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Short Communication

Vagal stimulation and cardiac chronotropy in rats

Gary G. Berntson, Karen S. Quigley, Vincent J. Fabro and John T. Cacioppo

Department of Psychology, Ohio State University, Columbus, Ohio, USA

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Abstract

Increasing frequencies of electrical stimulation of the right vagus nerve in the rat yielded a progressive lengthening of heart periods. Stimulation was capable of driving vagal control of cardiac chronotropy over its full physiological dynamic range, to the point of sinus block. The steady-state transfer function between vagal stimulation frequency and cardiac chronotropy was approximately linear, with a slope of 7.4 ms/Hz. The linearity of the stimulation-heart period function is consistent with previous reports in dogs, rabbits and humans, although the slope of the function was considerably lower in the rat.

Since the classic studies of the Weber brothers in the 1800s, it has been recognized that electrical stimulation of the vagus nerve yields a characteristic slowing of the beat of the heart. With some exceptions [21], a quasi-linear relationship between vagal stimulation frequency and heart period has been observed in humans, rabbits and dogs [3–5,7,12,14,18,19]. Moreover, a relatively linear function has been reported for the relationship between heart period and endogenous vagal activity in the dog [10,11]. According to the quantitative model of Dexter et al. [4], this linearity arises from two underlying nonlinear processes. Specifically, the model maintains that accumulation of acetylcholine (ACh) at cardiac effector synapses is a negatively accelerating function of vagal activity, while the effect of ACh on cardiac chronotropy is a positively accelerating function of concentration. The result of these two nonlinear processes approximates the observed linear relationship between vagal frequency and heart period. This linearity may thus be adventitious, and contingent on a symmetry in the forms of the underlying nonlinear processes.

Since functions relating nerve activity to ACh release and ACh levels to chronotropic effects probably vary with species, there may be no single relationship between central vagal outflow and cardiac chronotropy that applies universally across species. Moreover, while linear functions relating stimulation frequency to heart period have been found across species, the slopes of these functions differ from species to species. While most systematic vagal stimulation studies have employed the dog, the rat is increasingly being used in cardiovascular research, especially for studies of central mechanisms. Moreover, the results of these studies are often expressed in measures of

Correspondence to: G.G. Berntson, Ohio State University, 1885 Neil Avenue, Columbus, OH 43212, USA.

cardiac effects, rather than autonomic outflows. For these reasons, it is increasingly important to clarify the relationship between vagal activity and functional effects on the rat heart. In the present study, we examined the chronotropic effects of vagal stimulation in the rat over a wide range of stimulation frequencies.

Experiments were performed on five Sprague-Dawley rats (90–120 days of age, 250–450 g; Zivic Miller, Zellenople, PA). Experimental animals were anesthetized with urethane (1.4 g/kg), and supplemented if necessary. Subjects breathed spontaneously throughout the procedures. The left common carotid artery of each animal was cannulated (Vascular Access Port, Model SLA, Norfolk Medical Products, Skokie, IL), the left vagus nerve was transected, and the right vagus was isolated by careful blunt dissection. The

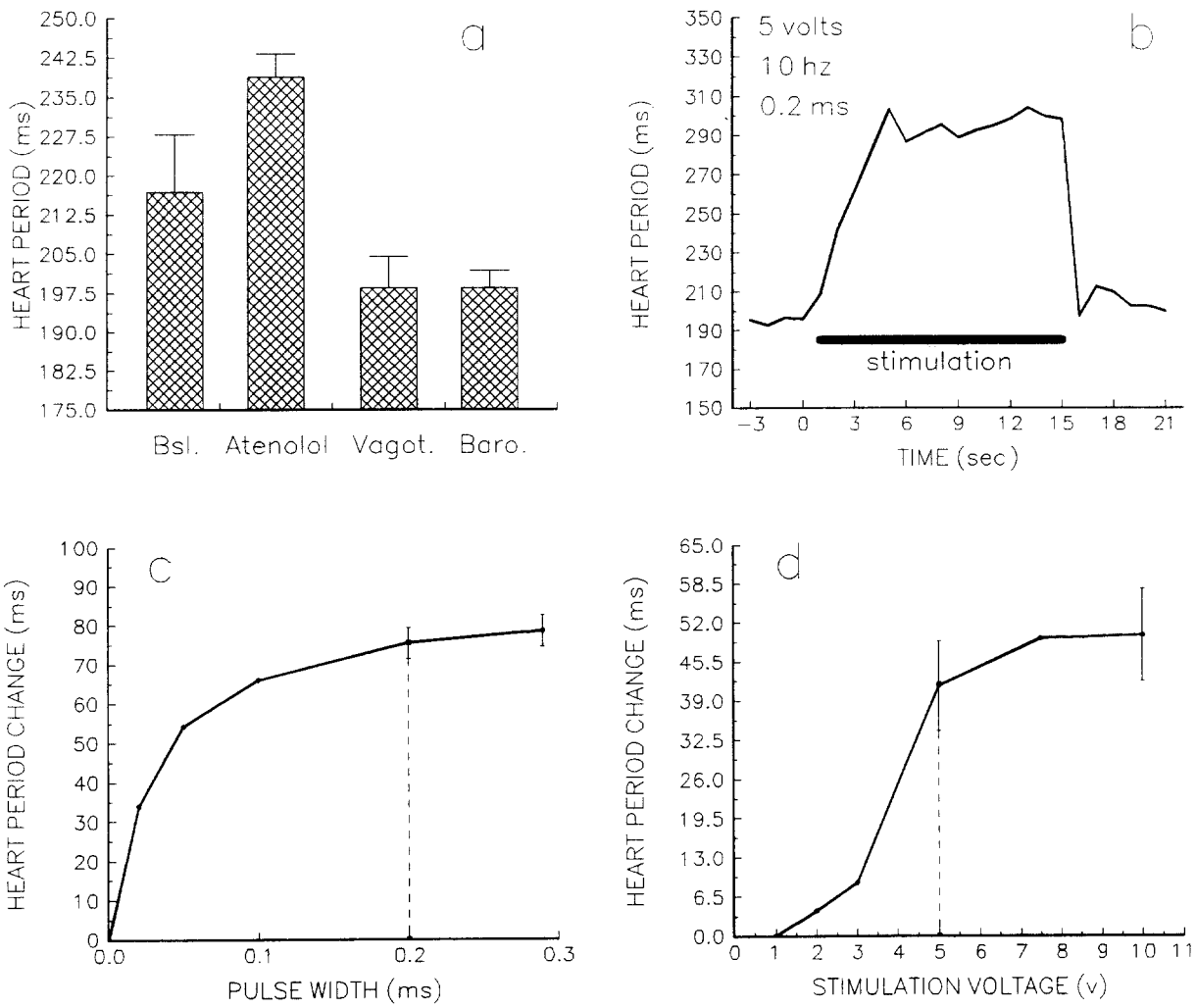

Fig. 1. **(a)** Basal heart period after experimental manipulations (means and standard errors, $n = 3$). Bsl: Mean heart period under urethane anesthesia and after left vagal transection. Atenolol: Mean heart period in the same animals after subsequent administration of atenolol (10 mg/kg). Vagot: Mean heart period after section of the right vagal nerve. Baro: Heart period change after nitroprusside-induced hypotension. **(b)** Time course of heart period change during stimulation of the right vagus nerve at an intermediate frequency. **(c)** Heart period change during vagal stimulation, as a function of stimulus pulse width (5 volts at 10 Hz). **(d)** Heart period change during vagal stimulation as a function of stimulation voltage (0.2 ms pulse width at 10 Hz).

catheter was coupled to a Spectramed TNF-R pressure transducer (Spectramed, Oxnard, CA), and then to a Grass Model 7 polygraph. ECG signals were recorded via transthoracic Grass subdermal electrodes. Both signals were recorded on chart paper (30 mm/s) and, for time-critical measures, were digitized (Metrabyte DAS-8, 500 Hz, 12 bit) and stored for subsequent analysis.

Baseline heart period and blood pressure were recorded after cannulation and section of the left vagal nerve. To minimize confound from sympathetic control of the heart, the animals received the β_1 receptor antagonist atenolol (10 mg/kg, SC), and physiological measures were again recorded. Atenolol was employed because of its cardioselectivity, minimal direct effects on the heart, and minimal central actions [1,6]. At that point, the right vagal nerve was ligated and sectioned, and basal measures were again obtained. The distal end of the transected right vagus was placed over a stainless steel hook electrode (0.2 mm diameter), and the stimulation field was drenched with mineral oil to prevent tissue drying and to minimize spread of stimulation. Monophasic cathodal pulses from a Grass Model S6 stimulator (Grass Instruments, Quincy, MA) were delivered via the stimulation electrode, referenced to a remote indifferent.

As illustrated in Fig. 1a, administration of atenolol resulted in the expected increase in heart period, whereas subsequent sectioning of the remaining right vagal nerve yielded the expected decrease. Atenolol produced an effective blockade of sympathetic chronotropic control, as documented by the administration of nitroprusside at the conclusion of the vagal stimulation regimen.

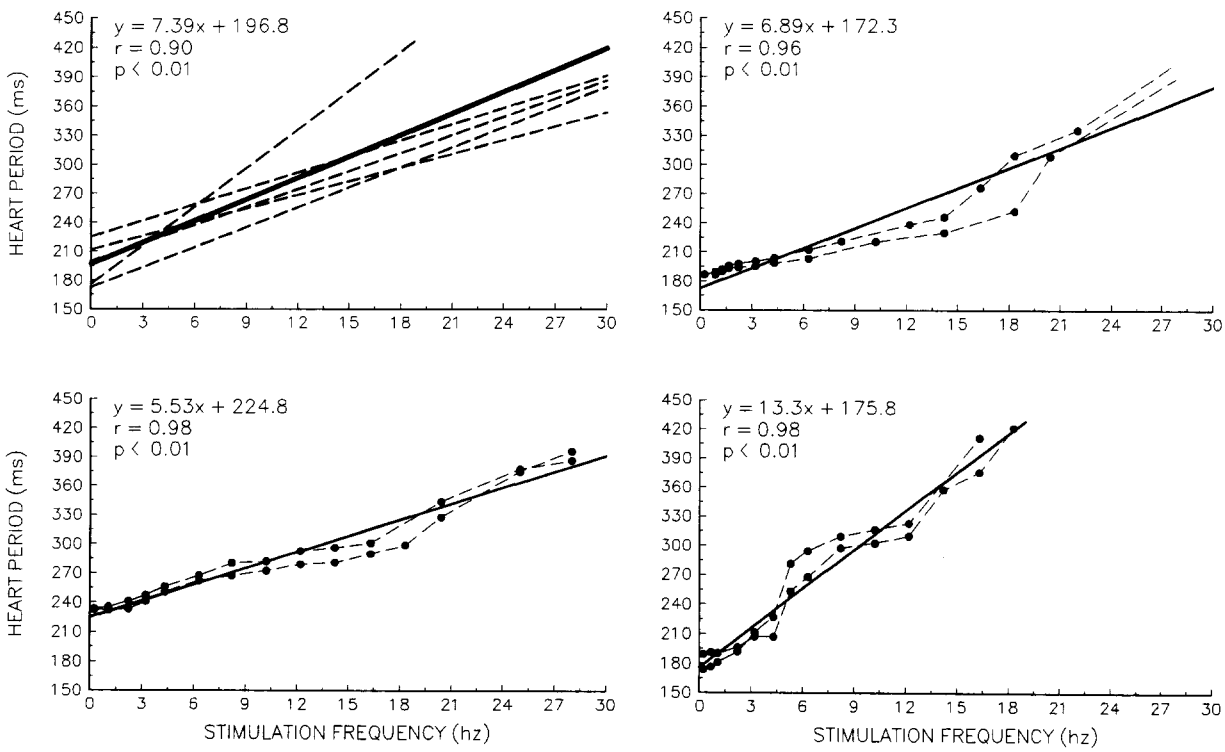

Fig. 2. Heart period response functions during vagal stimulation. Upper Left Panel: Individual (dashed) and overall (solid) regression functions relating heart period to vagal stimulation frequency. Remaining panels: Illustrative data from three animals for whom replicate stimulation series are available. Individual dotted lines depict results from the two separate stimulation series, and the solid line illustrates the linear best fit line. Stimulation consisted of 0.2 ms monophasic cathodal pulses at 5 volts.

Although nitroprusside resulted in a notable decrease in mean arterial pressure (mean = −45.5 mmHg), no baroreflex-mediated increase in heart period was observed (Fig. 1a).

As shown in Fig. 1b, vagal stimulation yielded a rapid lengthening of heart period that commenced within the first second of stimulation, approached asymptotic levels within 3–4 s, and rapidly returned to baseline after termination of stimulation. For the experimental testing series, vagal stimulation was delivered over 15-s epochs, with continuous recording of cardiovascular measures. Heart periods were allowed to return to baseline between stimulations. Stimulation was delivered at a voltage (5 v) and pulse duration (0.2 ms) that yielded asymptotic effects in preliminary studies (Fig. 1c,d). A wide range of stimulation frequencies was employed, extending from below threshold (0.2 Hz) to a level at which sinus block or other severe arrhythmias were manifest (maximum = 30 Hz). For three of the subjects, two separate stimulation series were given to evaluate replicability of the results and maintained integrity of the vagal nerve. For analysis, mean heart period and blood pressure were determined from 10–15 s after stimulation onset, and from the 5 s period immediately prior to stimulation.

Increasing frequencies of vagal stimulation yielded progressive increases in heart period. The relationship between heart period and stimulation frequency was closely approximated by linear functions in all subjects (to a level where sinus block or severe arrhythmias were apparent). Figure 2 illustrates the regression functions for each subject, and the overall regression function. Also plotted are individual data for the three subjects who received two stimulation series, illustrating the high replicability of the results. Although distinct nonlinearities were apparent in the heart period functions, the regression R^2 (mean = 0.97 ± 0.02 S.D.), revealed that linear trends accounted for 93–99% of the heart-period variance for all five animals. As generally reported, vagal stimulation also produced a progressive decrease in mean arterial pressure with increasing stimulation frequencies (mean at maximal stimulation frequencies = −38 mm Hg). This was attributable to a modest decrease in systolic pressure, and a more extreme decline in diastolic levels over the extended interbeat intervals.

As illustrated in Fig. 2, baseline heart periods of the subjects differed somewhat, and some subject-to-subject variation was seen in the slope of the frequency-heart period functions. Nevertheless, the overall regression function of heart period on stimulation frequency across all animals accounted for approximately 80% of the variance in heart period data ($F = 176.5$, $df = 1,45$, $P < 0.01$). Moreover, despite differences in baseline heart period, there was a striking consistency across animals in the heart period level at which heart block or other severe arrhythmias became apparent (390–420 ms). It is of note that this approximates the lowest survivable heart rate obtainable by potent reflexive manipulations (e.g. dive reflex) in the intact rat [9,15]. Thus, it appears that stimulation of the right vagus is capable of driving the full dynamic range of vagal control of the heart in this species. In fact, higher frequency stimulation could yield (arrhythmic) heart periods as long as 860 ms. These extremes are outside the normal physiological range, however, and are not illustrated in Fig. 2.

The present results are in general accord with previous vagal stimulation studies in dogs, rabbits and humans [3–5,7,18,19], which found an approximately linear increase in heart period with increasing stimulation frequency, up to arrhythmic or asymptotic levels. While the vagal influence on cardiac chronotropy can be approximated by a linear function across diverse species, differences in the slope of these functions are apparent. Previous reports indicate a reasonable correspondence in the slope of the regression functions for stimulation of the left vagus in humans (26 ms/Hz [3]) and dogs (19 ms/Hz [18]), with stimulation of the right vagus in both species yielding considerably higher slopes than left vagal stimulation (26–38 ms/Hz in the dog [7,18]). In contrast, the rabbit evidences a somewhat lower slope (11–16 ms/Hz [5,19]), which is not consistently different for the right and left nerves [19]. Finally, the slope of the function for right vagal stimulation in the rat (7.4 ms/Hz) is substantially lower than any of these species. It is unlikely that

the lower slope in the rat was attributable to suboptimal stimulation or to an unstable nerve preparation, because (a) asymptotic stimulation voltage and pulse durations were employed; (b) results were replicable across stimulation series; and (c) the full dynamic range of control was obtained in most animals. Rather, species differences in slope appear to be systematically related to basal heart period, with increasing basal heart period being associated with higher slopes of the stimulation-frequency × response functions. Thus, while slope estimates across species vary by over five-fold, a unit (Hz) change in stimulation frequency yields a more consistent change in heart period across species when expressed as a percentage of basal values (approximately 3–7%).

Phasic or burst patterns of vagal firing associated with cardiac, phrenic, or other rhythms may introduce additional complexities in the transfer functions relating vagal frequency to heart period change [2,11,13,16,20]. Thus, a discrete vagal stimulus can attenuate the chronotropic effect of a second stimulus, and this attenuation may extend for up to 20–30 s [16]. Moreover, entrainment of the sinus rhythm can be seen with stimulation frequencies close to the basal heart period [13]. While both of these effects would be expected within the range of stimulation frequencies employed in the present study, they did not appear to introduce appreciable nonlinearities in the heart period functions. Burst-like patterns of vagal activity in the intact animal, and their timing in the cardiac cycle, would certainly be expected to have an impact on the control of cardiac chronotropy [13,17,20]. Unless the temporal distribution of these burst patterns is explictly altered by experimental or contextual variables, however, the burst-like character of vagal discharge would be expected to manifest primarily in the slope, rather than the shape, of the transfer functions. Thus, direct physiological recordings in the dog have revealed a rather close linear relationship between endogenous vagal activity and cardiac chronotropy, over a wide range of heart period fluctuations [11].

Many physiological influences over the chronotropic state of the heart evidence a sigmoidal response function, with a dynamic range that is considerably more restricted than that obtainable with direct vagal stimulation. An example is the baroreflex in the rat, where the vagal contribution is a sigmoidal function of blood pressure, with a dynamic range of approximately 70 ms [8]. In contrast, direct vagal stimulation in the present study yielded an essentially linear function over a dynamic range of approximately 200 ms. These results indicate that the sigmoidal plateaus of the baroreflex function are not attributable to dynamic limits on vagal control of the heart. Rather, the baroreflex sigmoid appears to arise from nonlinearities in the baroreceptors and/or the central integrative substrates.

In summary, right vagal stimulation in the rat is capable of driving vagal control of cardiac chronotropy over its full physiological dynamic range. The relationship between vagal stimulation frequency and cardiac chronotropy in this species can be approximated by a linear function with a slope of 7.4 ms/Hz. Although vagally-mediated reflex influences on the chronotropic state of the heart may evidence sigmoidal activation functions with a limited dynamic range, it appears that these functions arise largely from constraints of the reflex networks rather than limitations of vagal cardiac effectors.

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