Medetomidine, an $\alpha_2$-Adrenergic Agonist, Activates Cardiac Vagal Nerve Through Modulation of Baroreflex Control

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**Background:** Although $\alpha_2$-adrenergic agonists have been reported to induce a vagal-dominant condition through suppression of sympathetic nerve activity, there is little direct evidence that they directly increase cardiac vagal nerve activity. Using a cardiac microdialysis technique, we investigated the effects of medetomidine, an $\alpha_2$-adrenergic agonist, on norepinephrine (NE) and acetylcholine (ACh) release from cardiac nerve endings.

**Methods and Results:** A microdialysis probe was implanted into the right atrial wall near the sinoatrial node in anesthetized rabbits and perfused with Ringer's solution containing eserine. Dialysate NE and ACh concentrations were measured using high-performance liquid chromatography. Both 10 and 100 $\mu$g/kg of intravenous medetomidine significantly decreased mean blood pressure (BP) and the dialysate NE concentration, but only 100 $\mu$g/kg of medetomidine enhanced ACh release. Combined administration of medetomidine and phenylephrine maintained mean BP at baseline level, and augmented the medetomidine-induced ACh release. When we varied the mean BP using intravenous administration of phenylephrine, treatment with medetomidine significantly steepened the slope of the regression line between mean BP and log ACh concentration.

**Conclusions:** Medetomidine increased ACh release from cardiac vagal nerve endings and augmented baroreflex control of vagal nerve activity. (Circ J 2012; 76: 152–159)

**Key Words:** Acetylcholine; Norepinephrine; Sinoatrial node; Sympathetic nervous system; Vagus nerve

The selective $\alpha_2$-adrenergic agonist, dexmedetomidine, is widely used for sedation in intensive care units because it has a less respiratory depressive effect. In addition, several benefits of dexmedetomidine that favor its use in intensive care have been reported, such as reduced opioid dosage requirement. In animal studies, Hayashi et al reported that dexmedetomidine prevented epinephrine-induced arrhythmias in halothane-anesthetized dogs. This antiarrhythmic effect of $\alpha_2$-adrenergic agonists may be partly ascribed to vagal activation. It has already been reported that central sympathetic inhibition by an $\alpha_2$-adrenergic agonist, guanfacine, augmented the sleep-related ultradian rhythm of parasympathetic tone in patients with chronic heart failure. Although $\alpha_2$-adrenergic agonists are widely recognized as inducing a vagal-dominant condition through the suppression of sympathetic nerve, there is little direct evidence that they directly increase cardiac vagal nerve activity, because such activity has been assessed only by indirect methods, such as heart rate variability, in most studies.

Vanoli et al reported that vagal stimulation after an acute ischemic episode effectively prevented ventricular fibrillation in dogs. Their group also indicated that the dogs that developed ventricular fibrillation during the acute ischemic episode had a significantly lower baroreflex-mediated heart rate response, suggesting the importance of the baroreflex in controlling vagal function. If an $\alpha_2$-adrenergic agonist is able to activate the cardiac vagal nerve directly or via modulation of the baroreflex function, it will provide a new therapeutic option for life-threatening arrhythmias after myocardial ischemia.

Medetomidine is a racemic mixture of 2 stereoisomers, dexmedetomidine and levomedetomidine. However, because it has already been reported that levomedetomidine has no effect on cardiovascular parameters and causes no apparent sedation or analgesia, the pharmacokinetics of dexmedetomidine and racemic medetomidine are almost similar. We hypothesized that medetomidine can activate the cardiac vagal nerve...
through a central action and improve the baroreflex control of vagal nerve activity. We have established a cardiac microdialysis technique for separate monitoring of neuronal norepinephrine (NE) and acetylcholine (ACh) release to the rabbit sinoatrial (SA) node in vivo. Using this microdialysis technique, we investigated the effects of medetomidine on cardiac autonomic nerve activities innervating the SA node.

Methods

Surgical Preparation

Animal care was provided in accordance with the “Guiding principles for the care and use of animals in the field of physiological sciences” published by the Physiological Society of Japan. All protocols were approved by the Animal Subject Committee of the National Cerebral and Cardiovascular Center.

In this study, 31 Japanese white rabbits weighing 2.3–3.0 kg were used. Anesthesia was initiated by an intravenous injection of pentobarbital sodium (50 mg/kg) via the marginal ear vein, and then maintained at an appropriate level by continuous intravenous infusion of a-chloralose and urethane (16 mg · kg⁻¹ · h⁻¹ and 100 mg · kg⁻¹ · h⁻¹) through a catheter inserted into the femoral vein. The animals were intubated and ventilated mechanically with room air mixed with oxygen. Respiratory rate and tidal volume were set at 30 cycles/min and 15 ml/kg, respectively. Systemic arterial pressure was monitored by a catheter inserted into the femoral artery. Esophageal temperature, which was measured by a thermometer (CTM-303, Terumo, Japan), was maintained between 38°C and 39°C using a heating pad.

With the animal in lateral position, a right lateral thoracotomy was performed and the right 3rd to 5th ribs were partially resected to expose the heart. After incising the pericardium, a dialysis probe was implanted as described below. Three stainless steel electrodes were attached around the thoracotomy incision for recording body surface electrocardiogram (ECG). The heart rate was determined from the ECG using a heart-rate monitor. Heparin sodium (100 IU/kg) was administered intravenously to prevent blood coagulation. At the end of the experiment, the animal was killed humanely by injecting an overdose of pentobarbital sodium. In the postmortem examination, the right atrial wall was resected en bloc with the dialysis probe. The inside of the atrial wall was observed macroscopically to confirm that the dialysis membrane was not exposed to the right atrial lumen.

Dialysis Technique

The materials and properties of the dialysis probe have been described previously. A dialysis fiber of semipermeable membrane (length 4 mm, outer diameter 310 µm, inner diameter 200 µm, PAN-1200, molecular weight cutoff 50,000, Asahi Chemical, Tokyo, Japan) was attached to both ends to polyethylene tubes (length 25 cm, outer diameter 500 µm, inner diameter 200 µm). A fine guiding needle (length 30 mm, outer diameter 510 µm, inner diameter 250 µm) with a stainless steel rod (length 5 mm, outer diameter 250 µm) was used for the implantation of the dialysis probe. A dialysis probe was implanted into the right atrial myocardium near the junction of the superior vena cava and the right atrium. After implantation, the dialysis probe was perfused with Ringer’s solution (in mmol/L: NaCl 147, KCl 4, CaCl₂ 3) containing a cholinesterase inhibitor eserine (100 μmol/L) at a speed of 2 μl/min using a microinjection pump (CMA/102, Carnegie Medicin, Sweden). Experimental protocols were started 120 min after implantation of the dialysis probe. The dead space between the dialysis membrane and the sample tube was taken into account at the beginning of each dialysate sampling. In protocols 1 and 2 as described below, 8 μl of phosphate buffer (pH 3.5) was added to each sample tube before dialysate sampling, and each dialysate sampling period was set at 20 min (1 sample volume=40 μl). Half of the dialysate sample was used for ACh and the other half for NE measurements. In protocol 3, 2 μl of phosphate buffer was added to each sample tube before dialysate sampling, and each dialysate sampling period was set at 5 min (1 sample volume=10 μl). In protocol 4, 4 μl of phosphate buffer was added to each sample tube before dialysate sampling, and each dialysate sampling period was set at 10 min (1 sample volume=20 μl). Dialysate NE and ACh concentrations were analyzed separately by high-performance liquid chromatography as described previously.

Experimental Protocols

Protocol 1 (n=7) Baseline dialysate was sampled before the injection of medetomidine. Thereafter, a low dose (10 μg/kg) of medetomidine was injected intravenously via the femoral vein. After allowing 20 min for hemodynamic stabilization, dialysate was sampled for 20 min (40 μl). When the hemodynamics had recovered to the baseline level, a high dose (100 μg/kg) of medetomidine was injected intravenously and another 20-min dialysate sample was collected after hemodynamic stabilization. Finally, the vagal nerves were sectioned bilaterally at the neck and a dialysate sample was collected immediately after vagotomy. In 4 rabbits, an α₂-adrenergic antagonist, atipamezole (2.5 mg/kg), was intravenously administered before euthanasia and hemodynamic responses were recorded.

Protocol 2 (n=7) To prevent possible interference of medetomidine-induced hypotension with vagal nerve activity, intravenous infusion of an α₁-adrenergic agonist, phenylephrine, was started simultaneous to intravenous injection of medetomidine. Baseline dialysate sample was collected for 20 min before medetomidine injection. Simultaneous to intravenous injection of high-dose (100 μg/kg) medetomidine, intravenous infusion of phenylephrine was started (6.6±1.2 μg · kg⁻¹ · min⁻¹) to maintain the mean blood pressure (BP) at baseline level. After hemodynamic stabilization, dialysate was sampled for 20 min. Finally, dialysate was again sampled immediately after bilateral cervical vagotomy.

Protocol 3 To investigate the effect of medetomidine on baroreflex-induced vagal ACh release, we varied the mean BP by changing the dose of intravenous phenylephrine in both the control (n=5) and medetomidine-treated (n=7) groups. In the control group, Ringer’s solution was infused intravenously at 1.0 ml · kg⁻¹ · h⁻¹ throughout the experiment. In the medetomidine-treated group, medetomidine was initially injected intravenously at a dose of 60 μg/kg, and thereafter continuously infused at a dose of 60 μg · kg⁻¹ · h⁻¹ or a rate of 1.0 ml · kg⁻¹ · h⁻¹. After baseline dialysate sampling, mean BP was increased in a stepwise manner by altering the dose of intravenous phenylephrine (maximal dose: 32.2±5.5 μg · kg⁻¹ · min⁻¹ in the control group and 18.6±2.1 μg · kg⁻¹ · min⁻¹ in the medetomidine-treated group). Dialysate samples were collected for 5 min at 4–7 different mean BP levels. Relations of log ACh concentrations vs. mean BP were plotted and regression lines for each animal were calculated.

Protocol 4 (n=5) We investigated the peripheral effects of medetomidine on heart rate and dialysate ACh concentration under electrical stimulation of the right cervical vagal nerve. Bilateral vagal nerves were exposed through a midline cervical incision and sectioned at the neck. A pair of bipolar stainless steel electrodes was attached to the efferent side of the
right vagal nerve. The nerve and electrode were covered with warmed mineral oil for insulation. After the baseline dialysate sampling, the right efferent vagal nerve was stimulated at the frequency of 20 Hz by a digital stimulator (SEN-7203, Nihon Kohden, Japan). The pulse duration and amplitude of nerve stimulation were set at 1 ms and 10 V. Thereafter, a low dose (10 μg/kg) of medetomidine was injected intravenously via the femoral vein. After hemodynamic stabilization, dialysate was sampled for 10 min under the 20-Hz electrical stimulation of vagal nerve. Finally, a high dose (100 μg/kg) of medetomidine was injected intravenously and another 10-min dialysate sample was collected under the 20-Hz electrical stimulation.

Statistical Analysis
All data are presented as mean ± standard error. Heart rate and mean BP were compared by 1-way repeated measures analysis of variance (ANOVA) followed by a Tukey’s test.¹⁴ Dialysate NE and ACh concentrations were also compared by 1-way repeated measures ANOVA followed by a Tukey’s test. Comparisons of data between protocols 1 and 2 were conducted using unpaired t-test (Student’s or Welch’s t-test). In protocol 3, the average slopes and intercepts of the regression lines were compared using unpaired t-test. Differences were considered significant at P<0.05.

Results

Protocol 1
Intravenous injection of medetomidine significantly decreased heart rate (Figure 1A) and mean BP (Figure 1B) in a dose-dependent manner (280±10 beats/min and 92±4 mmHg, respectively, at baseline; 251±10 beats/min and 69±3 mmHg at 10 μg/kg; and 193±11 beats/min and 47±4 mmHg at 100 μg/kg, P<0.01 for all comparisons). Vagotomy increased heart rate to 222±7 beats/min but did not affect mean BP (Figures 1A, B).

Low-dose medetomidine significantly decreased dialysate NE concentration (Figure 2A) from 0.72±0.06 to 0.59±0.04 nmol/L (P<0.01) but did not affect dialysate ACh concentration (Figure 2B) compared with baseline. High-dose medetomidine also decreased dialysate NE concentration (to 0.52±0.05 nmol/L) similar to low-dose medetomidine (Figure 2A) and significantly increased dialysate ACh concentration from 7.2±1.3 nmol/L at baseline to 12.1±1.6 nmol/L (P<0.01, Figure 2B). Dialysate NE concentration was not changed by vagotomy, whereas dialysate ACh concentration recovered to the baseline level immediately after vagotomy (Figures 2A, B).

In 4 rabbits treated with atipamezole, heart rate and mean BP recovered to the baseline levels immediately after the injection (276±18 beats/min and 88±6 mmHg, respectively, at baseline; and 280±11 beats/min and 83±6 mmHg after the injection).

Protocol 2
Intravenous injection of high-dose medetomidine combined with phenylephrine decreased heart rate (Figure 3A) and the decrease was significantly greater than that observed in protocol 1 (140±9 vs. 193±11 beats/min, P<0.01), while mean BP was maintained at the same level as baseline (Figure 3B). Medetomidine combined with phenylephrine decreased dialysate NE concentration from 0.85±0.09 at baseline to 0.68±0.10 nmol/L (Figure 4A), and the decrease was not significantly different from that of medetomidine alone (protocol 1). However, medetomidine combined with phenylephrine increased dialysate ACh concentration (Figure 4B) to a significantly and markedly higher level than that observed in protocol 1 (26.8±5.4 vs. 12.1±1.6 nmol/L, P<0.05). Dialysate ACh concentration recovered to the baseline level immediately after vagotomy.

Protocol 3
The change in mean BP by phenylephrine administration affected dialysate ACh concentration only slightly in the control group (Figure 5A), whereas the elevation of mean BP

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**Figure 1.** Intravenous administration of low and high doses of medetomidine decreased heart rate (A) and mean blood pressure (B). Medetomidine decreased heart rate in a dose-dependent manner. Vagotomy partially restored the decrease in heart rate but had no effect on mean blood pressure under administration of 100 μg/kg of medetomidine. **P<0.01.

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**Figure 2.** Intravenous administration of medetomidine significantly decreased dialysate NE concentration (Figure 2A) from 0.72±0.06 to 0.59±0.04 nmol/L (P<0.01) but did not affect dialysate ACh concentration (Figure 2B) compared with baseline. High-dose medetomidine also decreased dialysate NE concentration (to 0.52±0.05 nmol/L) similar to low-dose medetomidine (Figure 2A) and significantly increased dialysate ACh concentration from 7.2±1.3 nmol/L at baseline to 12.1±1.6 nmol/L (P<0.01, Figure 2B). Dialysate NE concentration was not changed by vagotomy, whereas dialysate ACh concentration recovered to the baseline level immediately after vagotomy (Figures 2A, B).

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**Figure 3.** Intravenous injection of high-dose medetomidine combined with phenylephrine decreased heart rate (Figure 3A) and the decrease was significantly greater than that observed in protocol 1 (140±9 vs. 193±11 beats/min, P<0.01), while mean BP was maintained at the same level as baseline (Figure 3B). Medetomidine combined with phenylephrine decreased dialysate NE concentration from 0.85±0.09 at baseline to 0.68±0.10 nmol/L (Figure 4A), and the decrease was not significantly different from that of medetomidine alone (protocol 1). However, medetomidine combined with phenylephrine increased dialysate ACh concentration (Figure 4B) to a significantly and markedly higher level than that observed in protocol 1 (26.8±5.4 vs. 12.1±1.6 nmol/L, P<0.05). Dialysate ACh concentration recovered to the baseline level immediately after vagotomy.

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**Figure 4.** Intravenous injection of high-dose medetomidine combined with phenylephrine decreased heart rate (Figure 3A) and the decrease was significantly greater than that observed in protocol 1 (140±9 vs. 193±11 beats/min, P<0.01), while mean BP was maintained at the same level as baseline (Figure 3B). Medetomidine combined with phenylephrine decreased dialysate NE concentration from 0.85±0.09 at baseline to 0.68±0.10 nmol/L (Figure 4A), and the decrease was not significantly different from that of medetomidine alone (protocol 1). However, medetomidine combined with phenylephrine increased dialysate ACh concentration (Figure 4B) to a significantly and markedly higher level than that observed in protocol 1 (26.8±5.4 vs. 12.1±1.6 nmol/L, P<0.05). Dialysate ACh concentration recovered to the baseline level immediately after vagotomy.

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**Figure 5.** Intravenous administration of medetomidine significantly decreased heart rate (Figure 5A) and mean blood pressure (Figure 5B). Medetomidine decreased heart rate in a dose-dependent manner. Vagotomy partially restored the decrease in heart rate but had no effect on mean blood pressure under administration of 100 μg/kg of medetomidine. **P<0.01.
markedly increased dialysate ACh concentration in the medetomidine-treated group (Figure 5B). The average slopes of the regression lines between mean BP and log dialysate ACh concentration were 0.0018±0.0004 in the control and 0.0062±0.0006 in the medetomidine-treated group. The slope was significantly steeper in the medetomidine-treated group than that in the control (P<0.01). However, the intercept did not differ significantly between the control (0.59±0.05) and medetomidine-treated (0.68±0.07) groups.

Protocol 4

The 20-Hz electrical stimulation of the right vagal nerve significantly decreased heart rate from 271±11 beats/min at the baseline to 112±6 beats/min and increased dialysate ACh concentration from 7.1±0.9 nmol/L at the baseline to 38.5±7.2 nmol/L (P<0.01). However, both 10 and 100 μg/kg of me-
**Figure 4.** Effects of simultaneous intravenous administration of medetomidine and phenylephrine on dialysate NE (A) and ACh concentrations (B). Medetomidine combined with phenylephrine decreased dialysate NE concentration (A), and the decrease was similar to medetomidine alone (protocol 1: Figure 2A). Medetomidine combined with phenylephrine caused a marked increase in dialysate ACh concentration (B) and the increase was significantly greater than medetomidine alone (protocol 1: Figure 2B). Vagotomy suppressed the increase in dialysate ACh concentration. **P<0.01; †P<0.05 vs. medetomidine alone (protocol 1: Figure 2B).** ACh, acetylcholine; NE, norepinephrine.

**Figure 5.** Regression lines of dialysate ACh concentration vs. MBP in control group (A) and medetomidine-treated group (B). In the control group (n=5), the increase in MBP had little effect on dialysate ACh concentration (A). In the medetomidine-treated group (n=7), dialysate ACh concentration was elevated with increase in MBP (B). Average regression line for control group: log[ACh]=0.0018xMBP+0.59. Average regression line for medetomidine-treated group: log[ACh]=0.0062xMBP+0.68. Each symbol represents the data of 1 animal. ACh, acetylcholine; MBP, mean blood pressure.
detomidine did not affect heart rate and dialysate ACh concentration under the electrical stimulation (106±5.9 beats/min and 37.1±6.1 nmol/L at 10 μg/kg, 108±15 beats/min and 41.6±7.7 nmol/L at 100 μg/kg).

**Discussion**

We have elucidated the effects of medetomidine on cardiac sympathetic and vagal nerve activities simultaneously using cardiac microdialysis technique. Intravenous administration of 10 μg/kg of medetomidine significantly decreased sympathetic NE release to the SA node, while intravenous administration of 100 μg/kg of medetomidine significantly increased vagal ACh release to the SA node in addition to sympathetic suppression.

**α2-Adrenergic Agonist and Cardiac Sympathetic Nerve Activity**

It is well-documented that α2-adrenergic agonist suppresses sympathetic nerve activity.15 Oku et al reported that dexmedetomidine suppressed renal sympathetic nerve discharge in baroreceptor-denervated rabbits.16 In the present study, low-dose medetomidine decreased heart rate and mean BP through inhibiting sympathetic nerve activity, without affecting cardiac vagal nerve activity. High-dose medetomidine also suppressed NE release to the same level as low-dose medetomidine.

Several mechanisms may be involved in the sympathoinhibitory effect of α2-adrenergic agonist. The rostral ventrolateral medulla has been reported to serve as an important site in mediating the hypotensive and sedative effects of α2-adrenergic agonist.17 McCallum et al reported that the central sympathoinhibitory effects of α2-adrenoceptor stimulation are augmented by peripheral inhibition of ganglionic transmission.18 The results obtained from protocol 1 indicate that low-dose medetomidine may induce a vagal-dominant condition through suppression of the cardiac sympathetic nerve without direct activation of the cardiac vagal nerve.

**α2-Adrenergic Agonist and Cardiac Vagal Nerve Activity**

Kamibayashi et al reported that the vagus nerve played an important role in the antidysrhythmic effect of dexmedetomidine.3 However, because it is difficult to selectively monitor cardiac vagal nerve activity, there is little direct evidence that α2-adrenergic agonists can directly increase cardiac vagal nerve activity. In the present study, high-dose medetomidine significantly decreased heart rate and mean BP compared with low-dose medetomidine in protocol 1, and analyses of NE and ACh release by microdialysis technique proved that these decreases in heart rate and mean BP were associated with an increase in vagal ACh release to the heart. Histocytological studies demonstrated the presence of α2-adrenergic receptors in the vagal dorsal motor nucleus and nucleus tractus solitarius.19 Therefore, it is possible that α2-adrenergic agonists directly activate the cardiac vagal nerve. It is also possible that intravenous medetomidine also modulates vagal ACh release through ganglionic transmission and the direct action to nerve endings. In protocol 4, however, medetomidine did not affect heart rate or the dialysate ACh concentration under electrical stimulation of the right efferent vagal nerve. Thus, in our experimental setting the peripheral effects of medetomidine on cardiac vagal nerve activity may be small compared with its central effects.

To exclude the possibility that medetomidine-induced hypotension affects local ACh concentrations, the mean BP was maintained constant by co-administration of phenylephrine in protocol 2. High-dose medetomidine combined with phenylephrine enhanced the decrease in heart rate and the increase in dialysate ACh concentration without medetomi-
dine-induced hypotension, indicating that hypotension occurring in protocol 1 had actually reduced ACh release in response to high-dose medetomidine. The results also suggest an interaction between baroreflex-induced and medetomidine-induced vagal nerve activation, which was extensively examined in protocol 3. In protocol 3, medetomidine steepened the slope of the regression line between mean BP and log dialysate ACh concentration, without affecting the intercept. In other words, medetomidine enhanced the baroreflex-induced ACh release from cardiac vagal nerve endings. Because the central pathway of baroreflex includes the vagal dorsal motor nucleus and nucleus tractus solitarius, in which α2-adrenergic receptors have been demonstrated, medetomidine may act on this pathway and modulate baroreflex-induced ACh release.

Clinical Implication
The selective α2-adrenergic agonist, dexmedetomidine, is widely used for sedation in intensive care units. Bradycardia and hypotension are known to be unfavorable events during dexmedetomidine sedation. Some cases of dexmedetomidine-induced atrioventricular block followed by cardiac arrest have been reported. This critical complication may be associated with direct vagal activation by the α2-adrenergic agonist. Compared with our previous results of electrical cervical vagal nerve stimulation in rabbits, intravenous administration of 100 μg/kg of medetomidine had an effect equivalent to electrical vagal stimulation at 10 Hz. Furthermore, when the mean BP was maintained constant using phylephrine, medetomidine had a stronger effect on cardiac vagal nerve activity, which is similar to 20-Hz electrical vagal stimulation, and this magnitude may sometimes cause atrioventricular block or sinus arrest.

Notwithstanding these adverse effects, vagal activation has several favorable cardioprotective effects. Our study proved that medetomidine, a selective α2-adrenergic agonist, is a strong activator of cardiac vagal nerve. Vanoli et al reported that vagal stimulation after acute ischemia can prevent ventricular fibrillation. Ando et al reported that efferent vagal nerve stimulation prevented ischemia-induced arrhythmias by preserving connexin 43 protein. Our results suggest that vagal activation in addition to sympathetic suppression probably contributes to the antiarrhythmic effect of medetomidine.

Because inhibition of the sympathetic nerve system has been the cornerstone of drug therapy for heart failure, a selective α2-adrenergic agonist may be a potential therapeutic option for heart failure. Recent studies have shown that electrical vagal nerve stimulation also improves the outcomes in patients with heart failure. Electrical stimulation of carotid baroreceptor has recently been reported to be a therapeutic option for heart failure. Sabbah et al reported that chronic electrical stimulation of the carotid sinus baroreflex improved left ventricular function and promoted reversal of ventricular remodeling in dogs with advanced heart failure. Our study demonstrated that medetomidine modulates baroreflex control to enhance vagal nerve activity, which may also induce further cardioprotective effects.

Study Limitations
First, ACh is degraded by ACh esterase immediately after release. Therefore, detection of in vivo ACh release requires the addition of eserine, a specific ACh esterase inhibitor, into the perfusate. The presence of eserine around the semipermeable membrane might have affected ACh release in the vicinity of the semipermeable membrane. Eserine could have activated regulatory pathways such as autoinhibition of ACh release via muscarinic receptors.

Second, medetomidine is a chiral imidazole derivative. Thus, imidazoline receptors may also be involved in the cardiac vagal activation by medetomidine. Further investigation is necessary to clarify the influence of imidazoline receptors on cardiac vagal nerve activity. However, because an α2-adrenergic antagonist, atipamezole, abolished the hemodynamic responses to medetomidine, we think that the cardiovascular effects of medetomidine are mainly related to the direct action of α2-adrenergic receptors.

Third, the interactive effects between sympathetic and vagal nerve endings remain uncertain in the present study. Thus, we need further investigations including the open-loop approach where baroreceptor input pressure is strictly controlled.

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References
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