Heart Rate–Independent Vagal Effect on End-Systolic Elastance of the Canine Left Ventricle Under Various Levels of Sympathetic Tone

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Background—Although we have shown that in rabbits the direct (heart rate [HR]–independent) vagal effect on left ventricular end-systolic elastance (Ees) was negligible under minimal sympathetic tone, how underlying sympathetic tone modulates the inotropic response to vagal stimulation remains unknown.

Methods and Results—We used an isolated canine heart preparation with functioning autonomic nerves. We examined the direct vagal inotropic effect by measuring Ees under fixed-rate atrial pacing with or without concomitant sympathetic nerve stimulation. Right and left vagal stimulation at 20 Hz decreased HR by 27±3% and 14±2%, respectively, and decreased Ees by 11±2% and 6±2%, respectively. When we fixed HR by atrial pacing, right and left vagal stimulation at 20 Hz did not decrease Ees (0.01±0.3% and 0.3±0.4%; NS). Concomitant left sympathetic nerve stimulation at 4 Hz enhanced direct vagal negative inotropism to −19±3% and −34±5% for 20-Hz right and left vagal stimulation (interaction, P<0.01).

Conclusions—Direct vagal negative inotropism was unobservable with minimal sympathetic tone in dogs but was enhanced with concomitant sympathetic stimulation. (Circulation. 2001;104:2277-2279.)

Key Words: vagus nerve ■ nervous system, sympathetic ■ contractility ■ inotropism

The effect of vagal stimulation on ventricular contractility has been controversial.1–3 Main factors contributing to the debate include (1) the effect on contractility of bradycardia accompanying vagal stimulation,4 (2) the effect of different levels of sympathetic tone, and (3) the various indexes used to represent contractility. To address this issue, Matsuura et al5 studied the direct (heart rate [HR]–independent) vagal effect on left ventricular function in rabbits and showed that the direct vagal inotropic effect was negligible under a minimal level of sympathetic tone. They used ventricular end-systolic elastance (Ees), which is known to be least sensitive to changes in loading condition,3 as an index of contractility. The study by Matsuura et al,5 however, did not fully resolve the controversy because (1) volumetry with a conductance catheter might have introduced some imprecision in evaluating Ees; (2) they used rabbits, which are known to have smaller amounts of cholinergic ventricular receptors than do dogs, which are more widely used; and (3) they did not investigate the direct vagal effect on Ees under different levels of sympathetic tone.

To circumvent these problems, we developed a new method to study the effect of autonomic nerves on contractility using isolated, blood-perfused canine hearts with preserved autonomic nerves.6 Because we can control and measure ventricular volume precisely with this method, estimated Ees is more precise. In addition, we examined pure vagal as well as vagosympathetic interactive effects. The results indicated that the direct inotropic effect of the vagus was also minimal for the canine ventricle, and the concomitant sympathetic tone enhanced the vagal inotropic effect.

Methods

Animal Preparation

Animal care was in strict accordance with the guiding principles of the Physiological Society of Japan. A total of 21 isolated canine ventricles were studied. Adult mongrel dogs of either sex, weighing 15 to 28 kg, were tranquilized with ketamine hydrochloride (5 mg/kg IM) and anesthetized with pentobarbital sodium (25 mg/kg IV). While respiration was maintained with a volume respirator (SNA-480-6, Shinano), the arterial and venous lines to perfuse coronary circulation were connected to the left subclavian artery and right atrial appendage, respectively, through a midline sternotomy. The vascular connections of the heart were ligated, and the heart was excised after coronary perfusion was initiated using a supported dog. The left ventricle was vented. The left atrium was opened, and the mitral leaflets were surgically excised. A ring adapter was sutured to the mitral annulus.7 A fluid-filled balloon attached to the outflow port of the ventricular volume servo pump system was fitted to the left ventricular cavity and was used to measure and control ventricular volume. Left ventricular pressure was measured with a catheter-tipped micromanometer (SPC 350, Millar Instrument) positioned...
within the balloon. To preserve autonomic nerves, the heart was excised en bloc with trachea and esophagus. The upper and lower poles of the left stellate ganglia and the branches from the spinal cord were cut. The vagus was cut at the distal cervical level.

Data Acquisition
Left ventricular volume, pressure, and ECG were recorded on a multichannel recorder (Omnicorder SM14, NEC Medical Systems). These data were also digitized online at 200 Hz by a 12-bit analog-to-digital converter (AD12-16D98, Contec) interfaced with a laboratory computer (PC9821Ap, NEC). The digitized data were stored on a hard disk for subsequent off-line analysis.

Vagal and sympathetic stimulations were performed with a pair of bipolar needle electrodes by an electrical stimulator (SEN-7203M, Nihon-Koden) via an isolator (SS-2023, Nihon-Koden). The vagus was stimulated with pulses of 2 V in amplitude and 10 ms in width. At voltages greater than this, atrioventricular block occurred when the vagus was stimulated at 20 Hz. To increase Ees by approximately 25%, we stimulated the (sympathetic) stellate ganglia at 4 Hz with pulses of 1 to 3 V in amplitude and 10 ms in duration. We maintained nerve stimulation for 2 minutes to reach a new steady state before we measured HR and Ees. Then we stopped nerve stimulation for 2 minutes and, after full recovery, proceeded to the next stimulation.

Protocol
We first obtained the volume (V0) at which peak pressure was atmospheric (0 mm Hg) under the control condition. We evaluated Ees under different autonomic nervous tones by subjecting the ventricle isovolumic contraction at a fixed volume level and calculating the ratio of the peak pressure to the stressed volume (fixed ventricular volume − V0).

First, we examined the total (including direct and bradycardia-mediated) effect of unilateral vagal stimulation on Ees. We altered stimulation frequency at 5, 10, 15, 20, and 30 Hz and measured HR and Ees. The sequence of stimulation frequency was randomized. Second, we removed the bradycardia-mediated effect on contractility by applying fixed-rate left atrial pacing. We selected the pacing rate of 160 bpm. We altered stimulation frequency at 5 and 20 Hz and measured Ees. Third, we examined the vagosympathetic interaction in controlling contractility under a fixed-rate pacing condition. We measured Ees with left sympathetic stimulation (with which Ees increased by 25%), before and after the stimulation of unilateral vagus. We performed the vagal stimulation at 5, 10, and 20 Hz to clarify the differences of vagosympathetic interactions at the wide range of stimulation frequency.

Statistical Analysis
Results were expressed as mean±SEM. The responses of Ees to vagal stimulation were evaluated with 1-way ANOVA for repeated measures. To evaluate vasovagal interaction, we calculated the ratio of the Ees attenuated by vagal stimulation to the Ees enhanced by left sympathetic stimulation and defined the relative change of Ees under the condition without vagal stimulation as 0%. Differences in vagosympathetic interaction by vagal stimulation frequency under sympathetic stimulation were analyzed with 1-way ANOVA for repeated measures and with the Bonferroni’s method for multiple comparisons. *P<0.05 was considered statistically significant.

Results
Illustrated in Figure 1A are the pooled data of changes in HR and in Ees by right vagal stimulation without pacing (n=12). As the frequency of the stimulation was increased, both HR and Ees decreased. At 20-Hz stimulation, HR decreased by 27±3% and Ees decreased by 11±2%. Illustrated in Figure 1B are changes in HR and in Ees by left vagal stimulation (n=9). Heart rate and Ees also decreased as the frequency of the stimulation increased. The magnitudes of the changes in HR and in Ees were, however, smaller in left vagal stimulation than in right. At 20 Hz, HR decreased by 14±2% and Ees decreased by 6±2%.

In Figure 2A and B, we depict the pooled data of the direct (HR-independent) effect of Ees with the right (A) and left (B) vagal stimulation under a fixed-rate pacing condition. When bradycardia-mediated effect was removed, Ees was virtually constant in right (0.01±0.3% at 20 Hz; NS; n=12) and left vagal stimulation (0.3±0.4% at 20 Hz; NS; n=8). Figure 2C and D show the pooled data of the vagosympathetic interactions. With the left sympathetic nerve stimulation (4 Hz; 2.2±0.6 V, with which Ees increased by 24±4%), the graduated right (C; n=7) and left (D; n=9) vagal stimulation decreased Ees. Although the frequency dependences were not clear between 5 Hz and 10 Hz, between 10 Hz and 20 Hz,
there was significant difference between 5 Hz and 20 Hz in
vagal frequency dependency.

Discussion

Direct and Bradycardia-Mediated Vagal Negative Inotropism

It is well known that HR itself could alter ventricular contractility (force-frequency mechanism).4 It seems, therefore, natural that vagal stimulation could lower ventricular contractility through 2 different mechanisms, the direct effect on one hand and the indirect effect via force-frequency mechanism on the other hand. Our group has clearly indicated that in rabbit hearts, vagal stimulation lowers contractility mainly through force-frequency mechanism. This result could be reconciled with larger direct vagal inotropism demonstrated in dog hearts8 in 2 ways. Because rabbits are unique in having a low concentration of muscarinic receptors in the ventricle, the difference might merely be due to the difference between species. Another explanation is that in canine experiments, the higher sympathetic tone might have modified the results. To explore which was the main cause of the difference in results, we conducted the present study. Our results indicated that under minimal sympathetic tone, vagal negative inotropism was mainly via bradycardia in dogs, as well. We conjectured that the vagosympathetic interaction was the main reason for the difference in results.

Study Limitations and Physiological Implications

There were no autonomic nervous activities in the absence of stimulation, and autonomic nerves were disconnected and were perfused only via the coronary circulation in our preparation. Therefore, our results are not equivalent to physiological responses in an intact animal. However, our results with both vagal and sympathetic stimulation are similar to physiological responses in an intact animal with enhanced sympathetic tone, in patients with pheochromocytoma, and in patients medicated by β-stimulant drugs with bronchial asthma and normal heart.

The results of this study indicated that the vagal stimulation attenuated the enhanced contractility by sympathetic nerve stimulation but did not decrease contractility in the absence of sympathetic tone. In other words, the enhanced sympathetic tone could increase gain of Ees control by the vagus. We conjectured that the negative inotropic effects of the vagal nerves are large in enhanced sympathetic subjects, such as patients with pheochromocytoma and patients medicated by β-stimulant drugs. In these subjects, cardiac function might be more sensitive to the changes in the vagal tone.

Acknowledgments

This study was supported by Grants-in-Aid for General Scientific Research (No. 05454281, 05770505, 06770530, 06770531, 06770533) from the Ministry of Education, Science, and Culture of Japan; by a Research Grant for Cardiovascular Diseases (5A-3, 6A-4, 7C-2) from the Ministry of Health and Welfare of Japan; by a grant from the Science and Technology Agency, Encourage System of COE; and by a grant from Sankyo Foundation of Life Science.

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Circulation. 2001;104:2277-2279
doi: 10.1161/hc4401.099448

Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/104/19/2277

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