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Accentuated antagonism of vagal heart rate control and less potent prejunctional inhibition of vagal acetylcholine release during sympathetic nerve stimulation in the rat

Toru Kawada[a,*], Takashi Sonobe[b], Yohsuke Hayama[a], Takuya Nishikawa[a], Tadayoshi Miyamoto[c], Tsuyoshi Akiyama[b], James T. Pearson[b], Masaru Sugimachi[a]

[a] *Department of Cardiovascular Dynamics, National Cerebral and Cardiovascular Center, Osaka 565-8565, Japan*
[b] *Department of Cardiac Physiology, National Cerebral and Cardiovascular Center, Osaka 565-8565, Japan*
[c] *Graduate School of Health Sciences, Morinomiya University of Medical Sciences, 559-8611, Japan*

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ABSTRACT

Complex interactions are known to occur between the sympathetic and parasympathetic controls of the heart. Although sympathetic nerve stimulation (SNS) usually augments the heart rate (HR) response to vagal nerve stimulation (VNS), exogenously administered norepinephrine (NE) can attenuate the HR response as well as the myocardial interstitial acetylcholine (ACh) release during VNS. To provide a basis for an integrative knowledge about the opposing adrenergic effects on the vagal control of the heart, we examined whether SNS significantly attenuates VNS-induced myocardial interstitial ACh release in the in vivo beating heart. In nine anesthetized rats, changes in HR and myocardial interstitial ACh release in response to 5- and 20-Hz VNS were examined in both the absence and presence of a 5-Hz background SNS. The SNS significantly enhanced the VNS-induced HR reduction during 20-Hz VNS (−101.2 ± 33.1 vs. −163.0 ± 34.9 beats/min, $P < 0.001$, a 60% augmentation). By contrast, the SNS significantly attenuated the ACh release during 20-Hz VNS (4.30 ± 0.72 vs. 3.80 ± 0.75 nM, $P < 0.01$, a 12% attenuation). In conclusion, SNS exerted only a moderate inhibitory effect on the VNS-induced myocardial interstitial ACh release in the in vivo beating heart.

1. Introduction

The heart is governed by the sympathetic and parasympathetic nervous systems, and complex interactions are known to occur between the sympathetic and vagal controls of heart rate (HR). The HR response to vagal nerve stimulation (VNS) is augmented in the presence of concomitant sympathetic activity. Levy [1971] termed this phenomenon *accentuated antagonism* of the vagal control of HR. The VNS-induced HR response can be augmented during exercise-induced sympathetic excitation in conscious dogs, which suggests the physiological importance of this interaction [Stramba-Badiale et al., 1991]. Accentuated antagonism is mainly attributable to β-adrenergic stimulation of the sinus nodal cells. In addition to β-adrenergic stimulation by isoproterenol, pharmacological interventions that lead to the intracellular accumulation of cyclic adenosine monophosphate (cyclic AMP) can also mimic the accentuated antagonism effect [Nakahara et al., 1998].

In contrast to β-adrenergic stimulation, α-adrenergic stimulation can reduce vagal effects on the heart. This inhibition is mediated by α-adrenergic receptors on the vagal nerves, and may be referred to as *presynaptic inhibition* or *prejunctional inhibition*. Local norepinephrine (NE) administration attenuates VNS-induced myocardial interstitial acetylcholine (ACh) release in the feline left ventricle, and this effect can be eliminated by local administration of an α-adrenergic antagonist phentolamine [Akiyama and Yamazaki, 2000]. Intravenous NE infusion also dose-dependently attenuates the dynamic HR response to VNS, and this attenuation does not occur when phentolamine is administered simultaneously with NE [Miyamoto et al., 2003]. This latter result suggests that, with respect to exogenous NE, the α-adrenergic receptor-mediated inhibitory effect outweighs the β-adrenergic receptor-mediated facilitatory effect on vagal control of HR.

Electrical cardiac sympathetic nerve stimulation (SNS) induces an endogenous NE release from the sympathetic nerve terminals in the heart [Yamazaki et al., 1997]. Cardiac SNS augments the dynamic HR response to VNS [Kawada et al., 1996], in agreement with the concept of accentuated antagonism. It may be of note that β-adrenergic blockade does not uncover an inhibitory effect of SNS on the dynamic

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[*] Corresponding author.
E-mail address: torukawa@ncvc.go.jp (T. Kawada).

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HR response to VNS [Miyamoto et al., 2004]. This result is in marked contrast with the exogenous NE-induced inhibitions of the myocardial interstitial ACh release [Akiyama and Yamazaki, 2000] and also the dynamic HR response to VNS [Miyamoto et al., 2003]. The prejunctional inhibition of the vagal effect has been well documented separately from the phenomenon of accentuated antagonism [Wetzel and Brown, 1985; McGrattan et al., 1987]. We thought it would be important to examine the prejunctional inhibition simultaneously with the accentuated antagonism to obtain an integrative knowledge about the interactions between the sympathetic and vagal systems in regulating the heart. We hypothesized that the prejunctional inhibition by SNS would not occur strongly in the in vivo beating heart. To test this hypothesis, we measured myocardial interstitial ACh release in response to VNS with and without SNS in anesthetized rats.

2. Materials and methods

The experimental animals were cared for in strict accordance with the Guiding Principles for the Care and Use of Animals in the Field of Physiological Sciences, which has been approved by the Physiological Society of Japan. The Animal Subjects Committee at the National Cerebral and Cardiovascular Center reviewed and approved the experimental protocols.

2.1. Surgical preparation

Nine male Wistar–Kyoto rats (402 ± 19 g, mean ± SD) were used in this experiment. Each rat was anesthetized by intraperitoneal injection (2 ml/kg) of an anesthetic mixture containing 40 mg/ml of urethane and 250 mg/ml of α-chloralose. All rats were mechanically ventilated with oxygen-enriched room air. The anesthesia was maintained by continuously administering an 18-fold diluted anesthetic mixture from a catheter inserted into the right femoral vein (2–3 $\text{ml}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$). Arterial pressure (AP) was measured from a catheter inserted into the right femoral artery, and HR was measured from the AP waveform. The body temperature of each animal was maintained at 38 °C using a heating pad and a lamp.

The myocardial interstitial ACh level was measured using cardiac microdialysis [Akiyama et al., 1994]. In a lateral position, the left fourth through sixth ribs were partially resected to expose the heart. The pericardium was incised, and two dialysis probes were implanted into the free wall of the left ventricle [Kawada et al., 2012, 2019]. Each dialysis probe was perfused with Ringer's solution containing a cholinesterase inhibitor eserine (100 μM) at a perfusion rate of 2 μl/min. The dialysate sampling was started at 2-h after implantation of the probes. The concentration of ACh in the dialysate sample was measured by a high-performance liquid chromatography system (Eicom, Japan). We note that the implanted dialysis probe was broken on a few occasions due to acute changes in ventricular contractions during SNS or VNS. When this happened, all dialysate samples from the broken probe were discarded; otherwise ACh concentrations measured from the two dialysate samples were averaged at each sampling period. It was confirmed by macroscopic examination at postmortem that the dialysis membrane was not exposed to the ventricular cavity.

The bilateral cervical sympathetic and vagal nerves were approached via a midline cervical incision. The nerves were sectioned, and a pair of stainless-steel wire electrodes (AS633, Cooner Wire, CA, USA) was attached to the cardiac end of each nerve for efferent stimulation [Mizuno et al., 2010]. The nerve and electrodes were secured with silicone glue (Kwik-Sil, World Precision Instruments, FL, USA). Four isolators and two electrical stimulators (SEN-7203, Nihon Kohden, Japan) were used to stimulate the sympathetic and vagal nerves.

2.2. Protocol

The sectioned sympathetic nerves were stimulated bilaterally by pulse trains of an amplitude of 10 V and a pulse duration of 100 μs. The sectioned vagal nerves were stimulated bilaterally by pulse trains of an amplitude of 5 V and a pulse duration of 100 μs. To establish stable stimulatory conditions, simultaneous 5-Hz SNS and 5-Hz VNS were conducted for 10 min, and a recovery time of > 10 min was allowed before starting dialysate sampling. Dialysate samples were collected for 10 min under the following six conditions: control (VNS0), 5-Hz VNS (VNS5), 20-Hz VNS (VNS20), 5-Hz SNS with no VNS (S-VNS0), 5-Hz SNS followed by 5-Hz VNS (S-VNS5), and 5-Hz SNS followed by 20-Hz VNS (S-VNS20). In S-VNS5 and S-VNS20 trials, SNS was started 1 min ahead of VNS to enable confirmation that the SNS increased HR. The antecedent SNS can cause an underestimation of the accentuated antagonism effect on the VNS-induced HR response [Yang and Levy, 1992], but nevertheless, we think the condition may be analogous to a study examining the effects of exogenous NE because NE is usually administered in advance of the VNS. An interval of 10 min with no stimulation was allocated between the consecutive trials. In all animals, the VNS0 trial was performed first, followed by the S-VNS0 trial. Thereafter, trials with and without SNS were conducted alternately, and the order of the remaining four trials was one among the following: {VNS5, S-VNS5, VNS20, S-VNS20}, {S-VNS5, VNS5, S-VNS20, VNS20}, {VNS20, S-VNS20, VNS5, S-VNS5}, and {S-VNS20, VNS20, S-VNS5, VNS5}. Although a possibility cannot be denied that VNS or SNS caused some declining of neurotransmitter release or receptor desensitization with elapsed time, the effects of VNS and SNS were relatively stable for the present experimental duration such that VNS20 and S-VNS20 trials showed lower HRs than VNS5 and S-VNS5 trials, respectively, irrespective the order of the protocols, except one out of nine animals.

2.3. Data and statistical analysis

The AP and HR data were recorded at 1000 Hz using a PowerLab system (PowerLab 8/30, ADInstruments, New Zealand). For statistical analyses, values of AP and HR were averaged every minute. Pre-stimulation baseline AP and HR were compared among the six trials using one-way repeated-measures analysis of variance (ANOVA). In each trial, changes in AP and HR relative to the pre-stimulation baseline values were tested using one-way repeated-measures ANOVA with *post-hoc* Dunnett's tests (Prism, GraphPad, CA, USA). The differences were considered significant at $P < 0.05$.

Representative data points were selected at the middle of the period of dialysate sampling to compare HR among the six trials. For the control trial (VNS0), the HR measured at the 5th min of the observation period was used. For the S-VNS0 trial, the HR measured at 5 min after the onset of the SNS was used. For the remaining trials (VNS5, VNS20, S-VNS5, and S-VNS20), the HR measured at 5 min after the onset of the VNS was used.

The effects of SNS and VNS on HR were examined using two-way ANOVA with repeated measurements on both factors. *Post-hoc* Bonferroni's tests were performed to examine the differences between trials with and without SNS at each condition of VNS. The ACh data were likewise analyzed. For the HR data, the magnitude of HR reductions from the corresponding trial without VNS (VNS0 or S-VNS0) were compared between VNS5 and S-VNS5 trials as well as between VNS20 and S-VNS20 trials, using paired *t*-tests with the Bonferroni correction.

3. Results

Changes in AP and HR pooled for each trial are depicted in Fig. 1. Pre-stimulation baseline AP and HR did not differ significantly among the six trials. In the control trial (VNS0, Fig. 1A), the AP and HR did not change significantly as time elapsed. In the VNS5 trial (Fig. 1B), VNS significantly decreased the HR from the pre-stimulation value. Although the AP showed some reduction after the onset of VNS, the change was not statistically significant relative to the pre-stimulation value. In the VNS20 trial (Fig. 1C), VNS again significantly decreased

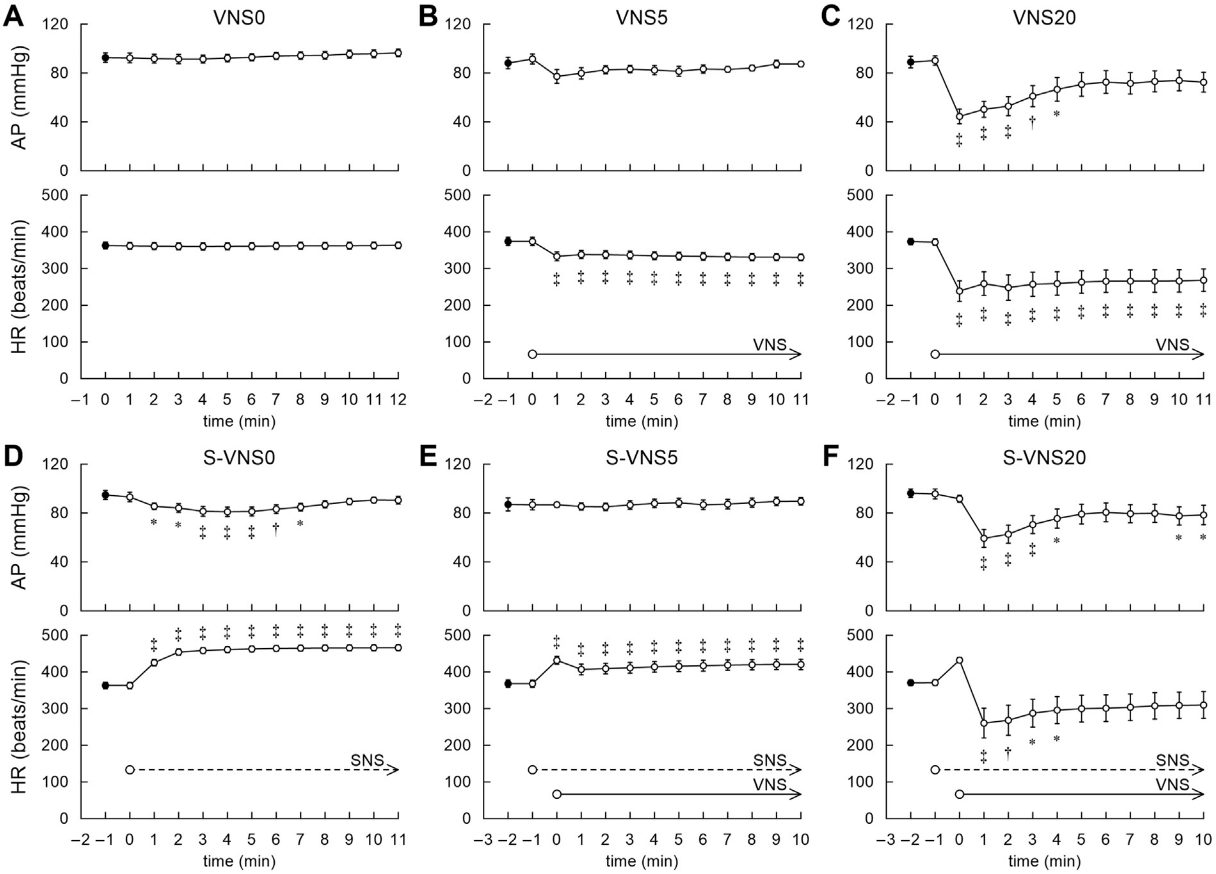

Fig. 1. Changes in arterial pressure (AP) and heart rate (HR) pooled for nine animals. **A**: The control trial in which no vagal nerve stimulation (VNS) was performed (VNS0). **B** and **C**: The trials of 5-Hz VNS (VNS5) and 20-Hz VNS (VNS20). **D**: The trial of sympathetic nerve stimulation (SNS) with no VNS (S-VNS0). **E** and **F**: The trials of 5-Hz VNS (S-VNS5) and 20-Hz VNS (S-VNS20) in the presence of the background SNS. The horizontal solid and broken arrows indicate the durations of VNS and SNS, respectively. The arrow tails are shown in open circles, which indicate that the stimulation was started just after the measurements of AP and HR at the corresponding time point. Data are means ± SE, $n = 9$. $^{*}P < 0.05$, $^{\dagger}P < 0.01$, and $^{\ddagger}P < 0.001$ by Dunnett's tests against the first data points (filled circles) following one-way repeated-measures analysis of variance (ANOVA) in each trial.

the HR from the pre-stimulation value. VNS also decreased the AP for the first 5 min. In the S-VNS0 trial (Fig. 1D), SNS significantly increased the HR, and a slight gradual decrease and recovery in AP was observed during the first 7 min. In the S-VNS5 trial (Fig. 1E), VNS attenuated the SNS-induced HR increase but did not completely counteract the sympathetic effect; i.e., the HR remained increased from the pre-stimulation value during the simultaneous SNS and VNS. In this trial, the AP did not change significantly. In the S-VNS20 trial, VNS predominated the sympathetic effect and decreased the HR from the pre-stimulation value. The HR decrease was significant for the first 4 min of the VNS. VNS decreased the AP for the first 4 min and also at the 9th and 10th min relative to the pre-stimulation value.

Fig. 2 summarizes the results of the two-way repeated-measures ANOVA. In the HR data (Fig. 2A), the effects of SNS and VNS were significant. There was also a significant interaction effect, which indicates that the magnitude of the VNS-induced HR reduction was different depending on the presence of SNS. The *post-hoc* analysis indicates that the HR was higher in the presence of SNS at each condition of VNS. The SNS did not significantly affect the HR reduction during 5-Hz VNS (ΔHR: −25.3 ± 7.8 vs. −47.2 ± 8.1 beats/min, $P = 0.099$), but it did significantly enhance the HR reduction during 20-Hz VNS (ΔHR: −101.2 ± 33.1 vs. −163.0 ± 34.9 beats/min, $P < 0.001$, an approximately 60% augmentation, depicted as vertical arrows in Fig. 2A).

In the ACh data, the effects of SNS and VNS were significant (Fig. 2B). The significant interaction effect indicates that the magnitude of the VNS-induced ACh release was different depending on the presence of SNS. The *post-hoc* analysis indicated that there was no significant difference in ACh values between VNS0 and S-VNS0 trials or between VNS5 and S-VNS5 trials. The SNS attenuated the ACh release during 20-Hz VNS by approximately 12% (4.30 ± 0.72 vs. 3.80 ± 0.75 nM, $P < 0.01$); however, the magnitude of this effect was modest compared with the amount of augmentation observed in the VNS-induced HR response.

4. Discussion

The present study is the first to demonstrate differential effects of SNS on the VNS-induced HR response and myocardial interstitial ACh release in an in vivo beating heart preparation. The SNS enhanced the VNS-induced HR response, which agrees with the concept of accentuated antagonism. By contrast, the SNS attenuated the VNS-induced myocardial interstitial ACh release. The magnitude of the ACh release suppression was, however, much smaller than that induced by exogenous NE [Akiyama and Yamazaki, 2000].

4.1. *Prejunctional inhibition by NE*

Wetzel and Brown [1985] demonstrated that exogenous NE inhibited ACh release from cardiac vagal nerve terminals by approximately 60% in an in vitro experiment using the rat right atria. Since they used superfusion of high potassium to directly depolarize the nerve terminals, they suggested that the inhibitory effect might have occurred independently of neuronal conduction or ganglionic transmission. On the other hand, Manabe et al. [1991] did not observe inhibition of ACh

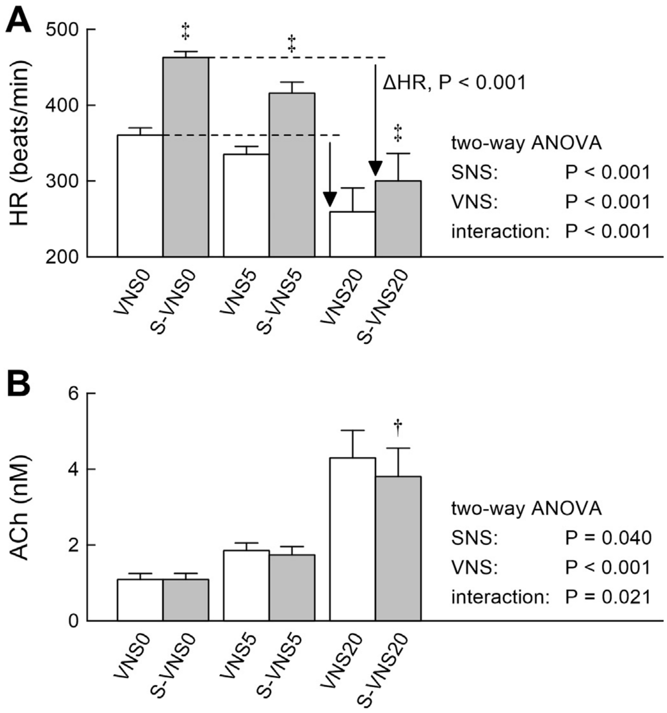

Fig. 2. Results of two-way analysis of variance (ANOVA) with repeated measurements on both factors [sympathetic nerve stimulation (SNS) and vagal nerve stimulation (VNS)]. **A**: Effects of SNS and VNS on heart rate (HR). Vertical arrows indicate the magnitude of HR reduction (ΔHR) from the corresponding trial without VNS. **B**: Effects of SNS and VNS on the concentration of myocardial interstitial acetylcholine (ACh). The significant interaction indicates that the effect of VNS differed depending on the presence of SNS. Data are mean and mean + SE, n = 9. $^{\dagger}P < 0.01$ and $^{\ddagger}P < 0.001$ between trials with and without SNS at each condition of VNS by *post-hoc* Bonferroni tests.

release by exogenous NE in an in vitro experiment using the guinea pig right atria. Because they used electrical field stimulation to depolarize the nerve terminals, it is possible that the field stimulation had maximally stimulated the sympathetic nerve terminals, leaving no room for an effect from exogenous NE. Manabe et al. [1991] also demonstrated that α_1- and α_2-adrenergic antagonists did not affect the ACh release during field stimulation. Hence, endogenous NE released by field stimulation might not have modulated the ACh release via the α-adrenergic mechanism. In addition to the differences in the mode of stimulation (high potassium versus field stimulation), species difference (rats versus guinea pigs) could account for the conflicting results from these in vitro experiments.

Akiyama and Yamazaki [2000] demonstrated that a local administration of NE causes an approximately 43% reduction of the VNS-induced myocardial interstitial ACh release in the in vivo feline ventricle (from 9.5 ± 1.8 to 5.4 ± 1.2 nM, $P < 0.05$). Miyamoto et al. [2003] demonstrated that an intravenous administration of NE causes a similar degree of attenuation in the dynamic gain of the VNS-induced HR response in rabbits (from 6.2 ± 0.8 to 3.9 ± 1.2 $\text{beats}\cdot\text{min}^{-1}\cdot\text{Hz}^{-1}$, $P < 0.05$, approximately 37% reduction). In these studies, the administration of an α-adrenergic antagonist phentolamine eliminated the ability of exogenous NE to attenuate the VNS-induced ACh release or the HR response. The results of these in vivo experiments support the inhibitory modulation of the vagal control of the heart via the α-adrenergic mechanism [McGrattan et al., 1987].

4.2. Prejunctional inhibition by SNS

The question remained unanswered as to whether SNS can exert the inhibitory effect on the vagal control of the in vivo beating heart. In contrast to the intravenous NE administration discussed above [Miyamoto et al., 2003], background sympathetic tone usually augments the VNS-induced HR response [Stramba-Badiale et al., 1991; Kawada et al., 1996; Mizuno et al., 2008], which suggests that the net effect of SNS on the VNS-induced HR response is facilitatory. In a previous study, we tested the hypothesis that β-adrenergic blockade would result in SNS inhibiting the vagal control of HR via the unblocked α-adrenergic mechanism [Miyamoto et al., 2004]; however, we found that SNS did not significantly affect the dynamic gain of the VNS-induced HR response under conditions of β-adrenergic blockade (3.7 ± 0.6 and 3.8 ± 0.5 $\text{beats}\cdot\text{min}^{-1}\cdot\text{Hz}^{-1}$ in the absence and presence of SNS, respectively). It was therefore concluded that SNS does not significantly inhibit the VNS-induced HR response via the α-adrenergic mechanism.

The above conclusion was, however, indirect with respect to the effect of SNS on the vagal nerve terminal function, because only HR change was examined. In the present study, SNS significantly augmented the VNS-induced HR response (Fig. 2A, ΔHR shown in vertical arrows) and simultaneously attenuated the VNS-induced myocardial interstitial ACh release (Fig. 2B). In VNS20 and S-VNS20 trials, AP significantly decreased (Fig. 1C and F), which must have increased systemic sympathetic tone via the arterial baroreflex. As we did not cut the cardiac sympathetic nerves from the thoracic spinal cord, the results of VNS20 and S-VNS20 trials might have been contaminated by the increased sympathetic tone. However, the comparison between VNS20 and S-VNS20 trials may be still valid because the HR was significantly higher in the S-VNS20 than in the VNS20 trial. The attenuation of the ACh release was observed only for the 20-Hz VNS, and it had a magnitude of approximately 12%. The degree of attenuation was much smaller than that observed during exogenous NE (43% attenuation) [Akiyama and Yamazaki, 2000], which suggests that SNS is not as effective as exogenous NE in suppressing the VNS-induced ACh release.

One possible explanation for the differential effects of SNS versus exogenous NE is the concentration gradient of NE around autonomic nerve terminals. The myocardial interstitial NE concentration during intravenous NE administration is much lower than the plasma NE concentration [Kawada et al., 1997]; and therefore, high plasma levels of NE do not easily increase HR as compared with cardiac SNS [Kawada et al., 2006]. According to electron microscopic investigations, adrenergic and cholinergic axon terminals are juxtaposed and share a common Schwann cell investment [Yamauchi, 1969]. Nevertheless, NE released from the sympathetic nerve terminals may be able to exert an inhibitory effect on the VNS-induced ACh release only after it spreads outside the neuroeffector junction with the sinus nodal cells. The NE concentration inside the neuroeffector junction may increase during SNS to produce the accentuated antagonism effect through activation of postjunctional β-adrenergic receptors on the sinus nodal cells. However, the NE concentration outside the neuroeffector junction may not become high enough to produce potent prejunctional modulation of the ACh release through stimulation of α-adrenergic receptors on the vagal nerve terminals.

4.3. Implications

The circadian variation indicates that the HR during the resting period is approximately 320 beats/min whereas the HR during the active period is approximately 380 beats/min in rats [Ohori et al., 2011]. Hence, the 20-Hz VNS, which lowered the HR to approximately 260 beats/min in the absence of SNS, might have been too strong for a physiological HR control. The SNS (5 Hz), which increased the HR to approximately 460 beats/min in the absence of VNS, might have been also too strong for the physiological HR control. Although the strong SNS and VNS can help clarify interactions between the sympathetic and vagal systems, the effects of interactions could be overestimated relative to the physiological HR control.

Heart rate variability (HRV) is often used to assess autonomic nervous activity. Decreased HRV can be a predictor of mortality after acute myocardial infarction [Kleiger et al., 1987], which is generally interpreted as reflecting increased sympathetic or decreased vagal tone. The present results suggest that increased sympathetic tone may increase the vagal HR response via the accentuated antagonism effect if the vagal tone is unchanged (Fig. 2A). In other words, the degree of vagal withdrawal may be underestimated in the presence of sympathetic activation. Further, the amount of vagally-induced ACh release in the ventricle may be attenuated to some extent during sympathetic activation (Fig. 2B). The ventricular effect of VNS is important for cardioprotection because the VNS prevents reperfusion injury through inhibiting mitochondrial permeability transition pore opening independent of the bradycardic effect [Katare et al., 2009]. Restoring the vagal tone, which cannot be attained by blocking the sympathetic effects, may be a key to treat cardiovascular diseases accompanying autonomic imbalance.

Chronic VNS has been shown to improve the survival of chronic heart failure rats after myocardial infarction [Li et al., 2004, 2018]. However, clinical translations of the VNS therapy for chronic heart failure do not show satisfactory results [De Ferrari et al., 2017; Gold et al., 2016] except one trial [Premchand et al., 2016]. Reasons for the discrepancy between the animal studies and clinical trials may include subjects' characteristics, the presence of pharmacotherapy, and the difference in the VNS conditions. We hope the understanding of the interactions between the sympathetic and vagal systems would aid the refinement of the VNS therapy. For instance, absolute HR values can dissociate from the myocardial interstitial ACh levels in the presence of the sympathetic tone, but the magnitude of the VNS-induced HR reduction may still correlate with the myocardial interstitial ACh levels (Fig. 2).

4.4. Limitations

First, only a single intensity of SNS was examined. Increasing the stimulation frequency of SNS may enhance its inhibitory effect on the vagal control of the heart by recruiting mechanisms such as those mediated by neuropeptide Y [Smith-White et al., 1999]. However, judging from the HR increase in the S-VNS0 trial, the stimulation frequency of SNS that we used here might have been already beyond the physiological HR response range. Second, eserine was added to the perfusate to avoid immediate breakdown of the released ACh. The accumulation of ACh in the vicinity of the dialysis probe can cause autoinhibition of ACh release via muscarinic receptors on the vagal nerve terminals [Wetzel and Brown, 1985; Manabe et al., 1991]. However, our comparisons across trials may be reasonable because the VNS-induced myocardial ACh releases were examined using the same perfusate. Third, NE released from the sympathetic nerve terminals were not measured, because of the limited sample volume of the dialysate. The amount of endogenous NE released by SNS could be lower during simultaneous VNS due to the prejunctional inhibition of the NE release by ACh [Manabe et al., 1991]. This attenuation of the release of endogenous NE might partly explain the less potent inhibitory effect on the VNS-induced ACh release by SNS, compared with that induced by exogenous NE. Measuring endogenous NE in the myocardial interstitium and plasma NE during VNS may be a future direction of the study. Fourth, the site of the inhibition by SNS of the VNS-induced myocardial interstitial ACh release was not restricted to the vagal nerve terminals and could be anywhere along the cardiac vagal pathway. The parasympathetic ganglia, however, might not be the site of inhibition because phenylephrine facilitates rather than inhibits the carbachol-induced vagal effect [Pardini et al., 1991]. Fifth, we were not able to measure the ACh release from the sinus node because of the thinness of the rat atrial wall around the sinus node relative to the size of the dialysis probe. Although both ventricular and atrial myocardial interstitial ACh concentrations increased in response to VNS in rabbits, the ACh concentration was much higher in the atrium than in the ventricle [Shimizu et al., 2009]. Relative changes in ventricular myocardial interstitial ACh concentrations can reflect vagal nerve terminal function [Kawada et al., 2001]. Nevertheless, further refinements of the microdialysis technique are required to measure the ACh release from the rat sinus node. Sixth, although VNS might have attenuated a positive inotropic response to SNS [Levy and Blattberg, 1976], we could not retrieve relevant data on this topic because we only measured peripheral AP and HR. Previous studies indicate that VNS can attenuate the ventricular contractility independently of HR in the presence of sympathetic tone [Nakayama et al., 2001] but not in the absence of sympathetic tone [Matsuura et al., 1997]. Finally, species differences need to be considered to reconcile diverse results reported in the modulation of ACh release from the vagal nerve terminals by adrenergic agents.

5. Conclusion

We conclude that complex interactions exist between the sympathetic and parasympathetic systems in regulating the heart. Although prejunctional inhibition of the vagal effect on the heart is confirmed in some experimental conditions [Wetzel and Brown, 1985; McGrattan et al., 1987; Akiyama and Yamazaki, 2000; Miyamoto et al., 2003], the present results indicate that SNS might exert only a moderate inhibitory effect on the VNS-induced myocardial interstitial ACh release, mainly at high-frequency VNS. The net effect of SNS on the VNS-induced HR response was facilitatory, reinforcing the concept of accentuated antagonism.

Conflict of interest

The authors declare that there are no conflicts of interest.

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