microtubule polymerization and unpolarized microtubule stabilization might affect the function of microtubules, which are required to establish cell polarity. We have shown that inhibiting CLIP-170 phosphorylation resulted in significant enlargement of focal adhesions, as detected with a paxillin antibody. Enlarged focal adhesions are similar to the phenotype observed in cells treated with paclitaxel or nocodazole, both of which disrupt microtubule dynamics. Microtubules bind to paxillin and help the cell adhesion system to destabilize focal adhesions and promote cell motility[21,22]. These functions suggest that microtubules play a key part in cell polarity and migration through interactions with focal adhesion molecules. Taken together, the results suggest that AMPK promotes the appropriate formation of focal adhesions, the subsequent establishment of cell polarity, and directional cell migration through efficient polymerization of microtubules, by phosphorylating CLIP-170 at Ser 311.

Under normal cell culture conditions, neither enhanced activation of AMPK by AICAR nor S311D CLIP-170–EGFP altered microtubule dynamics, indicating a high basal phosphorylation of CLIP-170. This might be caused by a high affinity of AMPK for CLIP-170, or colocalization of AMPK and CLIP-170.

The results of our broad substrate screening method suggest that CLIP-170 is one of the most important substrates of AMPK in various organs. We believe that observing microtubule dynamics is necessary to evaluate multiple functions of AMPK. Also, similarly to paclitaxel or nocodazole treatment, strong inhibition of microtubule dynamics by the CLIP-170 S311A mutant may have clinical implications. The interaction between AMPK and CLIP-170 might be a therapeutic target for treatment of conditions such as cancer, tumour angiogenesis and neointimal hyperplasia. □

## METHODS

Methods and any associated references are available in the online version of the paper at http://www.nature.com/naturecellbiology/

*Note: Supplementary Information is available on the Nature Cell Biology website.*

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AUTHOR CONTRIBUTIONS

A.N. designed and conducted the study, performed most of the experiments, and wrote the manuscript; S.T. designed and conducted the study, performed the biochemical experiments and wrote the manuscript; H.K. carried out immunoblot analysis; K.M. independently counted the number of cells; S.Y. helped to generate the plasmids; Y.A., O.S., S.H., Y.S., H.A., M.A. and T.M. discussed the results and reviewed the manuscript; T.W. and K.K. generated and provided antibodies and Vero cells and reviewed the manuscript; N.M. conducted and supported the biological experiments and wrote the manuscript; M.K. supervised all work.

COMPETING INTERESTS

The authors declare no competing financial interests.

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ORIGINAL CONTRIBUTION

# A histamine $H_2$ receptor blocker ameliorates development of heart failure in dogs independently of $\beta$-adrenergic receptor blockade

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**Abstract** Histamine has a positive inotropic effect on ventricular myocardium and stimulation of histamine $H_2$ receptors increases the intracellular cAMP level via Gs protein, as dose stimulation of $\beta$-adrenergic receptors, and worsens heart failure. To test whether a histamine $H_2$ receptor blocker had a beneficial effect in addition to $\beta$-adrenergic receptor blockade, we investigated the cardioprotective effect of famotidine, a histamine $H_2$ receptor blocker, in dogs receiving a $\beta$-blocker. We induced heart failure in dogs by rapid ventricular pacing (230 beats/min). Animals received no drugs (control group), famotidine (1 mg/kg daily), carvedilol (0.1 mg/kg daily), or carvedilol plus famotidine. Both cardiac catheterization and echocardiography were performed before and 4 weeks after the initiation of pacing. Immunohistochemical studies showed the appearance of mast cells and histamine in the myocardium after 4 weeks of pacing. In the control group, the left ventricular ejection fraction (LVEF) was decreased after 4 weeks compared with before pacing ($71 \pm 2$ vs. $27 \pm 2\%$, $p < 0.05$) and mean pulmonary capillary wedge pressure (PCWP) was increased ($8 \pm 1$ vs. $19 \pm 3$ mmHg). Famotidine ameliorated the decrease of LVEF and increase of PCWP, while the combination of carvedilol plus famotidine further improved both parameters compared with the carvedilol groups. These beneficial effects of famotidine were associated with a decrease of the myocardial cAMP level. Histamine $H_2$ receptor blockade preserves cardiac systolic function in dogs with pacing-induced heart failure, even in the presence of $\beta$-adrenergic receptor blockade. This finding strengthens the rationale for using histamine $H_2$ blockers in the treatment of heart failure.

**Keywords** Heart failure · Histamine · Histamine $H_2$ receptor blocker · $\beta$-Adrenergic receptor blocker

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## Introduction

Chronic heart failure (CHF) is one of the major causes of morbidity and mortality worldwide, and is characterized by neurohormonal imbalances that include activation of the sympathetic nervous system [9, 15]. $\beta$-Adrenergic receptor blockade is a established treatment of CHF because it protects the heart from the harmful effects of the sympathetic nervous system that are partly mediated via cyclic adenosine monophosphate (cAMP)-dependent pathways [2, 34]. Interestingly, histamine $H_2$ receptors are linked to Gs proteins that facilitate the production of cAMP (as are $\beta$-adrenergic receptors) and are expressed in the heart [18, 29, 33]. Histamine has a positive inotropic effect on human ventricular myocardium and chronotropic effects [3, 12], and also autonomic control of the heart [21]. Indeed, we previously reported that famotidine, a histamine $H_2$

receptor blocker, protected the heart against ischemia-reperfusion injury in dogs [1] and also improved both symptoms of CHF and ventricular remodeling in the clinical setting [16]. Although the maximum inotropic effects of substances acting through cAMP were decreased in diseased myocardium [6], famotidine, a histamine $H_2$ receptor blocker, exerts negative effects on cardiac performance [13], the roles of the histamine would have remained unclear in the state of heart failure.

In addition, it is still unclear whether histamine $H_2$ receptor blockers have a protective effect against CHF by reducing the myocardial accumulation of cAMP and whether there is an additive effect of histamine $H_2$ receptor blockade in the presence of $\beta$-adrenergic receptor blockade.

Therefore, we investigated the effect of a histamine $H_2$ receptor blocker on cardiac performance and myocardial cAMP accumulation in dogs with pacing-induced heart failure, and also investigated whether there was an additive effect of combined histamine $H_2$ receptor blocker and $\beta$-blocker therapy on cardiac performance.

## Methods

### Materials

The histamine $H_2$ receptor blocker famotidine was kindly provided by Astellas Pharma Inc. (Tokyo, Japan). Carvedilol, a $\beta$-adrenergic receptor blocker, was obtained from Sigma (St. Louis, MO, USA). Rabbit polyclonal anti-histamine antibody was obtained from Progen (Queensland, Australia).

### Animal preparation

Beagle dogs (Oriental Yeast Co. Ltd., Tokyo, Japan) weighing 8–10 kg were sedated with intravenous sodium pentobarbital at a dose of 25 mg/kg. After intubation with a cuffed endotracheal tube, anesthesia was maintained with 0.5–1% isoflurane and an equal mixture of air and oxygen. Ventilation was provided with a tidal volume of 22 mL/kg at a rate of 15 times per minute. A bipolar pacing lead (Model BT-45P, Star Medical Inc., Tokyo, Japan) was advanced under fluoroscopic guidance through the right jugular vein to the right ventricular (RV) apex and was connected to an external programmable pacemaker (VOO mode; Model SIP-501, Star Medical Inc., Tokyo, Japan) that was implanted in a subcutaneous pocket in the neck. The success of this procedure was confirmed by electrocardiography. Antibiotics were given after surgery, and the dogs were allowed to recover fully. Then heart failure was induced by rapid right ventricular pacing at a rate of 230 beats/min for 4 weeks as the model mimicking heart failure in human, as reported previously [22, 23, 27].

All procedures were performed in conformity with the Guide for the Care and Use of Laboratory Animals (NIH publication No. 85–23, 1996 revision) and were approved by the ethical committee for laboratory animal use of the National Cardiovascular Center in Japan.

### Echocardiography

Transthoracic echocardiography was performed by using an echocardiographic system equipped with a 2–4 MHz phased-array transducer (SONOS 5500, Hewlett Packard, Massachusetts, USA) in conscious dogs before pacemaker implantation and 30 min after the cessation of RV pacing at 4 weeks. Good two-dimensional short-axis views of the left ventricle were obtained at the level of the papillary muscles for guided M-mode measurement of the left ventricular (LV) end-diastolic dimension (LVDd), LV end-systolic dimension (LVDs), LV fractional shortening (LVFS), and LV ejection fraction (LVEF). All measurements were made by two observers, who were blinded to the source of the tracings.

### Hemodynamic studies

LV pressure and mean aortic pressure were measured by pressure amplifiers connected to a pig tail catheter (5F, Terumo Co. Ltd., Tokyo, Japan) that was inserted into the left ventricle from the left femoral artery. Pulmonary capillary wedge pressure (PCWP) was measured with a 7 Fr Swan-Ganz catheter (American Edwards Laboratories, California, USA). LV $dP/dt$ was analyzed using software (Data viewer, Yokogawa Electric Corporation, Tokyo, Japan). These studies were performed both before and after 4 weeks of RV pacing or 4 weeks after pacemaker implantation in the sham group.

### Measurement of the myocardial cAMP level

The myocardial cyclic AMP (cAMP) level was measured as described previously [8]. Briefly, a sample of frozen cardiac muscle was homogenized mechanically in 500 mL of frozen hydrochloric acid (0.1 N) with a mechanical homogenizer. The homogenate was thawed and centrifuged at $5{,}000\times g$ at room temperature for 15 min and then a 100 mL aliquot of the supernatant was employed to measure cAMP with a sensitive radioimmunoassay (cyclic AMP kit; Yamasa Shoyu Co., Choshi, Japan).

### Immunohistochemical analysis

Immunohistochemical analysis was performed as described previously [24]. Briefly, myocardial tissue samples were fixed in 10% formalin and embedded in paraffin. Then

5-μm-thick sections were cut and preincubated with 3% hydrogen peroxide. Rabbit polyclonal anti-histamine antibody (1:1,000 dilution) was added, and incubation was done at room temperature overnight. Next, the sections were incubated with biotinylated anti-rabbit immunoglobulin for 30 min and subsequently with horseradish peroxidase-labeled streptavidin solution for 30 min. The slides were rinsed in tris-buffered saline after each incubation step. Peroxidase activity was visualized by incubation with diaminobenzidine hydrochloride solution.

### Experimental protocols

*Protocol 1: effects of famotidine on cardiac performance and myocardial cAMP accumulation in dogs with pacing-induced heart failure*

After pacemaker implantation, the dogs were randomly assigned to a sham group ($n = 6$) without pacing, a control group ($n = 7$) with pacing only, and a famotidine group ($n = 5$) with pacing plus the daily oral administration of famotidine (1 mg/kg per day). The dose of famotidine was chosen on the basis of previous reports [30, 36]. Echocardiography and measurement of hemodynamic parameters were performed before and 4 weeks after pacemaker implantation. After the measurement of hemodynamic parameters, myocardial tissue samples were obtained and quickly placed into liquid nitrogen for storage at −80°C until measurement of cAMP levels.

*Protocol 2: effects of famotidine on cardiac performance in dogs with pacing-induced heart failure under β-adrenergic receptor blockade*

Next, we examined the additive effect of histamine $H_2$ receptor blockade on the development of CHF. After pacemaker implantation, the dogs were randomly assigned to a carvedilol group ($n = 6$) that received daily oral administration of carvedilol (0.1 mg/kg per day) or a carvedilol + famotidine group ($n = 6$) that received daily oral administration of both carvedilol (0.1 mg/kg per day) and famotidine (1 mg/kg per day).

### Statistical analysis

Results are expressed as the mean ± SEM. Comparison of time-course changes between the groups was performed by two-way repeated measures analysis of variance (ANOVA). For comparison of mast cell counts and cAMP levels between the groups, the Mann–Whitney $U$ test was performed. A $p$ value $< 0.05$ was considered to represent statistical significance.

## Results

### Mast cells and histamine expression

Mast cells were detected in the myocardium by toluidine blue staining. Consistent with previous reports [8, 26], we observed an increase of mast cells in the failing hearts compared with the number of cells in the sham group (Fig. 1a). Immunohistochemical analysis showed an increase of histamine expression indicating increased degranulation of mast cells in failing hearts compared with the level in the sham group (Fig. 1b).

### Effect of famotidine on cardiac performance and myocardial cAMP in dogs with pacing-induced heart failure

Both mean aortic pressure and heart rate before pacing were similar in the control group (104 ± 5 mmHg and

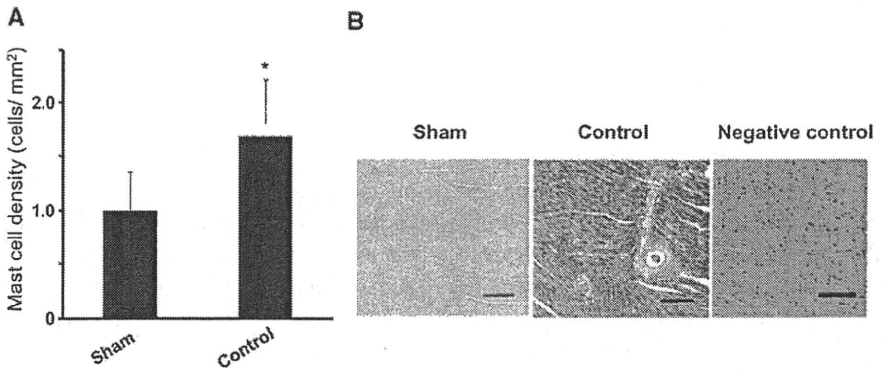


**Fig. 1** Mast cell density and histamine expression in the failing heart. **a** Mast cell density in the heart. Values are the mean ± SEM. *$p < 0.05$ versus the sham group. **b** Immunostaining with an anti-histamine antibody. **a** Representative staining of a heart from the sham group. **b** Representative staining of a heart from the control group. **c** Negative control section incubated without the primary antibody. The *scale bars* indicate 50 μm

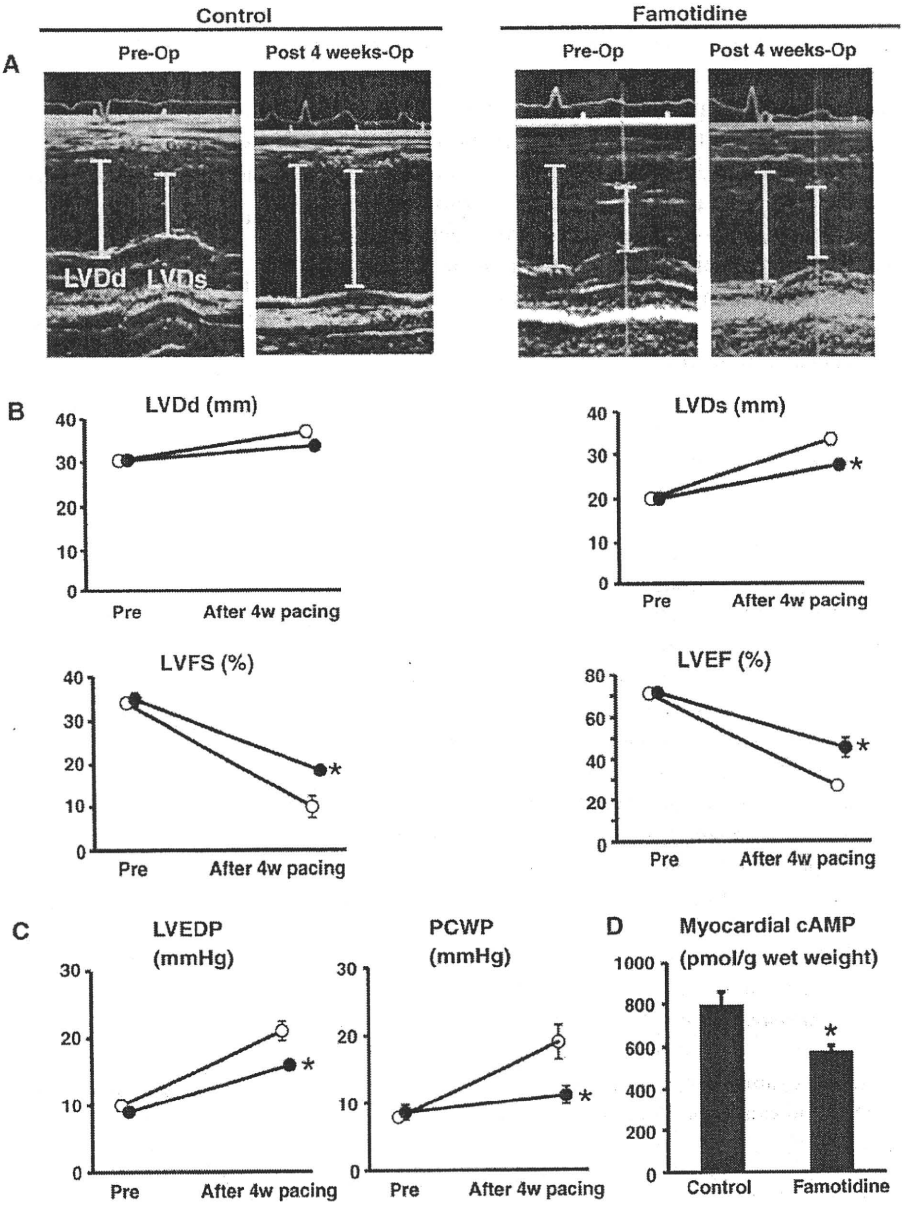


**Fig. 2** Effects of famotidine on echocardiographic and hemodynamic parameters and myocardial cAMP levels. **a** Representative 2D echocardiograms. **b** Quantitative analysis of echocardiographic parameters in the control and famotidine groups. *Open* and *closed circles* indicate the control group and the famotidine group, respectively. **c** LVEDP and mean PCWP in the control and famotidine groups. **d** Myocardial cAMP levels in each group. All values are the mean ± SEM. $*p < 0.05$ versus the sham group

117 ± 7 bpm, respectively), and the famotidine group (101 ± 3 mmHg and 115 ± 8 bpm, respectively). These parameters did not significantly differ among the groups. LV d*P*/d*t* was 3,592 ± 512 mmHg/s in the control group and 3,981 ± 528 mmHg/s in the famotidine group. Four weeks after surgery, neither hemodynamic nor echocardiographic data showed any changes compared with the preoperative values in the sham group (data not shown). Four weeks after rapid RV pacing, an administration of famotidine significantly limited the increase of both LVDd and LVDs, as well as the decrease of both LVFS and LVEF (33.4 ± 0.8 mm, 27.4 ± 1.2 mm, 18.5 ± 2.6% and 45.4 ± 4.8%, respectively), compared with the findings in the control group (37.0 ± 1.4 mm, 33.4 ± 1.4 mm, 9.9 ± 1.0% and 26.7 ± 2.4%, respectively) (Fig. 2a, b). Four weeks after RV pacing, LV end-diastolic pressure (LVEDP) and PCWP of the famotidine group (16 ± 2 and 11 ± 1 mmHg, respectively), were both significantly lower compared with the values in the control group (21 ± 2 and 19 ± 2 mmHg and, respectively) (Fig. 2c). LV d*P*/d*t* after RV pacing was significantly preserved higher in the famotidine group (2,601 ± 216 mmHg/s) compared with that in control group (2,077 ± 124 mmHg/s) ($p < 0.05$).

The myocardial cAMP level was significantly higher in the control group (796 ± 111 pmol/g wet weight) compared with that in the sham group (597 ± 77 pmol/g wet

weight), while it was significantly lower in the famotidine group (577 ± 28 pmol/g wet weight) compared with the control group ($p < 0.05$) (Fig. 2d).

### Additive effects of famotidine and a β-blocker on cardiac performance in dogs with pacing-induced heart failure

Before pacing, mean aortic pressure and heart rate were both similar in the carvedilol group (101 ± 7 mmHg and 111 ± 8 bpm, respectively), and the famotidine + carvedilol group (93 ± 2 mmHg, 106 ± 7 bpm, respectively), and these parameters did not significantly differ among the groups. LV $dP/dt$ was 3,672 ± 417 mmHg/s in the carvedilol group and 3,941 ± 284 mmHg/s in the famotidine + carvedilol group. After rapid RV pacing for 4 weeks, both LVDd and LVDs were decreased and both LVFS and LVEF were increased (33 ± 0.4 mm, 25 ± 0.7 mm, 28 ± 2%, and 54 ± 3%, respectively), in the famotidine + carvedilol group compared with the respective values in the carvedilol group (34 ± 1 mm, 28 ± 1 mm, 23 ± 1%, and 38 ± 5%, respectively) (Fig. 3a).

Four weeks after RV pacing, LVEDP and PCWP of the carvedilol + famotidine group (12 ± 3 and 10 ± 4 mmHg, respectively), were both significantly reduced compared with the respective values in the carvedilol group (16 ± 2 and 15 ± 1 mmHg, respectively) (Fig. 3b). LV $dP/dt$ after RV pacing was preserved higher in the famotidine + carvedilol group (3,382 ± 252 mmHg/s) compared with that in carvedilol group (2,740 ± 321 mmHg/s) ($p < 0.05$).

Furthermore, the myocardial cAMP level was significantly lower in the carvedilol + famotidine group (488 ± 45 pmol/g wet weight) compared with that in the carvedilol group (615 ± 28 pmol/g wet weight) (Fig. 3c).

## Discussion

In the present study, we demonstrated that (1) myocardial histamine expression was increased by pacing-induced heart failure, (2) the histamine $H_2$ receptor blocker famotidine improved cardiac performance (gauged by echocardiographic and hemodynamic parameters) along with a reduction of myocardial cAMP accumulation, and (3) there was an additive effect of combined histamine $H_2$ receptor and β-adrenergic receptor blockade on cardiac performance in dogs with pacing-induced heart failure.

### Impact of histamine blockade on cardiac failure

Histamine is one of the autacoids, and is stored and released by mast cells in the human heart, as well as in other organs or

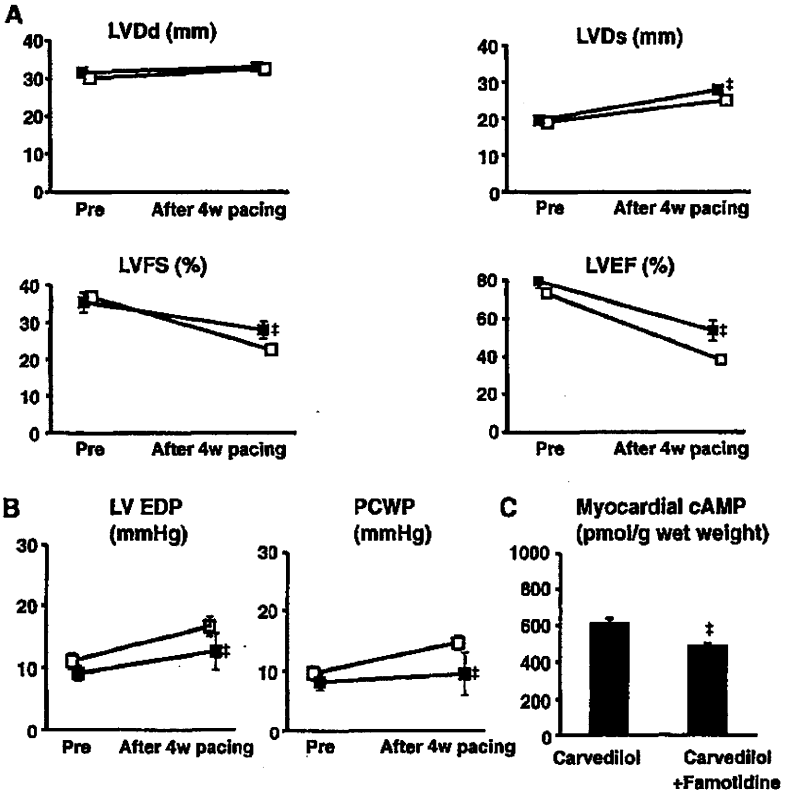


**Fig. 3** Additive effect of famotidine and carvedilol on cardiac performance. **a** Quantitative analysis of echocardiographic parameters in the carvedilol and carvedilol + famotidine groups. *Open* and *closed squares* indicate the carvedilol group and the carvedilol + famotidine group, respectively. **b** LVEDP and mean PCWP in the carvedilol group and carvedilol + famotidine group. *Open* and *closed squares* indicate the carvedilol group and the carvedilol + famotidine group, respectively. **c** Myocardial cAMP levels in each group. All values are the mean ± SEM. *$p < 0.05$ versus the carvedilol group

tissues [8]. Recently, the number of mast cells and the myocardial histamine level were found to increase in the hearts of patients with idiopathic dilated cardiomyopathy or ischemic cardiomyopathy [26]. Histamine modulates various cellular functions via the activation of four different G-protein-coupled receptors ($H_{1-4}$ receptors) [29]. As is well known, histamine $H_2$ receptors located on gastric cells promote the production of gastric acid [10] and histamine $H_2$ receptor blockers have been widely used for the treatment of peptic ulcer. Interestingly, histamine $H_2$ receptors are also expressed in canine and human ventricular myocardium [14, 18], although the expression levels were different among species [18]. Consisted with the previous studies, we confirmed the presence of histamine $H_2$ receptor in the canine heart using quantitative reverse-transcriptase PCR (data not shown).

In the present study, the precise locations of histamine receptors in myocytes or vessels in the canine hearts remained unclear. However, since famotidine did not decrease blood pressure in this study, the accumulating lines of evidence would suggest that histamine $H_2$ blockers did not have the potent effect on the vessels compared with cardiomyocytes. On the other hand, since histamine $H_1$ receptors are abundantly expressed in the vessels in most animal species [29], histamine $H_1$ receptor blocker has the effects on vessels. In addition, stimulation of $H_2$ receptors transduces the intracellular signals via Gs protein, as does the stimulation of $\beta$-adrenergic receptors. Moreover, histamine has a positive inotropic effect on human ventricular myocardium and has been suggested to have a role in cardiovascular diseases [3, 11]. Although it was reported that the maximum inotropic effects of histamine receptor stimulation were less than those mediated by beta-adrenergic receptors [5, 35], the roles of histamine receptor blockade have remained unclear compared with those of beta-adrenergic receptor.

Based on these backgrounds, we previously proposed that histamine $H_2$ receptor blockers could provide a novel therapeutic strategy for heart failure, and we have reported that histamine $H_2$ blocker treatment may have a cardioprotective effect in patients with chronic heart failure [16]. In the present study, we found that myocardial histamine expression was increased in dogs with pacing-induced heart failure compared with that in sham dogs on immunohistochemical analysis. Also, the histamine $H_2$ receptor blocker, famotide, prevented the development of heart failure induced by rapid RV pacing and lessened the myocardial accumulation of cAMP. These findings suggest that histamine $H_2$ receptor blockade exerts a cardioprotective effect along with the amelioration of myocardial cAMP accumulation. Recently it was reported that increased cardiac adenylyl cyclase expression is associated with mortality after myocardial infarction in rats [31]. It has been controversial for the role of myocardial cAMP in the heart failure [17]. Although increased cAMP acutely induced the improvement of ventricular function, several trials with either beta-adrenergic agonists or PDE inhibitors have revealed an increase in mortality [17, 20]. In the present study, in the dogs with the drugs that decreases myocardial cAMP levels, the development of heart failure was substantially attenuated, however further investigation will be needed to solve the roles of cAMP in the onset and progression of heart failure.

### Additive effects of histamine $H_2$ blocker and $\beta$-blocker therapy on cardiac performance

$\beta$-Blockers have long been established as useful agents for chronic heart failure [2, 7, 25, 32]. These drugs act by preventing intracellular Ca overload, because $\beta$-adrenergic stimulation promotes Ca overload via Gs protein [34]. Histamine $H_2$ receptor blockade also prevents Ca overload [28], so we hypothesized that the combination of a histamine $H_2$ receptor blocker and a $\beta$-blocker would exert a stronger cardioprotective effect than either agent alone. Almost all of the patients in our earlier study, which showed that histamine $H_2$ receptor blockers were effective for the treatment of CHF, were also on $\beta$-blocker therapy [16], suggesting that there was an additive effect of histamine $H_2$ receptor and $\beta$-adrenergic receptor blockade in patients with CHF. In the present study, we demonstrated that the combination of a histamine $H_2$ receptor blocker and a $\beta$-blocker prevented the development of heart failure compared with $\beta$-blocker monotherapy. Thus, histamine $H_2$ receptor blockers have a potential clinical role in the treatment of CHF.

### Rationale of the present study

The present study provided strong experimental evidence that histamine $H_2$ receptor blockade improves the pathophysiology of CHF. We have already reported about the beneficial effects of famotidine in patients with heart failure [16]. However, clinical research may be confounded by unexpected errors due to (1) the influence of other drugs being used by patients with CHF, (2) variation in the severity of CHF between patients, and (3) variation in the duration of CHF. Therefore, it was important to prove that histamine $H_2$ receptor blockade improves CHF in a controlled experimental setting (canine cardiomyopathy model) to support the clinical use of famotidine for CHF. Furthermore, to determine the merit of famotidine in heart failure patients, the present study would be a basis to design a prospective randomized double-blinded study.

## Limitations

There are several limitations in this study. First, carvedilol blocks alpha$_1$-, beta$_1$-, beta$_2$- receptors, decreased the cardiac effects of norepinephrine, and has additional antioxidant and antiproliferative effects [4, 19]. In the present study, we did not address that carvedilol has the pleiotropic effects and is not just a beta-blocker.

Second, we did not measure cardiac output as a cardiac contractive index in the present study. However, we have previously reported that our tachycardia-induced heart failure model in dogs using the same procedure of the present study, revealed the low output state that mimics heart failure in human [27]. Our untreated dogs in the heart failure group were strongly suggested in the low output state because of decreased the level of ejection fraction as much as our previous study. In the present study, we analyzed the values of $dP/dt$ as the index of contractility by measuring LV pressure using a pig tail catheter.

In summary, despite these limitations, we demonstrated that the histamine $H_2$ receptor blockade preserves cardiac systolic function in dogs with pacing-induced heart failure, even in the presence of $\beta$-adrenergic receptor blockade. This finding strengthens the rationale for the beneficial effects of histamine $H_2$ blockers in the treatment of heart failure.

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**Conflict of interest** None.

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