Circulation Journal
Official Journal of the Japanese Circulation Society
http://www.j-circ.or.jp

ORIGINAL ARTICLE
Heart Failure

Medetomidine Suppresses Cardiac and Gastric Sympathetic Nerve Activities but Selectively Activates Cardiac Vagus Nerve

Shuji Shimizu, MD, PhD; Tsuyoshi Akiyama, MD, PhD; Toru Kawada, MD, PhD; Atsunori Kamiya, MD, PhD; Michael James Turner, PhD; Hiromi Yamamoto, MD, PhD; Toshiaki Shishido, MD, PhD; Mikiyasu Shirai, MD, PhD; Masaru Sugimachi, MD, PhD

Background: To identify a pharmacological agent that can selectively activate cardiac vagus nerve for potential use in vagal activation therapy against heart failure, the effects of medetomidine on autonomic nerve activities in both the heart and stomach were examined.

Methods and Results: In anesthetized rabbits, microdialysis probes were implanted into both the right atrial and gastric walls. Dialysate acetylcholine (ACh) and norepinephrine (NE) concentrations were measured by high-performance liquid chromatography. First, the effects of 100 μg/kg of intravenous medetomidine on vagal ACh and sympathetic NE releases were examined. Medetomidine significantly increased cardiac ACh release (4.7±1.1 to 7.8±0.9 nmol/L, P<0.05), but suppressed gastric ACh release (8.0±2.6 to 3.5±1.5 nmol/L, P<0.01). In contrast, medetomidine suppressed both cardiac and gastric NE releases. Second, the effects of medetomidine on ACh releases induced by electrical vagus nerve stimulation (VNS; 10 Hz) were examined. Electrical VNS significantly increased both cardiac (6.7±1.2 to 14.8±1.8 nmol/L, P<0.01) and gastric (3.8±0.8 to 181.3±65.6 nmol/L, P<0.01) ACh releases. Medetomidine did not alter the VNS-induced increases in ACh release.

Conclusions: Medetomidine suppresses both cardiac and gastric sympathetic nerve activities. In contrast, medetomidine activates cardiac vagus nerve but inhibits gastric vagal activity. Medetomidine might be one of the potential pharmacological agents for vagal activation therapy against heart failure without the risk of gastric adverse effects. (*Circ J* 2014; **78:** 1405–1413)

Key Words: Acetylcholine; α_2-adrenergic agonist; Norepinephrine; Sympathetic nerve activity; Vagus nerve activity

Electrical vagus nerve stimulation (VNS) has remained a therapeutic option for epileptic seizures for several decades. Recently, electrical VNS has also been evaluated as a direct method to correct autonomic imbalance (activated sympathetic nerve system and suppressed vagus nerve activity) in patients with chronic heart failure (CHF).[1] The effect of VNS in CHF patients is being studied in an on-going multicenter international clinical trial, the INcrease Of VAgal TonE in Heart Failure study (INOVATE-HF).[2] However, from the clinical experience of using electrical VNS in patients with epileptic seizures, electrical stimulation of the cervical vagus nerve might cause several gastrointestinal adverse effects such as nausea and diarrhea.[3,4]

To avoid these adverse effects, selective activation of cardiac vagus nerve might be favorable. We have reported that a selective α_2-adrenergic agonist, medetomidine (a racemic mixture of dexmedetomidine and levomedetomidine), suppresses sympathetic norepinephrine (NE) release and enhances vagal acetylcholine (ACh) release to the sinoatrial node.[5] In contrast, dexmedetomidine has been reported to have an antiulcerative effect on indomethacin-induced gastric ulcers,[6] suggesting that medetomidine or dexmedetomidine might be able to activate cardiac vagus nerve without undesirable gastrointestinal effects. Thus, medetomidine might be one of the potential pharmacological agents for vagal activation therapy against CHF.

We have established a microdialysis technique for in vivo monitoring of neuronal NE and ACh releases to the rabbit sinoatrial node.[7,8] Local sampling of the microdialysis technique

Received November 28, 2013; revised manuscript received March 4, 2014; accepted March 6, 2014; released online April 11, 2014 Time for primary review: 13 days

Department of Cardiovascular Dynamics (S.S., T.K., A.K., M.J.T., T.S., M. Sugimachi), Department of Cardiac Physiology (T.A., M. Shirai), National Cerebral and Cardiovascular Center, Suita; and Division of Cardiology, Department of Medicine, Faculty of Medicine, Kinki University, Osaka-sayama (H.Y.), Japan

Mailing address: Shuji Shimizu, MD, PhD, Department of Cardiovascular Dynamics, National Cerebral and Cardiovascular Center, 5-7-1 Fujishiro-dai, Suita 565-8565, Japan. E-mail: shujismz@ri.ncvc.go.jp

ISSN-1346-9843 doi:10.1253/circj.CJ-13-1456

Table. Doses of Intravenous Phenylephrine Infusion Used to Maintain Mean Arterial Pressure at Baseline Level

(n=6)	Baseline after vagotomy	Bil. VNS	Medetomidine + Bil. VNS	Medetomidine + Bil. VNS + C6
Phenylephrine ($\mu g \cdot kg^{-1} \cdot min^{-1}$)	0	13.1±1.6	9.1±1.8	9.0±2.5

Bil. VNS, bilateral vagus nerve stimulation; C6, hexamethonium.

allows monitoring of ACh and NE releases as indices of organ-specific autonomic nerve activities. In the present study, we applied the microdialysis technique to both the heart and stomach of rabbits and examined the effects of medetomidine on organ-specific autonomic nerve activities.

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. Twenty-four Japanese white rabbits weighing 2.5–3.2 kg were used in this study. 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 α-chloralose and urethane ($16 mg \cdot kg^{-1} \cdot h^{-1}$ and $100 mg \cdot kg^{-1} \cdot h^{-1}$, respectively). An adequate anesthesia level was confirmed by loss of the ear pinch response. The animals were intubated and ventilated mechanically with room air mixed with oxygen. The 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. Heparin sodium ($10 IU \cdot kg^{-1} \cdot h^{-1}$) was infused to prevent blood coagulation in the femoral artery catheter. Esophageal temperature was maintained between 38 and 39°C using a heating pad.

With the animal in the supine position, a right lateral thoracotomy was performed and the right 3rd to 5th ribs were partially resected to expose the heart. After incision of the pericardium, a dialysis probe was implanted into the atrial wall near the sinoatrial node, as described in the *Dialysis Technique* section below. A midline laparotomy was also performed to expose the stomach. Another dialysis probe was implanted into the anterior wall of the stomach as described in the *Dialysis Technique* section.

Three stainless steel electrodes were attached around the thoracotomy incision for recording body surface electrocardiogram. The heart rate was determined from the electrocardiogram or arterial pressure waveform using a cardiotachometer.

At the end of the experiment, the animal was euthanized by injecting an overdose of pentobarbital sodium. In a post-mortem examination, the right atrial wall and the anterior wall of the stomach were resected en bloc with the dialysis probes. The internal surfaces of the atrial and gastric walls were examined macroscopically to confirm that the dialysis membranes were not exposed to the right atrial lumen and the gastric cavity.

Dialysis Technique

The materials and properties of the dialysis probe have been described previously.[7–10] A dialysis fiber of a semipermeable membrane (length 4 mm, outer diameter 310 μm, inner diameter 200 μm, PAN-1200, molecular weight cut-off 50,000; Asahi Chemical, Tokyo, Japan) was attached at both ends to polyethylene tubes. One dialysis probe was implanted into the right atrial myocardium near the sinoatrial node, which has the richest vagal innervation in the heart.[11] Another dialysis probe was implanted into the anterior wall of the stomach because peristaltic movement was relatively smaller than the other part of the gastrointestinal tract. After implantation, these dialysis probes were perfused with Ringer's solution (NaCl 147 mmol/L, KCl 4 mmol/L, $CaCl_2$ 3 mmol/L) alone for NE measurement, or with Ringer's solution containing a cholinesterase inhibitor, eserine (100 μmol/L), for ACh measurement, at a speed of 2 μl/min using a microinjection pump (CMA/102; Carnegie Medicin, Stockholm, Sweden). Experimental protocols were started 2 h after implantation of the dialysis probes. The dead space between the dialysis membrane and the sample tube was taken into account at the beginning of each dialysate sampling. Four microliters of phosphate buffer (pH 3.5) was added to each sample tube before dialysate sampling, and each dialysate sampling period was set at 10 min (1 sample volume=20 μl). In the supplementary protocol, 8-μl of phosphate buffer was added to each sample tube, and each dialysate sampling period was set at 20 min (1 sample volume=40 μl). The dialysate ACh or NE concentration was analyzed by high-performance liquid chromatography, as described previously.[9,10]

Experimental Protocols

Protocol 1 (n=13) We investigated the effects of intravenous medetomidine on both cardiac and gastric vagal ACh (n=7) and sympathetic NE (n=6) releases. First, 10-min baseline dialysate samples were collected under baseline conditions. Thereafter, 100 μg/kg of medetomidine, which has been shown to increase the cardiac dialysate ACh concentration,[5] was injected intravenously via the femoral vein. After hemodynamic stabilization, dialysate samples were collected for 10 min (20 μl). Immediately after the second sampling, intravenous infusion of phenylephrine was started ($4.3 \pm 0.7 \mu g \cdot kg^{-1} \cdot min^{-1}$) to restore mean arterial pressure to the baseline level because a decrease in mean arterial pressure during the second sampling might have affected sympathetic and vagal outflows through the arterial baroreflex. After hemodynamic stabilization, dialysate samples were again collected for 10 min.

Protocol 2 (n=6) We investigated the effect of medetomidine on both cardiac and gastric ACh releases induced by electrical stimulation of bilateral cervical vagus nerves. Because there was a difference in vagal innervations between the right atrium and anterior wall of the stomach, bilateral vagus nerves were exposed through a midline cervical incision and sectioned at the neck region to perform a simultaneous stimulation to both the heart and stomach. A pair of bipolar stainless steel electrodes was attached to the efferent side of each vagus nerve. The nerves and electrodes were immobilized using a quick-curing silicone gel (Kwik-Sil; World Precision Instruments, Inc, FL, USA). After sampling the baseline dialysates, bilateral efferent vagus nerves were simultaneously stimulated at a frequency of 10 Hz using a digital stimulator (SEN-7203; Nihon Kohden, Japan). The pulse duration and amplitude of nerve stimulation were set at 1 ms and 10 V, respectively. To main-

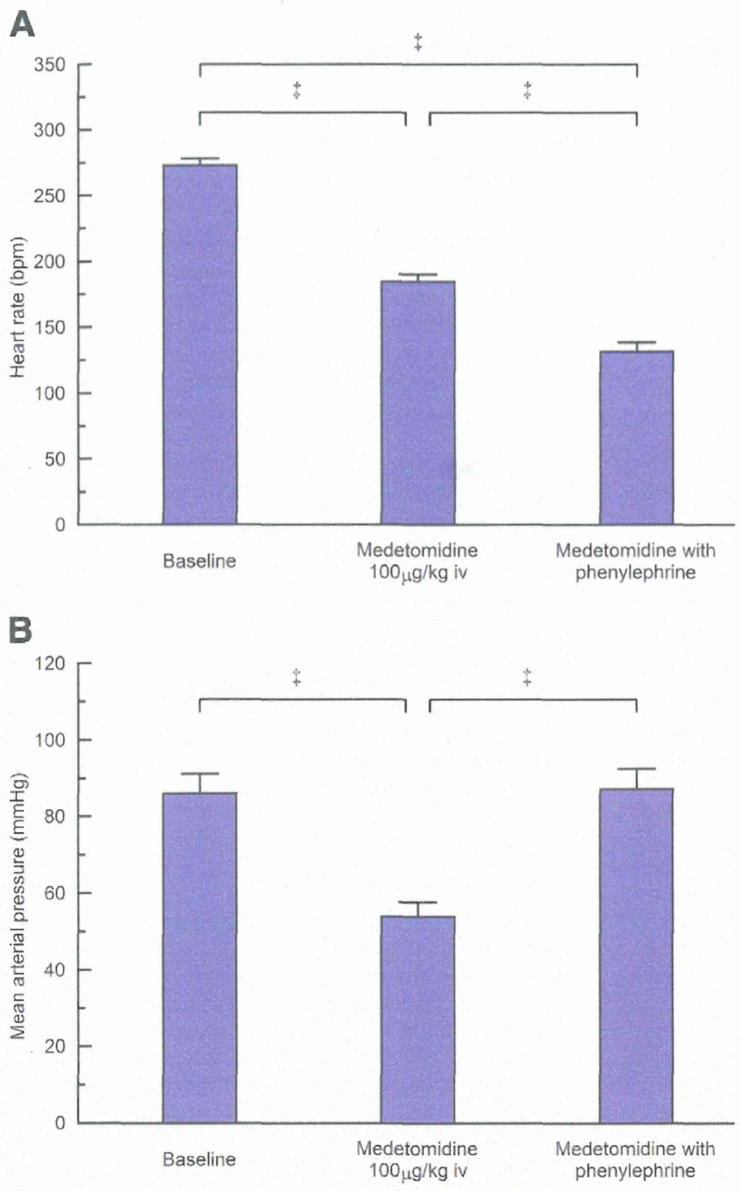

Figure 1. Effects of 100 μg/kg of medetomidine on heart rate (**A**) and mean arterial pressure (**B**) (n=13). All data are presented as mean±SE. iv, intravenous injection. ‡P<0.01.

tain mean arterial pressure at the baseline level, intravenous infusion of phenylephrine was started simultaneously to VNS (**Table**), and dialysates were sampled for 10 min (20 μl). Thereafter, to examine the effect of medetomidine on electrical VNS-induced ACh releases from nerve endings, 100 μg/kg of medetomidine was injected intravenously via the femoral vein. After hemodynamic stabilization, dialysate samples were again collected for 10 min under 10-Hz electrical VNS. Finally, a ganglionic blocker, hexamethonium bromide (30 mg/kg), was injected intravenously and 10-min dialysate samples were collected under 10-Hz VNS.

Supplementary protocol (n=5) We investigated the effects of intravenous atipamezole, an α_2-adrenergic antagonist, on both medetomidine-induced cardiac and gastric vagal ACh and sympathetic NE responses. First, 20-min baseline dialysate samples were collected under baseline conditions. Thereafter, 100 μg/kg of medetomidine was injected intravenously via the femoral vein. After hemodynamic stabilization, dialysate samples were collected for 20 min (40 μl). Immediately after the second sampling, 2.5 mg/kg of atipamezole was injected intravenously. After hemodynamic stabilization, dialysate samples were again collected for 20 min.

Statistical Analysis

All data are presented as mean±standard error. Heart rate and mean arterial pressure were compared by using one-way repeated measures analysis of variance (ANOVA) followed by a Holm's test.[12] After logarithmic transformation, dialysate ACh and NE concentrations were also compared by using one-way repeated measures ANOVA followed by a Holm's test. In the supplementary protocol, after logarithmic transformation, dialysate ACh and NE concentrations were compared by using one-way repeated measures ANOVA followed by a Dunnett's test against baseline values. Differences were con-

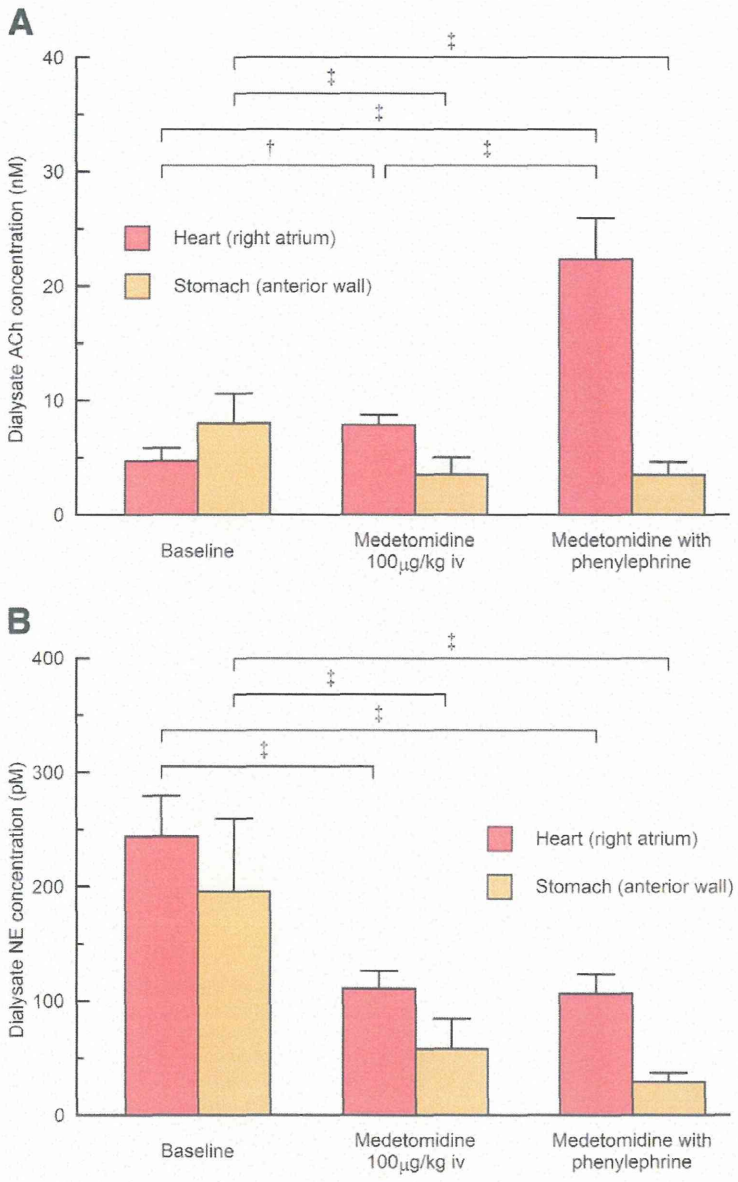

Figure 2. Effects of 100 μg/kg of medetomidine on dialysate acetylcholine (**A**, n=7) and norepinephrine (**B**, n=6) concentrations. All data are presented as mean±SE. ACh, acetylcholine; NE, norepinephrine; iv, intravenous injection. †P<0.05; ‡P<0.01.

sidered significant at P<0.05.

Results

Protocol 1

An intravenous injection of 100 μg/kg of medetomidine significantly decreased the heart rate from 273±5bpm at baseline to 185±6bpm (P<0.01) (**Figure 1A**), and the mean arterial pressure from 87±3 mmHg to 52±2 mmHg (P<0.01) (**Figure 1B**). This dose of medetomidine significantly increased the cardiac dialysate ACh concentration from 4.7±1.1 nmol/L at baseline to 7.8±0.9 nmol/L (P<0.05), but decreased the gastric dialysate ACh concentration from 8.0±2.6 nmol/L at baseline to 3.5±1.5 nmol/L (P<0.01) (**Figure 2A**). The medetomidine injection significantly decreased the cardiac dialysate NE concentration from 244±36 pmol/L at baseline to 111±16 pmol/L (P<0.01) and also the gastric dialysate NE concentration from 196±64 pmol/L at baseline to 58±27 pmol/L (P<0.01) (**Figure 2B**).

After the medetomidine injection and dialysate sampling, infusion of phenylephrine restored the mean arterial pressure to the baseline level (90±3 mmHg, not significant vs. baseline) and decreased the heart rate significantly (132±7 bpm, P<0.01 vs. medetomidine alone) (**Figures 1A,B**). After the medetomidine injection, infusion of phenylephrine increased the cardiac dialysate ACh concentration to a significantly higher level than that of medetomidine alone (22.3±3.6 nmol/L, P<0.01), but it did not change the gastric dialysate ACh concentration (3.5±1.1 nmol/L, not significant vs. medetomidine alone) (**Figure 2A**). Infusion of phenylephrine subsequent to the medetomidine injection did not change the cardiac or gastric dialysate NE concentration (not significant vs. medetomidine alone), and both cardiac (106±17 pmol/L, P<0.01 vs. baseline) and gastric dialysate NE concentrations (29±8 pmol/L, P<0.01

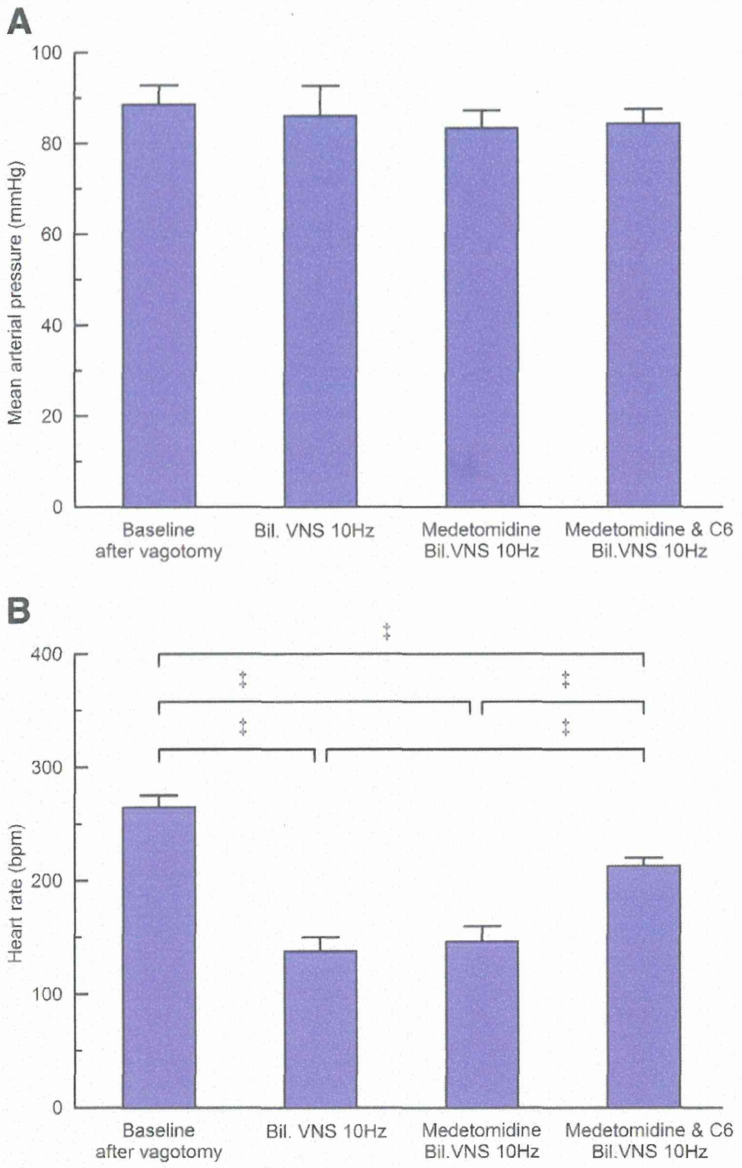

Figure 3. Effects of medetomidine injection on mean arterial pressure (**A**) and heart rate (**B**) in rabbits undergoing bilateral electrical vagus nerve stimulation (10 Hz, 1 ms, 10 V) and intravenous infusion of phenylephrine (n=6). All data are presented as mean±SE. Bil., bilateral; VNS, vagus nerve stimulation; C6, hexamethonium. ‡P<0.01.

vs. baseline) remained significantly reduced compared to baseline levels (**Figure 2B**).

Protocol 2

The mean arterial pressure was maintained at the same level as that of the baseline by intravenous infusion of phenylephrine during VNS throughout the experiment (**Figure 3A**). Bilateral electrical VNS at a frequency of 10 Hz significantly decreased the heart rate from 265±10bpm at baseline to 138±12 bpm (P<0.01) (**Figure 3B**). The 10-Hz VNS significantly increased the cardiac dialysate ACh concentration from 6.7±1.2 nmol/L at baseline to 14.8±1.8 nmol/L (P<0.01) (**Figure 4A**) and the gastric dialysate ACh concentration from 3.8±0.8 nmol/L at baseline to 181.3±65.6 nmol/L (P<0.01) (**Figure 4B**). Under a 10-Hz electrical VNS, injection of 100-μg/kg medetomidine did not alter the heart rate (146±14bpm) (**Figure 3B**), and it did not affect the cardiac (13.6±2.2 nmol/L) or gastric (196.7±70.7 nmol/L) dialysate ACh concentration (**Figures 4A,B**).

Subsequent to the medetomidine injection under a 10-Hz vagal stimulation, an intravenous injection of 30 mg/kg hexamethonium bromide significantly reduced both the cardiac and gastric dialysate ACh concentrations (7.2±1.5 nmol/L and 9.7±2.7 nmol/L, respectively) (**Figures 4A,B**). However, the gastric dialysate ACh concentration remained higher than that of the baseline level (P<0.01 vs. baseline).

Supplementary Protocol

An intravenous injection of 100 μg/kg of medetomidine significantly increased the cardiac dialysate ACh concentration from 5.2±1.1 nmol/L at baseline to 8.4±1.4 nmol/L (P<0.01), but decreased the gastric dialysate ACh concentration from 6.4±1.8 nmol/L at baseline to 3.6±1.0 nmol/L (P<0.01) (**Figure 5A**). The medetomidine injection signifi-

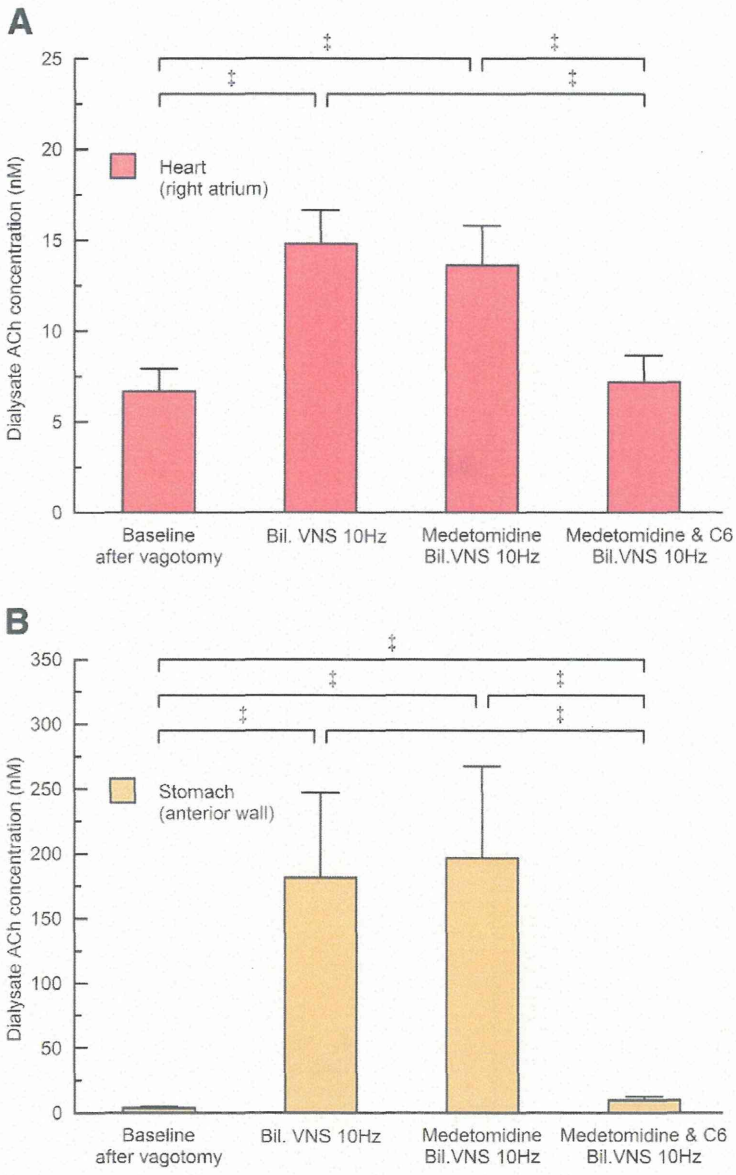

Figure 4. Effects of medetomidine injection on cardiac (**A**) and gastric (**B**) dialysate acetylcholine concentrations in rabbits undergoing bilateral electrical vagus nerve stimulation (10 Hz, 1 ms, 10 V) and intravenous infusion of phenylephrine. All data are presented as mean ±SE. ACh, acetylcholine; Bil., bilateral; VNS, vagus nerve stimulation; C6, hexamethonium. ‡P<0.01.

cantly decreased the cardiac dialysate NE concentration from 472±88 pmol/L at baseline to 266±47 pmol/L (P<0.05) and also the gastric dialysate NE concentration from 381±116 pmol/L at baseline to 130±29 pmol/L (P<0.05) (**Figure 5B**). An intravenous injection of 2.5 mg/kg of atipamezole restored both the cardiac and gastric dialysate ACh concentrations to the baseline levels (6.0±1.2 and 7.6±2.6 nmol/L, respectively) (**Figure 5A**). Atipamezole also restored both the cardiac and gastric dialysate NE concentrations to the baseline levels (436±102 and 385±130 pmol/L, respectively) (**Figure 5B**).

Discussion

Simultaneous monitoring of both the cardiac and gastric vagal ACh and sympathetic NE releases demonstrated that medetomidine enhanced cardiac ACh release but suppressed gastric ACh release to the stomach. In contrast, medetomidine suppressed both the cardiac and gastric NE releases.

Effects of Medetomidine on Vagal ACh Releases

The present study demonstrated that electrical stimulation of bilateral cervical vagus nerves at a frequency of 10 Hz significantly increased both the cardiac and gastric ACh releases. This result suggests that electrical stimulation of cervical vagus nerves might activate the whole vagal system. However, the extent of vagal activation might differ in various organs. Although baseline dialysate ACh concentrations in the heart and in the stomach did not differ (6.7±1.2 nmol/L and 3.8±0.8 nmol/L, respectively; not significant as found by an unpaired t-test), the dialysate ACh concentration during 10-Hz VNS was 12-times higher in the stomach (181.3±65.6 nmol/L) than that in the heart (14.8±1.8 nmol/L) (P<0.01 by an unpaired t-test). This difference in magnitude of a dialysate ACh response might reflect a difference in density of vagal innervation between the heart

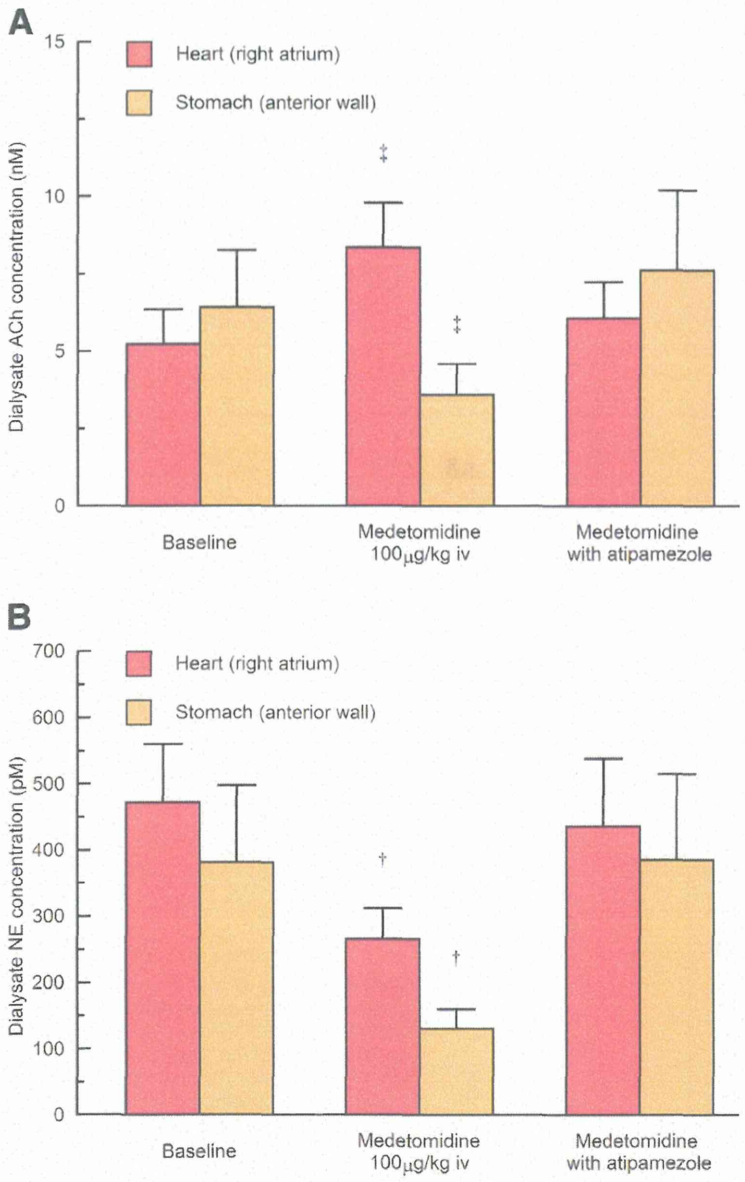

Figure 5. Effects of 2.5 mg/kg of atipamezole on medetomidine-induced acetylcholine (**A**) and norepinephrine (**B**) responses. All data are presented as mean±SE. ACh, acetylcholine; NE, norepinephrine; iv, intravenous injection. †P<0.05; ‡P<0.01.

and the stomach. In clinical settings, although weaker electrical VNS at a lower frequency and lower voltage compared to the present study is used, electrical VNS might cause unexpected vagal activation in non-target organs, resulting in adverse effects.

In contrast to electrical VNS, vagal response to medetomidine differs in the heart and in the stomach. In protocol 1, 100 μg/kg of medetomidine significantly increased the cardiac ACh release but suppressed the gastric ACh release. As shown in protocol 2, medetomidine scarcely affected VNS-induced cardiac and gastric ACh releases, suggesting that the effects of medetomidine on vagus ganglion and postganglionic vagus nerve terminals were small. Furthermore, hexamethonium, which blocks ganglionic transmission between preganglionic and postganglionic neurons, almost completely suppressed the VNS-induced ACh releases. This finding suggests that dialysate ACh concentration monitored by microdialysis mainly reflects ACh release from postganglionic vagus nerve endings, and the peripheral effects of medetomidine on nerve endings should be small. Furthermore, the supplementary protocol demonstrated that medetomidine-induced vagal ACh responses in both the heart and stomach were almost completely diminished by an α_2-adrenergic antagonist, atipamezole. Therefore, we think that medetomidine-induced vagal activation mainly depends on its central α_2-adrenergic action.

In protocol 1, restoring the mean arterial pressure to baseline level using phenylephrine enhanced medetomidine-induced cardiac ACh release. As we have already demonstrated that medetomidine enhances cardiac ACh release through modulation of the baroreflex control,[5] this vagotonic effect of medetomidine on the heart should be dependent on the baroreflex response. Robertson and Leslie showed that α_2-adrenergic receptors are densely distributed in the nucleus tractus solitaries (NTS), where baroreceptor afferent nerves terminate.[13] Grutu et al.

demonstrated that presynaptic α_2-adrenergic receptors in the nucleus ambiguus are involved in baroreflex bradycardia.[14] Thus, medetomidine might act on the NTS and/or the nucleus ambiguus, and exert this vagotonic effect on the heart. In contrast, the vagolytic effect on the stomach might depend on other pathways. Robertson and Leslie reported that α_2-adrenergic receptors are also distributed in the dorsal motor nucleus (DMN) of the vagus, which contains preganglionic neurons that control gastric motility and secretion.[13] As dexmedetomidine has been shown to inhibit gastric emptying and gastrointestinal transit in healthy volunteers,[15] medetomidine might act on the DMN and exert vagolytic effects on the stomach. Although further investigations are needed to elucidate the sites of medetomidine action, the organ-specific vagal responses to medetomidine might be associated with different actions of medetomidine on the nuclei.

Effects of Medetomidine on Sympathetic Nerve Activities

In protocol 1, 100 μg/kg of medetomidine significantly suppressed both the cardiac and gastric NE releases. Thus, medetomidine might suppress the whole sympathetic nerve activities. The rostral ventrolateral medulla (RVLM) is known to serve as an important site in mediating the hypotensive and sedative effects of α_2-adrenergic agonist, clonidine.[16] Furthermore, in the supplementary protocol, an α_2-adrenergic antagonist, atipamezole, blocked this medetomidine-induced suppression of sympathetic NE releases. Thus, medetomidine might act on α_2-adrenergic receptors in the RVLM and exert sympatholytic effects to both the heart and stomach.

Restoring the mean arterial pressure to baseline level by infusing phenylephrine scarcely affected medetomidine-induced suppression of NE release both in the heart and stomach. It is possible that 100 μg/kg of medetomidine has already suppressed sympathetic nerve activities to the lowest level, leaving no room for further baroreflex-induced sympathetic suppression. This strong sympatholytic effect might be useful for the treatment of CHF as described in the *Clinical implications* section.

Clinical Implications

Electrical VNS has recently become a new therapeutic option for CHF.[1] However, electrical VNS sometimes causes gastrointestinal adverse effects. Approximately 10% of patients receiving VNS therapy for epileptic seizures complain of nausea.[3] Sanossian and Haut reported chronic diarrhea associated with VNS.[4] As shown in protocol 2, electrical stimulation of the cervical vagus nerve increases both cardiac and gastric ACh releases, and the augmented gastric ACh release might cause nausea and diarrhea in clinical settings. Furthermore, in an animal study, Cho et al. reported that intermittent electrical stimulation of the left cervical vagus nerve induced a 100% incidence of hemorrhagic ulcers in the glandular mucosa of rat stomachs.[17] Thus, hemorrhagic gastric ulcer might be one of the most serious adverse effects caused by electrical VNS therapy in CHF patients, because CHF patients often receive concomitant anti-coagulation therapy, anti-platelet therapy, or both.

Organ-specific vagal activation is one strategy to reduce the above-mentioned adverse effects. Bianchi et al. have reported that endocardial atrioventricular vagal stimulation significantly reduces the ventricular rate acutely during atrial fibrillation in humans.[18] Thus, selective cardiac electrical VNS might be one of the most suitable approaches for vagal activation therapy in CHF patients, if the treatment effects of VNS are exclusively mediated by efferent vagal activation.

Another approach of vagal activation therapy in CHF patients is to use pharmacological agents. An ACh esterase inhibitor is a candidate for pharmacological vagal activation therapy.[19] Because ACh esterase degrades ACh immediately after its release, an ACh esterase inhibitor will block ACh degradation and increase ACh in the synaptic cleft. Kubo et al. have reported that donepezil, an ACh esterase inhibitor against Alzheimer's disease, significantly decreased plasma brain natriuretic peptide levels in patients with subclinical CHF.[20] However, an ACh esterase inhibitor might also cause gastrointestinal adverse effects. Nausea and diarrhea are the most common adverse events related to donepezil therapy,[21] which is similar to that caused by electrical VNS therapy. To prevent these gastrointestinal adverse events, we have to identify a new agent that activates cardiac vagus nerve without stimulating vagal activity in the gastrointestinal tract. However, there is a paucity of information on pharmacological agents that selectively activate the cardiac vagus nerve, partly because of the difficulty in selectively monitoring organ-specific vagus nerve activities. The microdialysis technique enables us to monitor organ-specific vagus nerve activities. The present study demonstrated that medetomidine selectively increased cardiac ACh release without augmenting gastric ACh release, suggesting that medetomidine is able to activate cardiac vagus nerve without stimulating gastric vagal activity. Medetomidine might be a more suitable agent than ACh esterase inhibitors for the treatment of CHF patients, although further investigations are required to examine the effects of medetomidine on other organs.

The sympatholytic effect of medetomidine might also be favorable for cardiac and gastric protection. Because sustained sympathetic overdrive contributes to progressive left ventricular dysfunction and promotes progressive left ventricular remodeling in CHF patients,[22] inhibition of the sympathetic nerve system has been the cornerstone of drug therapy for CHF.[23] Thus, we might be able to use medetomidine as well as β blockers to modify the augmented sympathetic tone in CHF. As medetomidine (or dexmedetomidine) has also been used as an anesthetic agent, sedation with medetomidine (or dexmedetomidine) might be beneficial for the intensive care of CHF patients. Furthermore, sympathetic overdrive also causes mucosal vasoconstriction and reduces the mucosal blood flow in the stomach, potentially leading to gastric ulcers. Because 50–100 μg/kg of dexmedetomidine has an antiulcerative effect equivalent to 25 mg/kg of famotidime,[6] sedation with medetomidine (or dexmedetomidine) might exert a gastroprotective effect in CHF patients while simultaneously conferring cardioprotection. Conversely, the sedative action of medetomidine might render this agent unsuitable for outpatient treatment.

Study Limitations

First, because ACh is degraded by ACh esterase immediately after release, the addition of eserine into the perfusate is required for measuring the in vivo release of ACh. The presence of eserine around the microdialysis fiber could have affected ACh release in the vicinity of the fiber.

Second, in protocol 2, gastric dialysate ACh concentration after hexamethonium injection was slightly but significantly higher than the post-vagotomy baseline level. Thus, the gastric dialysate ACh concentration might partly reflect ACh release from preganglionic nerves.

Third, medetomidine is a chiral imidazole derivative. Although hemodynamic and gastric secretory responses to medetomidine are known to be abolished by an α_2-adrenergic antagonist, atipamezole,[24,25] there is room for a possibility that imidazoline receptors might also be involved in the cardiac vagal activation by medetomidine.

Finally, we did not examine both cardiac and gastric functions

in the present study. According to previous papers, medetomidine can affect both cardiac and gastric functions as follows. Flacke et al. reported that the peak of the first derivative of systolic left ventricular pressure declined after intravenous administration of dexmedetomidine in anesthetized dogs.[26] Savola et al. reported that medetomidine inhibited basal gastric acid and fluid output in conscious rats in a dose-dependent manner while in anesthetized rats, no effect was observed when it was administered intravenously.[25] We need further investigations including chronic experiments to evaluate the effects of medetomidine on both cardiac and gastric functions in CHF conditions.

Conclusions

In the present in vivo study involving rabbits, while medetomidine suppressed both the cardiac and gastric NE releases, it enhanced cardiac ACh release but suppressed gastric ACh release through central actions. Medetomidine might be one of the potential pharmacological agents for vagal activation therapy against heart failure patients without the risk of causing gastric adverse effects.

Acknowledgments

This study was supported by a Grant-in-Aid for Scientific Research (23390415, 23592319) promoted by the Ministry of Education, Culture, Sports, Science and Technology of Japan, and by a Medical Research Promotion Grant from the Takeda Science Foundation, Japan.

Conflict of Interest

None declared.

References

1. Schwartz PJ. Vagal stimulation for heart diseases: From animals to men: An example of translational cardiology. *Circ J* 2011; **75:** 20–27.
2. Hauptman PJ, Schwartz PJ, Gold MR, Borggrefe M, Van Veldhuisen DJ, Starling RC, et al. Rationale and study design of the increase of vagal tone in heart failure study: INOVATE-HF. *Am Heart J* 2012; **163:** 954–962.
3. George MS, Sackeim HA, Rush AJ, Marangell LB, Nahas Z, Husain MM, et al. Vagus nerve stimulation: A new tool for brain research and therapy. *Biol Psychiatry* 2000; **47:** 287–295.
4. Sanossian N, Haut S. Chronic diarrhea associated with vagal nerve stimulation. *Neurology* 2002; **58:** 330.
5. Shimizu S, Akiyama T, Kawada T, Sata Y, Mizuno M, Kamiya A, et al. Medetomidine, an α_2-adrenergic agonist, activates cardiac vagal nerve through modulation of baroreflex control. *Circ J* 2012; **76:** 152–159.
6. Polat B, Albayrak Y, Suleyman B, Dursun H, Odabasoglu F, Yigiter M, et al. Antiulcerative effect of dexmedetomidine on indomethacin-induced gastric ulcer in rats. *Pharmacol Rep* 2011; **63:** 518–526.
7. Shimizu S, Akiyama T, Kawada T, Shishido T, Mizuno M, Kamiya A, et al. In vivo direct monitoring of interstitial norepinephrine levels at the sinoatrial node. *Auton Neurosci* 2010; **152:** 115–118.
8. Shimizu S, Akiyama T, Kawada T, Shishido T, Yamazaki T, Kamiya A, et al. In vivo direct monitoring of vagal acetylcholine release to the sinoatrial node. *Auton Neurosci* 2009; **148:** 44–49.
9. Akiyama T, Yamazaki T, Ninomiya I. In vivo detection of endogenous acetylcholine release in cat ventricles. *Am J Physiol* 1994; **266:** H854–H860.
10. Akiyama T, Yamazaki T, Ninomiya I. In vivo monitoring of myocardial interstitial norepinephrine by dialysis technique. *Am J Physiol* 1991; **261:** H1643–H1647.
11. Brown OM. Cat heart acetylcholine: Structural proof and distribution. *Am J Physiol* 1976; **231:** 781–785.
12. Glantz SA. Primer of Biostatistics, 6th ed. New York: McGraw-Hill, 2005.
13. Robertson HA, Leslie RA. Noradrenergic alpha 2 binding sites in vagal dorsal motor nucleus and nucleus tractus solitarius: Autoradiographic localization. *Can J Physiol Pharmacol* 1985; **63:** 1190–1194.
14. Gurtu S, Sharma DK, Sinha JN, Bhargava KP. Evidence for involvement of alpha 2-adrenoceptors in the nucleus ambiguous in baroreflex-mediated bradycardia. *Naunyn Schmiedebergs Arch Pharmacol* 1983; **323:** 199–204.
15. Iirola T, Vilo S, Aantaa R, Wendelin-Saarenhovi M, Neuvonen PJ, Scheinin M, et al. Dexmedetomidine inhibits gastric emptying and oro-caecal transit in healthy volunteers. *Br J Anaesth* 2011; **106:** 522–527.
16. Yamazato M, Sakima A, Nakazato J, Sesoko S, Muratani H, Fukiyama K. Hypotensive and sedative effects of clonidine injected into the rostral ventrolateral medulla of conscious rats. *Am J Physiol Regul Integr Comp Physiol* 2001; **281:** R1868–R1876.
17. Cho CH, Ogle CW, Dai S. Acute gastric ulcer formation in response to electrical vagal stimulation in rats. *Eur J Pharmacol* 1976; **35:** 215–219.
18. Bianchi S, Rossi P, Della Scala A, Kornet L. Endocardial transcatheter stimulation of the AV nodal fat pad: Stabilization of rapid ventricular rate response during atrial fibrillation in left ventricular failure. *J Cardiovasc Electrophysiol* 2009; **20:** 103–105.
19. Li M, Zheng C, Kawada T, Inagaki M, Uemura K, Shishido T, et al. Donepezil markedly improves long-term survival in rats with chronic heart failure after extensive myocardial infarction. *Circ J* 2013; **77:** 2519–2525.
20. Kubo T, Sato T, Noguchi T, Kitaoka H, Yamasaki F, Kamimura N, et al. Influences of donepezil on cardiovascular system--possible therapeutic benefits for heart failure--donepezil cardiac test registry (DOCTER) study. *J Cardiovasc Pharmacol* 2012; **60:** 310–314.
21. Shintani EY, Uchida KM. Donepezil: An anticholinesterase inhibitor for Alzheimer's disease. *Am J Health Syst Pharm* 1997; **54:** 2805–2810.
22. Sabbah HN. Biologic rationale for the use of beta-blockers in the treatment of heart failure. *Heart Fail Rev* 2004; **9:** 91–97.
23. Sata Y, Krum H. The future of pharmacological therapy for heart failure. *Circ J* 2010; **74:** 809–817.
24. Savola JM. Cardiovascular actions of medetomidine and their reversal by atipamezole. *Acta Vet Scand Suppl* 1989; **85:** 39–47.
25. Savola M, Savola JM, Puurunen J. Alpha 2-adrenoceptor-mediated inhibition of gastric acid secretion by medetomidine is efficiently antagonized by atipamezole in rats. *Arch Int Pharmacodyn Ther* 1989; **301:** 267–276.
26. Flacke WE, Flacke JW, Bloor BC, McIntee DF, Sagan M. Effects of dexmedetomidine on systemic and coronary hemodynamics in the anesthetized dog. *J Cardiothorac Vasc Anesth* 1993; **7:** 41–49.

frontiers in PHYSIOLOGY

PERSPECTIVE ARTICLE
published: 08 July 2014
doi: 10.3389/fphys.2014.00256

Systems physiology of the baroreflex during orthostatic stress: from animals to humans

Atsunori Kamiya *, Toru Kawada and Masaru Sugimachi

Department of Cardiovascular Dynamics, National Cerebral and Cardiovascular Center Research Institute, Suita, Japan

Edited by:
Qi Fu, The Institute for Exercise and Environmental Medicine and University of Texas Southwestern Medical Center, USA

Reviewed by:
Vaughan G. Macefield, University of Western Sydney, Australia
J. A. Taylor, Harvard University, USA

****Correspondence:***
Atsunori Kamiya, Department of Cardiovascular Dynamics, National Cerebral and Cardiovascular Center Research Institute, 5-7-1 Fujishirodai, Suita, Osaka 565-8565, Japan
e-mail: kamiya@ri.ncvc.go.jp

The baroreflex is a key mechanism involved in the control of arterial pressure (AP) during orthostasis in humans. However, the baroreflex is a closed-loop feedback system, from baroreceptor pressure input to systemic AP, and therefore requires open-loop experiments to identify its system characteristics. The requirement limits our ability to identify baroreflex system characteristics in humans. Open-loop research in animals has revealed dynamic and static characteristics of the two baroreflex subsystems: the neural and peripheral arcs. The neural arc, from baroreceptor pressure input to sympathetic nerve activity (SNA), has high-pass dynamic characteristics, indicating that more rapid change in input AP causes greater response in SNA. In contrast, the peripheral arc, from SNA input to systemic AP, has low-pass characteristics. Orthostasis increases the gain of the neural arc, which compensates for the lower transfer gain of the peripheral arc and in turn maintains total baroreflex function. Here, I discuss the possibility that baroreflex subsystem characteristics identified in animals can be applicable to the human sympathetic response to orthostasis, with a focus on loading speed-dependence of orthostatic sympathetic activation.

Keywords: baroreflexes, systems analysis, sympathetic nerve activity, autonomic nervous system, integrative physiology

INTRODUCTION

The maintenance of arterial pressure (AP) under orthostatic stress from gravitational fluid shift is of great importance in humans (Eckberg and Sleight, 1992) and animals (rats, rabbits etc.) that spend most of their time in a head-up posture and that frequently stand during their daily life. The baroreflex is a key mechanism involved in the control of AP during orthostasis since baroreflex failure leads to severe orthostatic hypotension (Cooke et al., 1999; Fu et al., 2009). The baroreflex is a negative-feedback closed-loop system, from baroreceptor pressure input to systemic AP, and therefore needs open-loop surgical operation to identify its system characteristics (Ikeda et al., 1996), which is fundamentally impossible in human research. Animal research that has used open-loop baroreflex and white-noise input techniques (system identification) have clarified dynamic and static transfer characteristics of the two baroreflex subsystems, the neural arc and peripheral arc, during orthostasis as described below.

Regarding the dynamic transfer characteristics, the neural arc, from baroreceptor pressure input to SNA, has high-pass dynamic characteristics, which means that a more rapid change in AP results in a greater response in SNA, whereas the peripheral arc, from SNA input to systemic AP, has low-pass dynamic characteristics (Kawada et al., 2002; Kamiya et al., 2005c). The open-loop transfer function of the neural arc is able to predict time-series SNA responses to drug-induced AP changes with an r^2 of 0.9, whereas the closed-loop-spontaneous transfer function cannot with a negative r-value (the inverse of measured responses) (Kamiya et al., 2011). In addition, orthostatic stress, caused from movement from a horizontally supine position, increases the transfer function gain of the neural arc, which helps compensate for the lower transfer function gains of the peripheral arc during orthostasis. This in turn helps maintain total baroreflex function (Kamiya et al., 2008). Regarding the static transfer characteristics, orthostatic stress resets the neural arc (baroreceptor pressure-SNA curve) to a higher SNA level (in the kidney and the heart), which compensates for the reduced presser responses to an increase in SNA in the peripheral cardiovascular system and helps prevent postural hypotension (Kamiya et al., 2005b, 2010).

Although system identification of the baroreflex is a useful tool for understanding baroreflex function in a variety of physiological and pathophysiological conditions, it requires surgical operation to open the baroreflex loop. The requirement of an open-loop experimental condition limits its application in human research. Therefore, the system characteristics of the baroreflex identified in animals, particularly the dynamic transfer function characteristics, have not been related to human baroreflex physiology.

Here, I discuss the possibility that baroreflex subsystem characteristics identified in animals can be applicable to the human sympathetic response to orthostasis. I will focus on the high-pass filter dynamic transfer function characteristics identified in muscle, cardiac and renal SNA of anesthetized rabbits (Kamiya et al., 2005c). I will also discuss whether transfer function characteristics identified in animals can explain the previously reported finding in humans that slow head-up tilt causes lower activation of muscle SNA (MSNA): loading speed-dependence of orthostatic sympathetic activation in humans (Kamiya et al., 2009).