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

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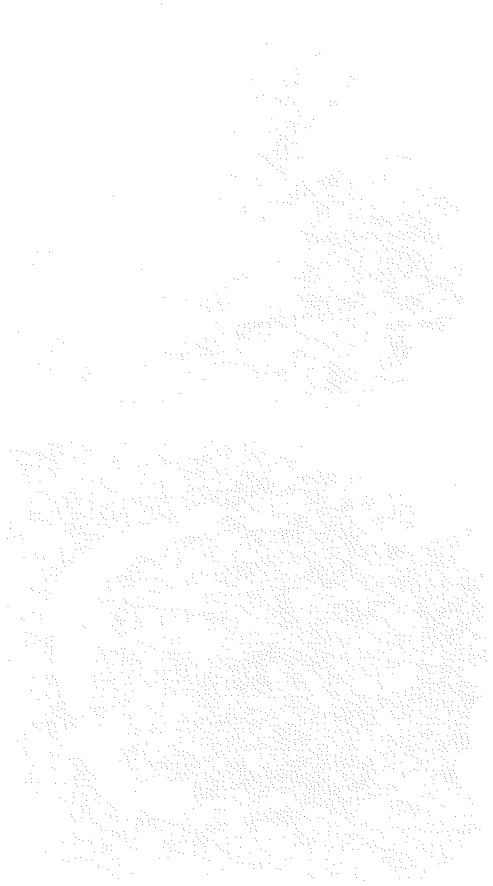
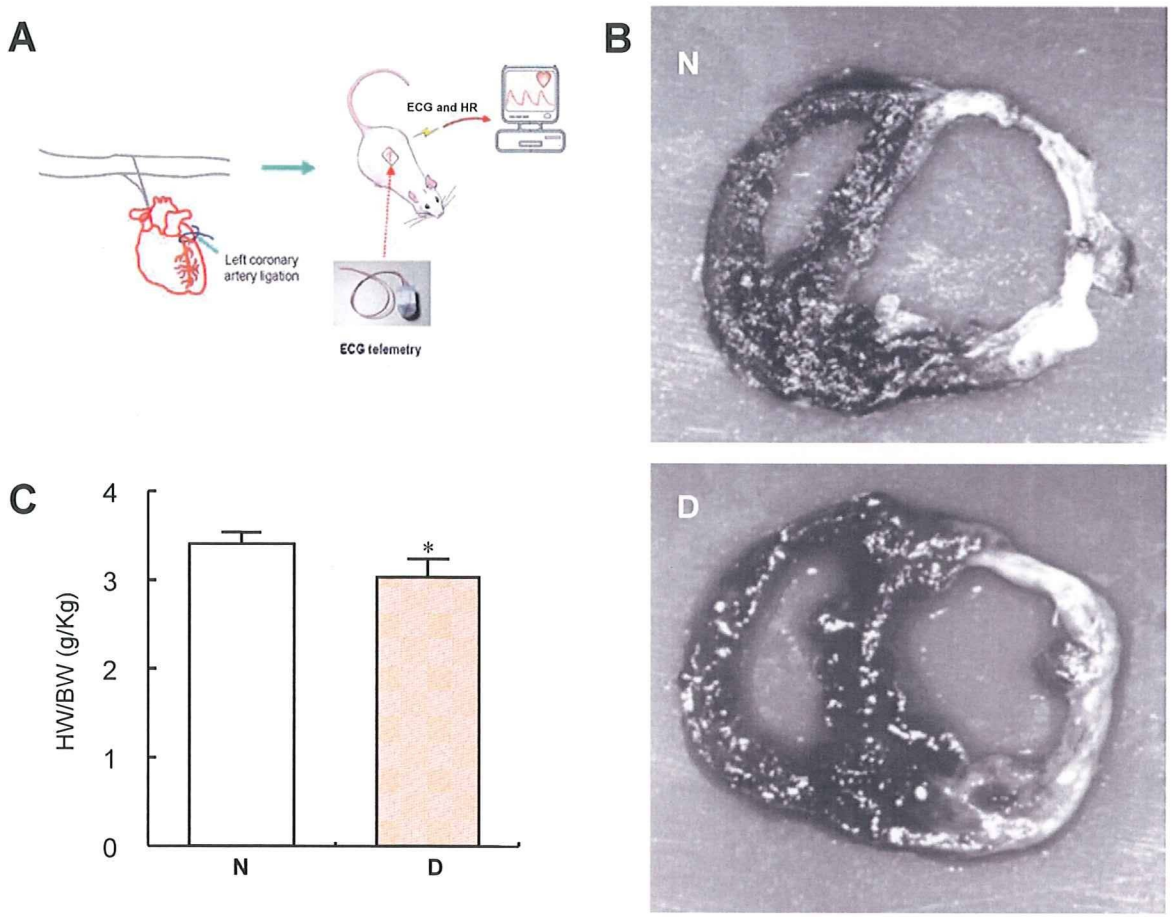


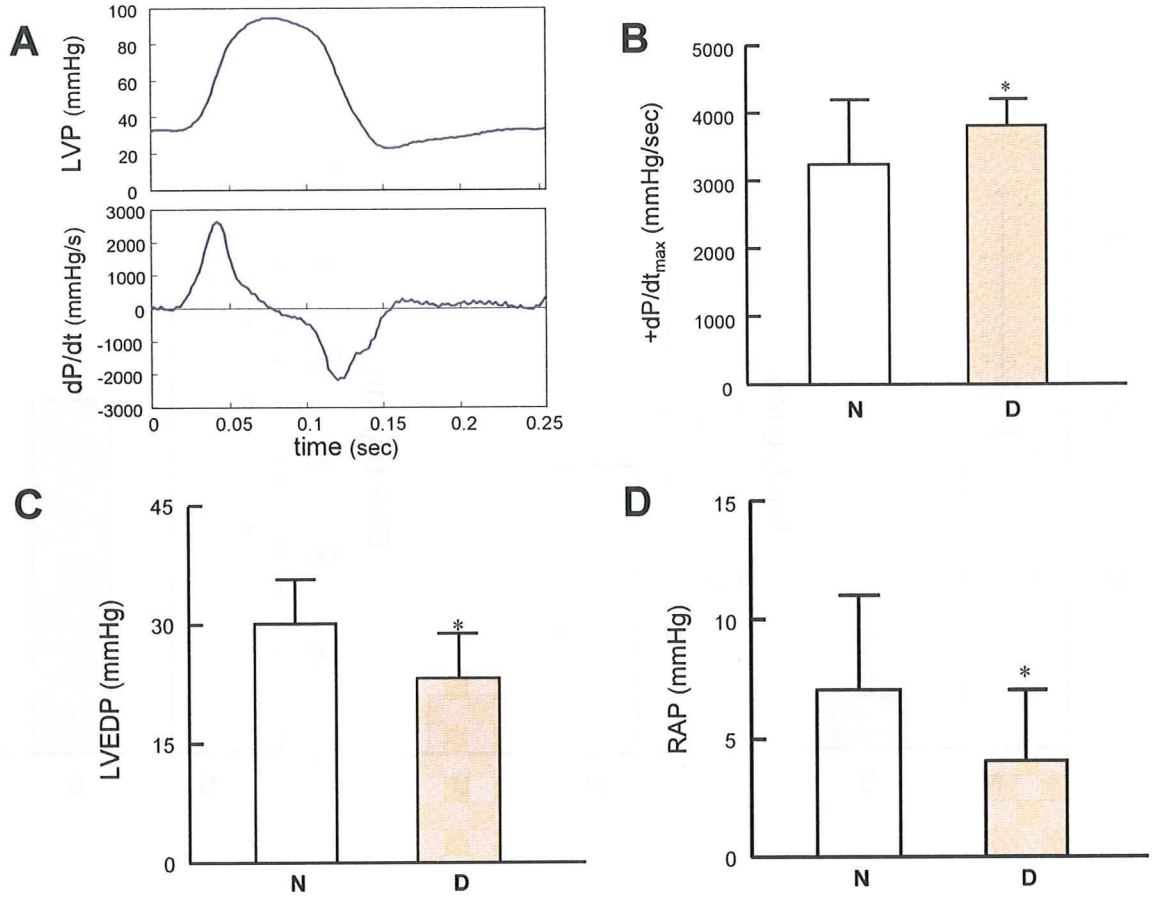
Figure legends

Figure 1.



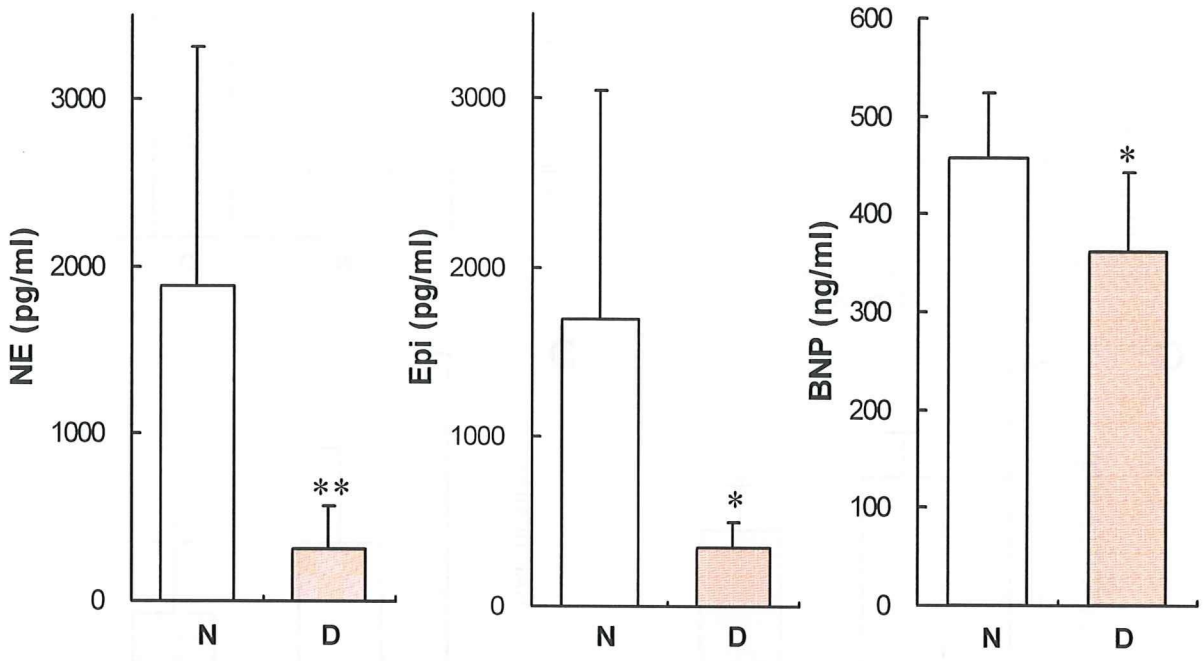
A: Schematic representation of the experimental design. Electrocardiogram was recorded continuously using a telemetric system. **B:** Ventricular sections of representative animals at week 6 of treatment. No significant difference in the size of myocardial infarction is observed between the donepezil group and the nontreated group. Compared with the nontreated heart (N), the donepezil-treated heart (D) showed 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 in the donepezil group (shaded bar, D) compared to the nontreated group (open bar, N). *: $p<0.05$

Figure 2.



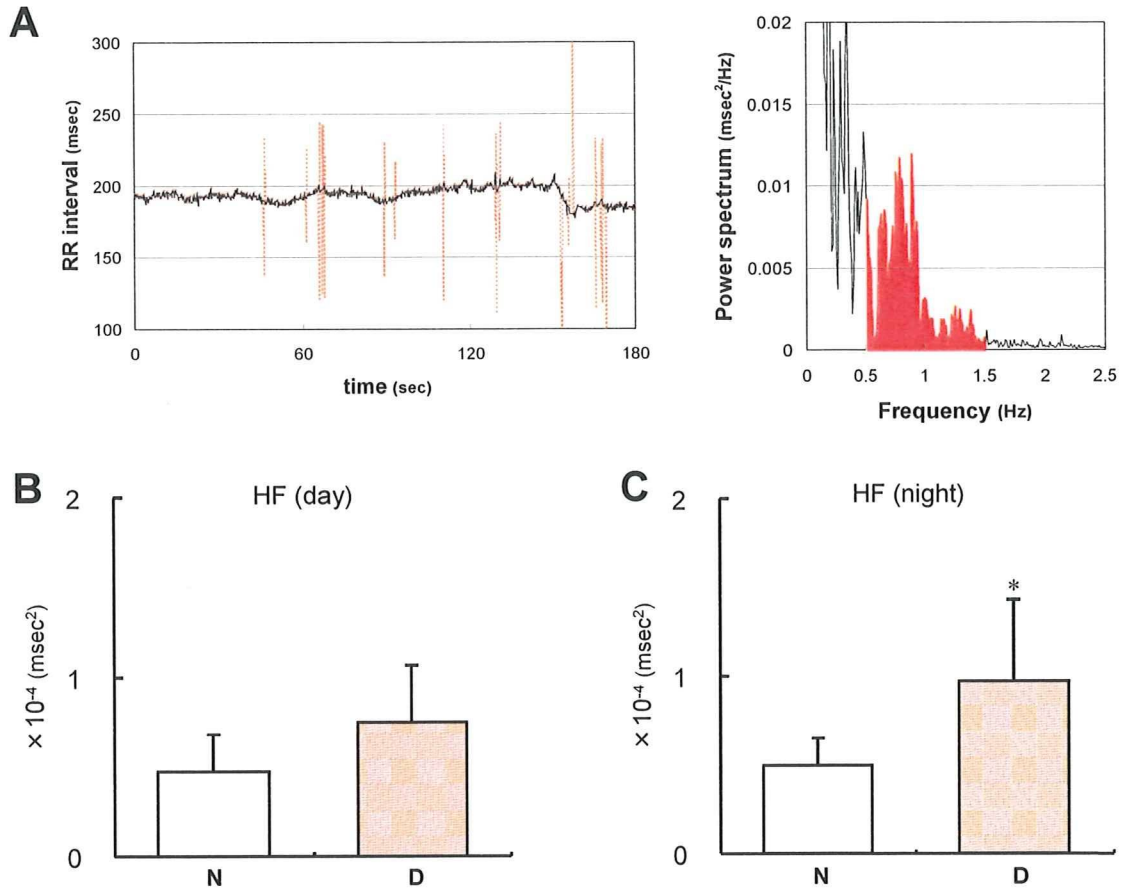
A: A representative example of left ventricular pressure waveform and its derivative in a nontreated rat. **B:** Maximal first derivative of left ventricular pressure (dP/dt_{max}) at week 6 of treatment. A significant increase in dP/dt_{max} was observed in the donepezil group (shaded bar, D) compared to the nontreated group (open bar, N). *: $p<0.05$ **C:** Left ventricular enddiastolic pressure (LVEDP) at week 6 of treatment. A significant decrease in LVEDP was observed in the donepezil group (shaded bar, D) compared to the nontreated control group (open bar, N). *: $p<0.05$ **D:** Right atrial pressure (RAP) at week 6 of treatment. A significant decrease in RAP was observed in the donepezil group (shaded bar, D) compared to the nontreated control group (open bar, N). *: $p<0.05$

Figure 3.



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

Figure 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 judged as extrasystoles or post-extrasystoles and were removed before calculating power spectrum. Solid area indicates high frequency component (HF). **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:** High frequency component (HF) of heart rate variability during the night. A significant increase in nocturnal HF value was observed in the donepezil group (shaded bar, D) compared to the nontreated group (open bar, N). *: $p<0.05$ by t-test using log(HF) values

Metformin Prevents Progression of Heart Failure in Dogs
Role of AMP-Activated Protein Kinase

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Background—Some studies have shown that metformin activates AMP-activated protein kinase (AMPK) and has a potent cardioprotective effect against ischemia/reperfusion injury. Because AMPK also is activated in animal models of heart failure, we investigated whether metformin decreases cardiomyocyte apoptosis and attenuates the progression of heart failure in dogs.

Methods and Results—Treatment with metformin (10 μmol/L) protected cultured cardiomyocytes from cell death during exposure to H_2O_2 (50 μmol/L) via AMPK activation, as shown by the MTT assay, terminal deoxynucleotidyl transferase–mediated dUTP nick-end labeling staining, and flow cytometry. Continuous rapid ventricular pacing (230 bpm for 4 weeks) caused typical heart failure in dogs. Both left ventricular fractional shortening and left ventricular end-diastolic pressure were significantly improved in dogs treated with oral metformin at 100 $mg \cdot kg^{-1} \cdot d^{-1}$ (n=8) (18.6±1.8% and 11.8±1.1 mm Hg, respectively) compared with dogs receiving vehicle (n=8) (9.6±0.7% and 22±0.9 mm Hg, respectively). Metformin also promoted phosphorylation of both AMPK and endothelial nitric oxide synthase, increased plasma nitric oxide levels, and improved insulin resistance. As a result of these effects, metformin decreased apoptosis and improved cardiac function in failing canine hearts. Interestingly, another AMPK activator (AICAR) had effects equivalent to those of metformin, suggesting the primary role of AMPK activation in reducing apoptosis and preventing heart failure.

Conclusions—Metformin attenuated oxidative stress–induced cardiomyocyte apoptosis and prevented the progression of heart failure in dogs, along with activation of AMPK. Therefore, metformin may be a potential new therapy for heart failure. (***Circulation*. 2009;119:2568-2577.**)

Key Words: AMP-activated protein kinase ■ heart failure ■ metformin ■ nitric oxide

Metformin is widely used as an antidiabetic drug with an insulin-sensitizing effect. A large-scale clinical trial (the UK Prospective Diabetes Study [UKPDS] 34) has shown that metformin therapy decreased the risk of cardiovascular death and the incidence of myocardial infarction associated with diabetes mellitus,[1] suggesting that this drug may be useful for patients who have both cardiovascular disease and diabetes mellitus. Eurich and colleagues[2] recently reported the results of a meta-analysis showing that metformin was the only antidiabetic agent to reduce all-cause mortality without causing any harm in patients who had heart failure and diabetes mellitus. These results suggest that a tight link exists between cardiovascular disease and diabetes mellitus and that metformin has a cardioprotective effect. Metformin is known to activate AMP-activated protein kinase (AMPK),[3–5] which is expressed in various tissues, including the myocardium, and plays a central role in the regulation of energy metabolism under stress conditions.[6] AMPK is activated by ischemia/reperfusion,[7–9] as well as in hearts with pressure overload hypertrophy[10] and subsequent heart failure.[11,12] In addition, Russell et al[9] have demonstrated that isolated hearts of AMPK-deleted mice show increased apoptosis and dysfunction after ischemia/reperfusion. Activation of AMPK by adiponectin also has been reported to protect cardiomyocytes against apoptosis and to attenuate myocardial ischemia/reperfusion injury in mice.[8] Furthermore, metformin has been reported to increase the production of nitric oxide (NO),[13–15] which is known to have various beneficial cardiovascular

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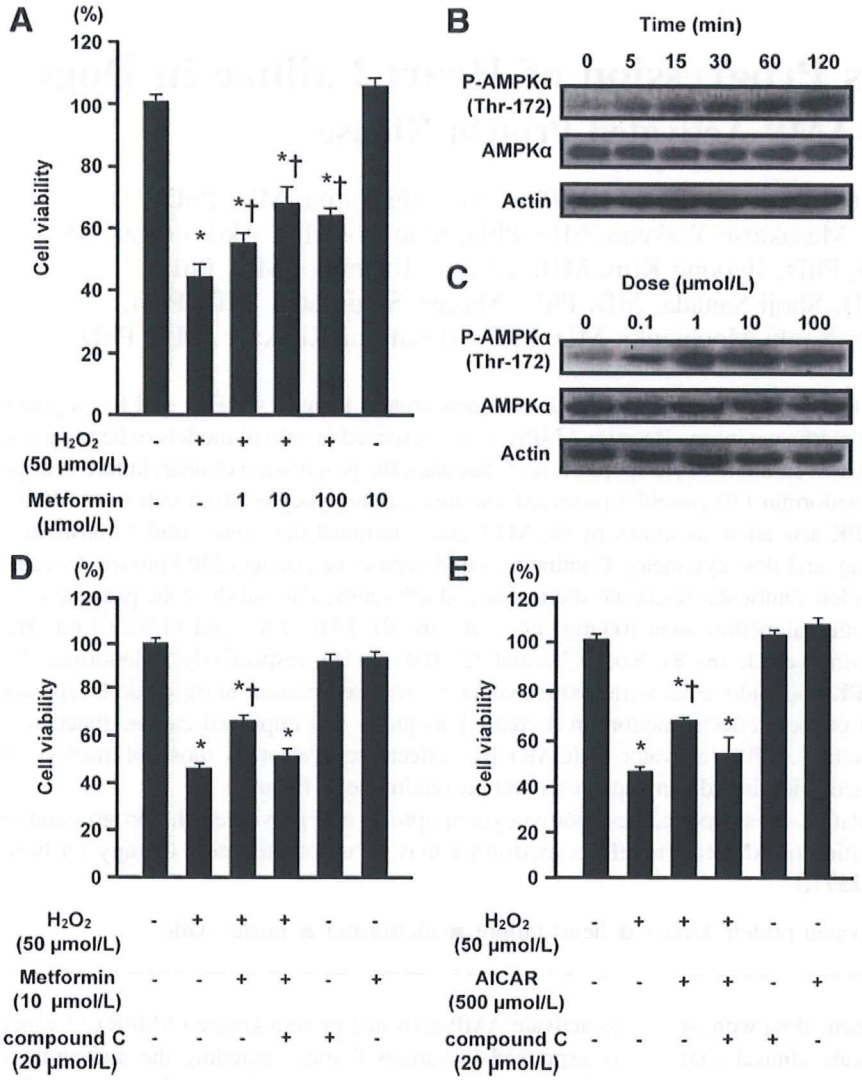

Figure 1. Effect of metformin on oxidative stress–induced cell death via AMPK activation in cultured rat cardiomyocytes. A, Cardiomyocyte viability after treatment with metformin (1, 10, or 100 μmol/L) and exposure to H_2O_2 (50 μmol/L). B, Time (0, 5, 15, 30, 60, 120 minutes) -dependent changes in AMPK phosphorylation in cardiomyocytes after treatment with metformin (10 μmol/L). C, Dose-dependent changes in AMPK phosphorylation in cardiomyocytes after treatment with metformin (0.1, 1, 10, or 100 μmol/L). D, Effect of an AMPK inhibitor (compound C; 20 μmol/L) on cardiomyocyte viability after treatment with metformin (10 μmol/L). E, Effect of an AMPK activator (AICAR; 500 μmol/L) on cardiomyocyte viability after treatment with metformin (10 μmol/L). Values are mean±SEM. P-AMPKα indicates phosphorylation of AMPKα. *$P<0.05$ vs no treatment; †$P<0.05$ vs H_2O_2 (50 μmol/L) treatment.

effects[16] and may alleviate mechanical or neurohormonal stress on the heart.

Clinical Perspective on p 2577

These findings led us to hypothesize that activation of AMPK by metformin may exert a cardioprotective effect under stress conditions. Accordingly, metformin might be a potential new treatment for cardiac failure because it activates AMPK and increases NO production. Therefore, we investigated the influence of metformin on apoptosis, an important feature of heart failure, using cultured neonatal cardiomyocytes exposed to H_2O_2 and the effect of metformin on the progression of pacing-induced heart failure in dogs, along with activation of AMPK.

Methods

Experimental procedures are described in the online-only Data Supplement.

Statistical Analysis

Results are expressed as mean±SEM. Comparison of changes between groups over time was performed by 2-way repeated-measures ANOVA. Other data were compared between groups by 1-way fractional ANOVA. The Tukey-Kramer test was used to correct for multiple comparisons. In all analyses, values of $P<0.05$ were considered to indicate statistical significance.

The authors had full access to and take full responsibility for the integrity of the data. All authors have read and agree to the manuscript as written.

Results

Metformin Attenuates Oxidative Stress–Induced Cell Death and Apoptosis in Cultured Cardiomyocytes via AMPK Activation

Cell viability was decreased in the presence of H_2O_2, as shown by the MTT assay, but this change was blunted by treatment with metformin in a dose-dependent manner (Figure 1A). Treatment with metformin (10 μmol/L) stimulated phosphorylation of AMPK in cultured cardiomyocytes in a time- and dose-dependent manner (Figure 1B and 1C). The effect of metformin on cell viability was blunted by cotreatment with compound C, an AMPK inhibitor (20 μmol/L) (Figure 1D). 5-Amino-4-imidazole-1-β-D-carboxamide ribofuranoside (AICAR; another AMPK activator) had an effect similar to metformin on cardiomyocyte viability after exposure to H_2O_2 (Figure 1E). These results suggested that

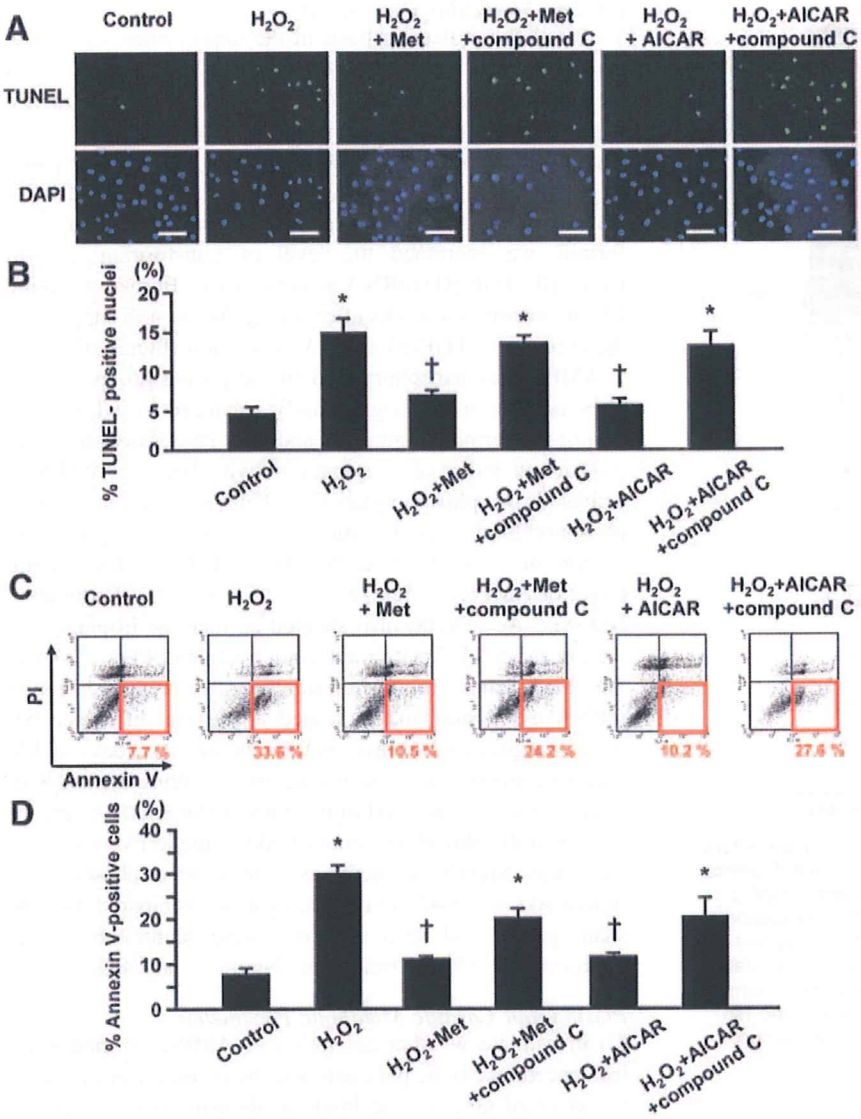

Figure 2. Effect of metformin on oxidative stress–induced apoptosis via AMPK activation in cultured rat cardiomyocytes. Representative (A) and quantitative (B) data on cardiomyocyte apoptosis obtained by TUNEL staining (n=3 in each experiment). Representative (C) and quantitative (D) data on cardiomyocyte apoptosis obtained by flow cytometry (n=3 in each experiment). Values are mean±SEM. PI indicates propidine iodide. *$P<0.05$ vs control; †$P<0.05$ vs H_2O_2 (50 μmol/L) treatment.

activation of AMPK protected cardiomyocytes against damage caused by H_2O_2.

H_2O_2 also increased cardiomyocyte apoptosis, as shown by the terminal deoxynucleotidyl transferase–mediated dUTP nick-end labeling (TUNEL) staining and flow cytometry (annexin V–positive and propidine iodide–negative cells) (Figure 2A through 2D). Metformin pretreatment significantly reduced the extent of cardiomyocyte apoptosis compared with that in untreated control cells (Figure 2A through 2D). Treatment with compound C inhibited the effects of metformin and AICAR (which was similar to that of metformin) on apoptosis in cardiomyocytes exposed to H_2O_2 (Figure 2A through 2D). These results suggested that the activation of AMPK by metformin could prevent apoptosis of cardiomyocytes induced by H_2O_2.

Effect of Metformin on Cardiac Function in Dogs With Pacing-Induced Heart Failure

Cardiac Physiological and Pathophysiological Parameters

Four weeks after the rapid right ventricular (RV) pacing, left ventricular (LV) end-diastolic dimension, LV end-systolic dimension, LV fractional shortening, and LV ejection fraction of the pacing group showed significant deterioration compared with the sham group (Figure 3A and 3B). Treatment with metformin significantly reduced both LV dimensions and increased both LV fractional shortening and LV ejection fraction compared with the pacing group (Figure 3A and 3B). Before RV pacing, both mean aortic pressure and heart rate were similar in all groups, and these parameters did not change throughout the study (Table). Four weeks after the RV pacing, pulmonary capillary wedge pressure, mean pulmonary artery pressure, and LV end-diastolic pressure were all significantly higher in the pacing group compared with the sham group (Figure 4A and 4B). Metformin treatment significantly reduced pulmonary capillary wedge pressure, mean pulmonary artery pressure, and LV end-diastolic pressure compared with the pacing group (Figure 4A and 4B). Furthermore, cardiac output was decreased and systemic vascular resistance was increased in the pacing group compared with the sham group, whereas metformin increased cardiac output and decreased systemic vascular resistance compared with the levels in the pacing group (the Table).

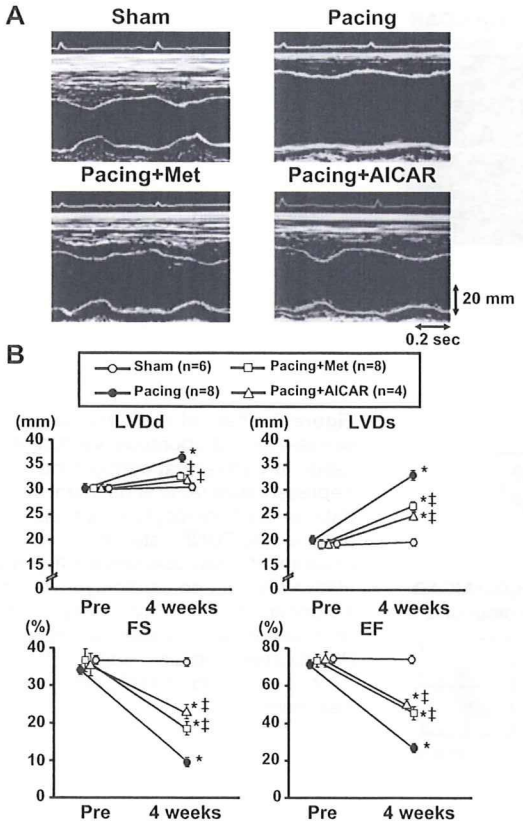

Figure 3. Effect of metformin on echocardiographic parameters. A, Representative M-mode echocardiograms obtained 4 weeks after sham surgery or after RV pacing. B, Echocardiographic parameters before and after sham surgery or after RV pacing in the sham group (n=6), pacing group (n=8), pacing plus metformin group (n=8), and pacing plus AICAR group (n=4). Values are mean±SEM. LVDd indicates LV end-diastolic dimension; LVDs, LV end-systolic dimension; LVFS, LV fractional shortening; and LVEF, LV ejection fraction. *$P<0.01$ vs sham group; ‡$P<0.01$ vs pacing group.

Importantly, the percentage of TUNEL-positive cells to total cells in LV myocardium in the pacing group increased compared with that in the sham group, which was blunted by treatment with either metformin or AICAR (Figure 5A through 5E).

Consistent with previous data,[17] no significant differences were found in body weight, the ratio of LV plus septal weight to body weight, and the ratio of RV weight to body weight among all groups (the Table).

To explore established markers of cardiac failure, we analyzed LV myocardial expression of the atrial natriuretic peptide and brain natriuretic peptide genes, which showed an increase in the pacing group, whereas metformin significantly suppressed this increase (Figure 6A and 6B). Metformin also significantly reduced the levels of angiotensin II and norepinephrine compared with the pacing group (the Table).

Pedometer counts were significantly reduced in the pacing group compared with the sham group, suggesting that heart failure led to reduced physical activities (the Table). Metformin increased the pedometer count compared with that in the pacing group. No differences in body fat were found among all groups (the Table).

Cardiac Molecular Parameters

To assess the molecular basis of the improvement in cardiac performance achieved by metformin administration for 4 weeks, we examined the collagen volume fraction in LV myocardium after staining with Masson's trichrome stain. Metformin reduced the collagen volume fraction compared with the pacing group (Figure 6C and 6D). To further investigate the mechanism of this antifibrotic effect of metformin, we examined the level of transforming growth factor-β1 (TGF-β1) mRNA associated with fibrosis in canine LV myocardium 4 weeks after pacing. Metformin suppressed the increase in TGF-β1 mRNA expression (Figure 6E).

AMPK was phosphorylated in the pacing group, and its phosphorylation was significantly enhanced by administration of metformin (Figure 7A and 7B). Phosphorylation was used as an index of enzymatic activity because AMPK is activated by phosphorylation.[18] This increase in AMPK phosphorylation was accompanied by augmented phosphorylation of acetyl-CoA carboxylase (ACC; a downstream target of AMPK) at Ser-79 (Figure 7A and 7C). Endothelial NO synthase (eNOS) also showed an increase in phosphorylation at Ser-1177 with metformin treatment (Figure 7A and 7D). Furthermore, metformin significantly upregulated eNOS mRNA expression and increased ΔNO (the difference between the plasma NO level before and after 4 weeks of RV pacing) compared with the pacing group (Figure 8A and 8B).

To investigate the level of insulin signaling in the heart, we examined the phosphorylation of Akt in the left ventricles in all groups. Significant increases were found in phosphorylation of Akt at Ser-473 in the pacing group compared with the sham group, and such increases were blunted by either metformin or AICAR treatment (Figure 8C and 8D).

Plasma and Cardiac Metabolic Parameters

To investigate whether activation of AMPK by metformin influenced metabolic parameters in the periphery or the heart, we assessed glucose and lipid metabolism after 4 weeks of pacing. Plasma free fatty acids tended to increase in the pacing group compared with the sham group, although no statistically significant difference was found. Fasting plasma levels of both glucose and lactate were similar among all groups (the Table). Both the fasting plasma insulin level and the homeostasis model assessment–insulin resistance value were significantly increased in the pacing group, whereas metformin reduced both parameters until they were similar to those of the sham group (the Table).

In the heart, both glucose extraction and the arterial–coronary sinus difference were increased in the pacing group compared with the sham group (the Table). In the pacing group, the free fatty acids extraction was not increased, but the arterial–coronary sinus difference tended to increase compared with the sham group (the Table). Lactate extraction and the arterial–coronary sinus difference were similar among all groups (the Table).

AICAR Mimics the Effect of Metformin in This Canine Pacing Model

To further confirm that activation of AMPK contributed to inhibition of the progression of heart failure, we administered

Table. Characteristics of the Dogs at 4 Weeks

	Sham Group (n=6)	Pacing Group (n=8)	Pacing+Metformin Group (n=8)	Pacing+AICAR Group (n=4)
Organ weight				
Body weight, kg	9.5±0.2	9.4±0.2	9.7±0.1	9.6±0.3
LV+septal weight, g	42±0.6	47.3±1.2	43.6±0.9	44.8±1.3
LV+septal weight/body weight ratio, g/kg	4.4±0.1	5.0±0.1	4.5±0.1	4.7±0.2
RV weight, g	14.7±0.5	15.6±0.6	15.0±1.2	14.7±1.0
RV weight/body weight ratio, g/kg	1.5±0.1	1.7±0.1	1.5±0.1	1.5±0.1
Hemodynamic parameters				
Mean aortic pressure, mm Hg	105±5	109±2	100±2	97±3.3
Heart rate, bpm	118±5	136±4	128±5	126±3.6
Cardiac output, L/min	2.6±0.1	1.6±0.1*	2.2±0.3†	2.2±0.3†
Systemic vascular resistance, dynes · s · cm^{-5}	3317±189	4769±235*	3775±334†	3763±237†
Plasma metabolic parameters				
Fasting glucose, mmol/L	5.3±0.3	5.3±0.1	5.3±0.1	5.3±0.2
Fasting insulin, μU/mL	14.2±3.3	67.6±13.7*	18.9±7.3†	24.4±10.5†
HOMA-IR	3.4±0.1	15.8±0.1*	4.4±0.1†	5.8±0.1†
Free fatty acids, μmol/L	305±67	716±68	554±101	595±69
Lactate, mmol/L	1.4±0.2	1.5±0.2	1.5±0.1	1.4±0.1
Cardiac metabolic substrates				
Glucose				
Arterial, mmol/L	5.8±0.1	6.4±0.2	6.6±0.1	6.6±0.4
Arterial–coronary sinus difference, mmol/L	0.6±0.1	1.6±0.3*	0.9±0.1	1.1±0.3
Extraction rate, %	10.5±1.2	28.6±4.7*	13.3±1.8	17.7±4.7
Free fatty acids				
Arterial, mmol/L	213.5±44.9	532.3±98.5*	312.8±56.6	294.5±22.8
Arterial–coronary sinus difference, mmol/L	90.4±13.2	153.7±20.6	99.0±9.1	103.2±20.6
Extraction rate, %	47.5±9.2	29.9±2.8	33.9±5.1	36.9±8.6
Lactate				
Arterial, mmol/L	1.8±0.1	1.9±0.3	2.3±0.7	1.8±0.8
Arterial–coronary sinus difference, mmol/L	1.2±0.3	1.0±0.2	1.3±0.5	1.1±0.4
Extraction rate, %	62.6±16.0	48.2±3.8	55.0±12.2	61.8±6.9
Plasma neurohormone levels				
Norepinephrine, pg/mL	34.9±13.0	195.9±21.3*	59.2±11.2†	79.3±8.9†
Angiotensin II, pg/mL	34.7±15.0	153.6±24.3*	78.1±14.8†	73.4±11.8†
Body fat and activity				
Body fat, %	13.7±1.2	18.7±2.9	16±1.2	14.3±0.8
Pedometer count	88 783±2899	64 541±2530*	78 423±3292†	77 716±1472†

HOMA-IR indicates homeostasis model assessment–insulin resistance. Values are mean±SEM.
*$P<0.05$ vs the sham group; †$P<0.05$ vs the pacing group.

another AMPK activator (AICAR at a dose of 5 mg/kg SC every other day) to dogs. As expected, AICAR reproduced the effects of metformin in this canine pacing model (Figures 3 through 8).

Discussion

To the best of our knowledge, this is the first study to demonstrate clearly that long-term (not short-term) oral administration of metformin, which is used as an antidiabetic agent worldwide, inhibits cardiac remodeling and prevents the progression of heart failure in dogs, along with increases in AMPK activation and NO production. Of course, we and others have previously shown that in rodent either AMPK activation or NO production attenuates myocardial ischemia/reperfusion injury in the ischemic model[7–9] and prevents cardiac remodeling in the pressure overload model.[11,12,19,20] However, it has been unclear whether AMPK or NO can modulate cardiac remodeling and inhibit the progression of heart failure in a canine model with another pathogenic mechanism that is not an ischemic or a pressure overload heart failure model. Therefore, we used a rapid pacing-induced heart failure dog model, which is considered to be similar to human dilated cardiomyopathy[21,22] and can be superimposed on translational study for human heart failure.