarction). Preliminary analysis indicated no differences in heart rate variability at 8 weeks after infarction.

Hemodynamic measurement

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

Neurohumoral factor measurements

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

Heart tissue examination

The left and right ventricles were excised and the total weight was measured. Next, both ventricles were sectioned into 3 mm-thick three slices, starting from the apex towards the base of the heart. Myocardial infarction size was assessed from the proportion of the length of 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 International, St. Paul, MN) were recorded on a recording software HEM (Notocord, Newark, NJ). From the data of the continuous recording (1 kHz sampling), an analysis software HRT10a1 (Notocord, Newark, NJ) was used to extract the RR intervals. All the RR intervals were extracted from 24-hour continuous recording data for the nontreated and the donepezil groups. The text data of 2-hour 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-hour data were combined to obtain 24-hour 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 the RR interval data to produce a sequence. Heart beats with RR intervals differing from the median value by 15 msec (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-hour data were divided into 6-minute data (with 50% overlap). After excluding the RR intervals associated with extrasystole, the valid RR interval data were resampled at intervals of 1/10 seconds using linear interpolation.

(4) Power spectral analysis

In the power spectral analysis, 1024 points of 1/10-second data were grouped into a segment (segment length = 100.24 seconds) for fast Fournier transformation (FFT). The power spectra obtained from 6 segments were ensemble averaged. Prior to FFT, 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 or more extrasystoles within 6 minutes were excluded from analysis.

(6) Definition of high frequency component (HF)

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. Power of HF component was determined during daytime (6:00 to 18:00) and nighttime (18:00 to 6:00).

Statistical analysis

All data are presented as mean ± SD. Continuous variables were compared using unpaired t-test between two groups. The differences were considered significant when $p < 0.05$.

Results

Hemodynamics

Figure 2 shows the results of hemodynamic parameters measured under anesthesia 6 weeks after the onset of donepezil administration. A left ventricular pressure waveform and its first derivative are exemplified in Figure 2A. In this example (in a nontreated rat) the maximal first derivative of left ventricular pressure (dP/dt_{max}) was markedly decreased. In the donepezil group, dP/dt_{max} was significantly increased compared to the nontreated group (3822 ± 389 versus 3256 ± 955 mmHg/s, $p < 0.05$, Figure 2B). Left ventricular enddiastolic pressure (LVEDP; 23.2 ± 5.7 versus 30.1 ± 5.6 mmHg, $p < 0.05$, Figure 2C) and right atrial pressure (RAP; 4.1 ± 2.9 versus 7.0 ± 4.0 mmHg, $p < 0.05$, Figure 2D) was significantly lowered by donepezil administration. The contractility index dP/dt_{max} is known as a heart rate- and preload-dependent index. Because heart rate was higher in the nontreated group (354 ± 37 vs. 324 ± 23 bpm, difference ~9%) and LVEDP was higher in the nontreated group, the difference in heart rate and preload would have underestimated the true difference in contractility. Moreover, decreased LVEDP with decreased RAP in 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. Donepezil administration resulted in significant decreases in blood norepinephrine (316 ± 248 versus 1885 ± 1423 pg/ml, $p < 0.01$), epinephrine (347 ± 153 versus 1694 ± 1355 pg/ml, $p < 0.05$) and BNP (362 ± 80 versus 457 ± 68 ng/ml, $p < 0.05$) concentrations. 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 starting two weeks after myocardial infarction did not reduce the infarct size, 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 compared to 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 Figure 4A shows a representative change in RR intervals with respect to time in a rat from the donepezil group. RR intervals connected with dotted lines were judged as extrasystoles or post-extrasystoles and were removed before spectral analysis. The right panel shows the result of spectral analysis from the same data. The area circumscribed by the thick lines was calculated as the HF component. The HF components during daytime (6:00 to 18:00, Figure 4B) and nighttime (18:00 to 6:00, Figure 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) was 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

Imbalance of the autonomic nervous system, particularly overactive sympathetic activity together with reduced vagal activity has been considered to be one of the major factors that aggravate heart failure. Our previous study has demonstrated that upstream treatment using electrical stimulation of the vagal nerve improves the survival rate in rats with heart failure after extensive healed myocardial infarction. Although pharmacological reproduction of the vagotonic treatment of heart failure would benefit clinically, no vagotonic drugs have successfully showed anti-remodeling, the most direct evidence against the progression of heart failure.

Our study results clearly demonstrated that donepezil treatment improved hemodynamics, ameliorated cardiac remodeling, and prevented neurohumoral activation. Because donepezil exerted no significant effects on infarct size, and donepezil was

administered after 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 to survival. Further studies on survival are needed for its clinical application.

We failed to prepare sham-operated rats that would serve as a true control. To make up for this, we have shown historical control values for hemodynamic measurements (dP/dt_{max}, 11237 ± 1389 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 except for 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 to maximize its 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 affected both the afferent and efferent pathways of the vagus nerve, and the detailed therapeutic mechanisms including which of the two pathways plays a greater role in the therapeutic effect has remained unclear. However, the drug with dual central and peripheral action was certainly inappropriate for deepening mechanistic insights.

Mechanistic study would be important as donepezil itself may not be clinically applicable. The dose in rats, which we aimed at decreasing heart rate by 10 %, was 50 times larger than dose used for Alzheimer's disease. Although the present study does not elucidate how large is the contribution of each of the effect of donepezil on the peripheral

vagus nerve, ganglion, and central nervous system, we would like to add some mechanistic discussion for designing future studies.

Regarding the mechanism downstream of the neuro-effector junction, the neurotransmitter acetylcholine per se may have some protective effect for cardiomyocytes. In fact, Sato et al. have obtained several lines of evidence supporting this hypothesis from acute studies. First, acetylcholine promotes the phosphorylation of connexin 43, a gap junction molecule located between cardiomyocytes. This 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 increased in the neuro-effector junction by vagal efferent activation possesses various functions that support the survival of cardiomyocytes. Further studies are required to study the contribution of acetylcholine in cardiomyocytes at molecular levels. Vagal enhancement at 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 have suggested the presence of abnormalities in the ganglia of the vagus nerve. For example, in rats with heart failure following myocardial infarction, the bradycardiac response to pre-ganglionic vagus stimulation was attenuated, while the bradycardiac response to acetylcholine was unchanged compared to control rats [22]. Furthermore, in dogs with heart failure induced by high frequency pacing, with pre-ganglionic vagus stimulation heart rate responses were attenuated, while postganglionic stimulation at the fat pad showed no difference in heart rate response compared to control dogs [23]. Taking together the above observations, donepezil may act on the ganglia of the vagus nerve in the present study.

Also, 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 high-frequency component (at respiratory frequency) reflected background vagal tone, and has been shown to be a strong prognostic determinant [15, 16]. Our results revealed that donepezil increased high frequency component (HF) of heart rate variability during the night, indicating enhanced vagal activity. On the other hand, HF of the heart rate variability tended to increase but 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 better outcome in these rats, as shown in e.g., the ATRAMI study [24, 25]. These issues require further investigations.

In summary, the present study suggests that donepezil treatment, similar to electrical stimulation of the vagus nerve, confers beneficial effects in the prevention of cardiac remodeling in rats with heart failure following myocardial infarction. It is worthy to 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**

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.** *BMJ* 330: 625

19. Ando M, Katare RG, Kakinuma Y, Zhang D, Yamasaki F, Muramoto K, Sato T (2005)