Effect of Constant and Intermittent Vagal Stimulation on the Heart Rate and Heart Rate Variability in Rabbits

Tetsu IWAO, Hidetoshi YONEMOCHI, Mikiko NAKAGAWA, Naohiko TAKAHASHI*, Tetsunori SAIKAWA, and Morio ITO

Department of Laboratory Medicine and * First Department of Internal Medicine, School of Medicine, Oita Medical University, Oita, 879-5593 Japan

Abstract: To examine whether the high-frequency (HF) component of heart rate variability (HRV) reflects fluctuation or tonic level of vagal outflow, we investigated the effects of vagal efferent nerve stimulation (VS) on the heart rate and HRV in anesthetized open-chest rabbit under artificial respiration at a rate of 52 breaths/min (0.86 Hz). A power spectral analysis was performed at baseline and during VS (stimuli at 2 ms, 1–10 V and 5–25 Hz). VS was applied using two different patterns. The first was constant VS; continuous stimulation at graded frequency or voltage to simulate changes in the level of vagal “tone.” The second pattern was intermittent VS; stimulation at 0.5 Hz of on-off cycle to simulate fluctuations in vagal efferent activity. The power spectrum at baseline showed a single narrow component at 0.86 Hz, identical to respiration rate. Both the constant and intermittent VS prolonged RR interval. The amplitude of the component at 0.86 Hz remained unaffected by either the constant or intermittent VS, whereas the latter evoked a distinct narrow component at 0.5 Hz, reflecting the on-off cycle of intermittent VS. Our results suggest that the HF component of the power spectrum of HRV measures the magnitude of fluctuations of vagal input associated with respiratory modulation. [Japanese Journal of Physiology, 50, 33–39, 2000]

Key words: heart rate variability, heart rate, autonomic nervous system, fluctuation.

Heart rate variability (HRV) has been used to evaluate cardiac autonomic activity and to predict the prognosis of patients with cardiovascular disease [1–11]. The power spectrum of HRV usually contains two major peaks. One is a high-frequency (HF) component (0.15–0.40 Hz) affected by respiratory modulation, and the other is a low-frequency (LF) component (0.04–0.15 Hz).

The HF component has been widely used as an index of cardiac vagal activity [2, 3, 9]. However, several studies have suggested that this component does not reliably measure vagal tone. Saul et al. [12] have reported that the respiratory component does not change with either increases or decreases in arterial blood pressure. Furthermore, Goldberger et al. [7] demonstrated that infusion of phenylephrine decreased HF in normal humans despite a significant prolongation of RR interval. These results lend support to the interesting theory proposed by Malik and Camm that HRV measures fluctuations in autonomic input to the heart rather than the mean level of autonomic “tone” [13]. However, this theory remains to be confirmed in in vivo studies since the technique used to alter vagal activity may affect both modulated vagal activity (fluctuation) and the level of vagal tone, and it is often difficult to separate the effects of these two activities on the power spectrum of HRV [2, 14]. Although there are a few studies that have examined the direct effects of vagal efferent nerve stimulation (VS) on HRV [14, 15], to our knowledge, no study has previously reported the effect of intermittent VS on HRV in the intact vagal nerve system. The present study was designed to investigate whether the HF component of HRV predominantly measures fluctuation or...
tone level in vagal outflow in rabbits with an intact nerve system using intermittent or constant VS.

METHODS

Animals. Adult Japanese white male rabbits (weight, 2.5 to 3.3 kg) were anesthetized with intraperitoneal secobarbital sodium (20 mg/kg) and injected with additional intravenous doses as needed to maintain a constant level of anesthesia throughout the course of the experiment. Animals were instrumented as described previously [16]. In brief, mechanical ventilation with room air was performed with a ventilator (SN-480-5, Shinano, Tokyo, Japan) through a tracheal cannula at a constant rate of 52 breaths/min (0.86 Hz). Adequacy of ventilation was confirmed by arterial blood gas analysis. The rectal temperature was maintained at 38 to 39°C with a heating pad. A saline-filled polyethylene catheter (4 Fr) was inserted into the femoral artery to record blood pressure (BP). A similar catheter was inserted into the femoral vein for drug administration.

After median sternotomy, the heart was exposed and suspended in a pericardial cradle. The standard limb-lead ECG (lead II) and femoral arterial BP were monitored. Monophasic action potentials (MAPs) from the left ventricular endocardium were recorded by the contact electrode technique as described previously [16].

The right vagus was carefully dissected out of the neck and cut. The distal end of the cut vagus nerve was stimulated using 2-ms square pulses at a frequency of 5 to 25 Hz and 1 to 10 V (SEN-7203, Nihon Kohden, Tokyo, Japan). Two different patterns for VS were used. In the first pattern, VS was applied continuously (constant VS, Fig. 1A). In the second pattern, VS was applied intermittently at a rate of 30/min (0.5 Hz, on-off frequency) (intermittent VS, Fig. 1B). The latter protocol consisted of alternating 1-s periods of stimulation and quiescence. All procedures conformed to the guidelines for animal experimentation of Oita Medical University, Japan, for the care and use of laboratory animals.

Experimental protocols. After a control recording was made for 3 min, one of the following three protocols was performed. In protocol 1, the effects of constant VS at three different frequencies (5, 10 and 25 Hz) were recorded (five rabbits). The stimulation voltage for VS at 10 Hz was set to achieve a 20 to 50% increase in the RR interval. In protocol 2, the
The effects of constant VS at a frequency of 10 Hz were determined at two stimulus voltages (five rabbits). The low-stimulus voltage was adjusted to achieve a 20 to 50% increase in the RR interval. An approximate doubling of the voltage was required for the high-level VS. The intensity of VS stimulation was altered by increasing the frequency as well as voltage. The latter increased the number of electrically-stimulated vagal efferent fibers and resulted in accelerated vagal stimulation because of incomplete separation of the vagal nerve fibers. In protocol 3, the effects of intermittent VS at 10 Hz were studied (five rabbits). The stimulus voltage was set to induce a 20 to 50% increase in the RR interval. Each pattern of VS was maintained for 3 min at a constant frequency and voltage to allow for stabilization of the response. A recovery period of 3 to 5 min was allowed after each 3-min VS before the subsequent VS was performed. Protocols 1 and 2 (constant VS) were designed to examine the effects of changing the level of vagal tone, while protocol 3 (intermittent VS) aimed to examine the effects of fluctuation (modulation) in vagal activity.

In three rabbits, the left and right vagal bundles were dissected out of the neck and the effects of bilateral vagotomy on HRV were examined.

**HRV analysis.** All recorded signals were stored on a PCM data recorder (RD-111T, TEAC, Tokyo, Japan) for off-line analysis. The MAP recordings were used for HRV analysis since the baseline drift in MAP recordings was much smaller than that of ECG (Fig. 1). MAP recordings were replayed off-line and digitized at a frequency of 500 Hz with an analog low-pass filter. We excluded the recordings indicative of instability, artifact or ectopic beats. The RR interval was measured as the interval between the upstrokes of successive MAPs. RR interval files were created from successive 2-min recordings. Spectral plots were computed in 2-min segments using a 512-point fast-Fourier transformation algorithm with a frequency resolution of 1/120. A Hanning windowing function was applied to minimize spectral leakage between segments without diminishing the frequency resolution. These files were used to compute the mean RR intervals and subjected to fast-Fourier transformation using commercially available software (PASHERVAR, Kissei Comtec Co., Ltd., Matsumoto, Japan) on a personal computer (PC-9801, NEC, Tokyo, Japan). We measured the power spectral components centered at the on-off frequency of the intermittent VS (0.40 to 0.60 Hz, component 1) and at the frequency of the artificial respirator (0.70 to 1.00 Hz, component 2).

**Statistical analysis.** Data are expressed as mean±SEM. Analyses of the amplitude of the power spectral components of HRV were performed using the natural logarithms of the data. Statistical analyses were performed by Student’s t-test or ANOVA. A p value less than 0.05 was considered statistically significant.

---

**Fig. 2.** Representative examples of the effects of constant vagal stimulation (VS) on the RR interval series (upper) and power spectrum for heart rate variability (lower). The power spectrum shows a single peak coincident with the respiratory frequency (0.86 Hz, component 2) during the control period (left lower) and VS (right lower). VS increased the mean RR interval from 250 to 280 ms, with minimal change in the amplitude of component 2 (comp 2).
RESULTS

Representative recordings of MAPs, ECG (II) and BP at the baseline and during constant or intermittent VS are shown in Fig. 1. At baseline, power spectra demonstrated a single narrow component centered at 0.86 Hz of the respiratory frequency (component 2; Figs. 2 and 3, left lower panels). This component shifted according to the change in the rate of artificial respiration and disappeared after bilateral vagotomy (data not shown). Constant VS prolonged the RR interval from 210 to 280 ms and induced a distinct component coincident with the on-off frequency of VS (0.50 Hz, component 1; right lower). However, the amplitude of component 2 remained almost unchanged. Comp 1, component 1; comp 2, component 2.

![Graphs showing effects of constant and intermittent VS on RR interval and spectral power of heart rate variability.](image)

**Fig. 3.** Representative examples of the effects of intermittent vagal stimulation (VS) on the RR interval series (upper) and the power spectrum for heart rate variability (lower). The power spectrum during the control period showed a single peak at the respiratory frequency (0.86 Hz, component 2) (left lower). VS increased the mean RR interval from 210 to 280 ms and induced a distinct component coincident with the on-off frequency of VS (0.50 Hz, component 1; right lower). However, the amplitude of component 2 remained almost unchanged. comp 1, component 1; comp 2, component 2.

**Fig. 4.** Effects of increasing the frequency of constant vagal stimulation (VS) on the RR interval and spectral power of heart rate variability. With increases in the frequency of VS from 5 to 25 Hz, the RR interval increased, but no significant change in the amplitude of components 1 and 2 was present. Values are mean±SEM (n=5). *p<0.05, **p<0.01 versus control.

**Fig. 5.** Effects of increasing stimulus voltage of constant vagal stimulation (VS) on the RR interval and spectral power of heart rate variability. With increasing stimulus voltage, the RR interval increased without a significant change in the amplitude of components 1 and 2. Values are mean±SEM (n=5). stim. voltage, stimulus voltage. *p<0.05, **p<0.01 versus control.
with increasing stimulus frequency and voltage on the RR interval and power spectra of HRV are summarized in Figs. 4 and 5. Constant VS significantly increased the RR interval in stimulus frequency- and voltage-dependent manners, but it did not induce significant changes in the amplitude of components 1 and 2. The effects of intermittent VS are shown in Fig. 6. Intermittent VS significantly increased the RR interval and amplitude of component 1, while the amplitude of component 2 showed no significant change.

**DISCUSSION**

The major finding of the present study was that intermittent but not constant VS increased the amplitude of the HF component (0.4–1.0 Hz) and produced a new peak (component 1) centered at the on-off cycle of intermittent VS but no significant change in the component reflecting respiratory modulation (component 2). These results suggest that the HF component in HRV may not reflect the intensity of VS or probably vagal tone, irrespective of the presence of an intact or denervated autonomic nerve system. In contrast, Kamath *et al.* [10] have reported that continuous VS for more than several hours increased HF power, but did not prolong the RR interval in only one patient with intractable epilepsy. The observed discrepancy may be attributable to setting, including anesthesia, denervation or stimulation protocol.

Malik and Camm [13] proposed a working hypothesis that the spectral power of HRV reflects the modulation (fluctuation) of vagal activity rather than the level of vagal tone. To test this, we examined the effects of fluctuation in vagal efferent nerve activity on HRV by intermittent VS. The present study with intact vagi in rabbits demonstrated that intermittent VS resulted in the appearance of a discrete component centered at the on-off cycle of intermittent VS (component 1), and produced no significant change in the respiration-induced component of the HRV power spectrum (component 2). These results were consistent with previous studies in denervated dogs and healthy humans. Odemuyiwa *et al.* [15] showed in denervated dogs that when the instantaneous stimulation frequency oscillated between 4 and 17 Hz during a 5-s period (similar to 0.2 Hz on-off frequency), stimulation of the efferent vagal nerve markedly increased HF power with a peak value corresponding to the frequency of modulation (0.20 Hz). Furthermore, Piepoli *et al.* [17] examined respiration- and baroreceptor-induced HRV separately in healthy volunteers by setting respiration and neck suction rates at distinct frequencies (0.25 and 0.20 Hz, respectively) and found that neck suction (i.e., manipulation of baroreceptor activity) caused the appearance of a distinct spectral component at 0.20 Hz, while the respiration-induced 0.25 Hz component remained unaffected. Thus, the HF component in the HRV power spectrum reflects the magnitude of fluctuations in efferent vagal input to...
the heart, originating from any level of the vagal nerve system such as afferent, central and efferent nerve levels. Fluctuations in efferent vagal nerve outflow may also be independent and additive to each other as suggested theoretically [13].

The HF component of HRV is thought to provide a quantitative index of cardiac vagal tone because it decreased by treatment with parasympathetic blockade [18, 19]. When the inspiratory level of cardiac activity diminishes to zero, the amplitude of HF component can reflect the expiratory level of vagal tone. Our results do not exclude the above concept and the possibility that the magnitude of modulation in vagal outflow and vagal tone are positively related in a number of physiological conditions. However, one may consider that this concept is not always true in certain pathological conditions [20].

The present study with the intact autonomic nerve system in rabbits may raise the consideration of interaction between vagal and sympathetic activity, as Kawada et al. [11, 21] recently demonstrated that tonic and dynamic stimulation of sympathetic and vagal systems bidirectionally augmented heart rate regulation. This interaction may contribute to the small graded increase in the amplitude of component 1 noted in constant VS. However, the interaction between the two components of the autonomic nervous system is expected to be smaller in low-voltage than high-voltage stimulus since graded RR prolongation by VS produces a graded reflex attenuation of sympathetic activity. We examined the effects of intermittent VS on the HRV power spectrum at a setting of low-voltage stimulus since graded RR prolongation of the RR interval, and thereby resulted in no significant blood pressure reduction. Hence, it is unlikely that the interaction between vagal and sympathetic activity critically influences our conclusions.

Clearly, our study has few limitations. Anesthesia has been shown to alter cardiac autonomic activity and HRV [12, 22]. Moreover, open-chest procedures and artificial respiration may also alter the autonomic control of the heart. However, Halliwell and Billman [8] reported that pentobarbital anesthesia caused little change in vagal control of the heart. Secobarbital, used here, may exert similar effects. The presence of component 2 modulated by respiration at baseline suggests that the vagal nerve system may be functional, at least in part, in the present preparation. Hence, it seems that the present results could not be attributed to the masking effect of anesthesia alone.

In conclusion, the present study demonstrated that the HF component of the HRV power spectrum increased with intermittent VS, but not with constant VS. Our results suggest that the HF component of the power spectrum of HRV measures the magnitude of fluctuations reflecting respiratory modulation.

REFERENCES


