Autonomic cardiac control. II. Noninvasive indices and basal response as revealed by autonomic blockades

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Abstract

Heart period, systolic time intervals, low and high frequency heart period variability, blood pressure, and respiration were measured in female subjects under three drug conditions (saline, atropine sulfate, metoprolol) while sitting and standing on three consecutive days. Following preinfusion baseline recordings, saline, metoprolol (14 mg), or atropine sulfate (2 mg) was infused for 15 min (by using a double-blind procedure). Recordings were taken during a postinfusion baseline and in response to an orthostatic stressor (standing versus sitting postures). At the end of the metoprolol session, atropine sulfate was infused and responses were monitored during the postinfusion (i.e., double blockade) baseline and during orthostatic stressor. Analyses of the blockade data revealed that the preejection period (PEP) reflected sympathetic but not vagal influences on the heart, and high frequency (HF, 0.12–0.40 Hz) heart rate variability (respiratory sinus arrhythmia) reflected vagal but not sympathetic influences on the heart. No other measure provided a specific index of the tonic sympathetic or vagal activation of the heart. Postinfusion PEP under saline predicted individual differences in postinfusion cardiac sympathetic activation, whereas postinfusion heart period (but not HF variability) under saline predicted individual differences in postinfusion cardiac vagal activation.

Descriptors: Autonomic nervous system, Autonomic blockade, Cardiac activity, Heart rate variability, Impedance cardiography, Posture, Preejection period, Respiratory sinus arrhythmia

Autonomic blockades have provided an important means of examining the autonomic control of the heart as well as the validity of noninvasive indices of cardiac control. We investigated the basal autonomic control of the heart in a sample of young healthy women using a within-subjects design, single and double autonomic blockades, and cardiac indices based on heart period variability and systolic time intervals. Our goals were to (a) determine the specificity of systolic time intervals (e.g., preejection period [PEP], electromechanical systole [EMS], and PEP/left ventricular ejection time [LVET]) and low-frequency heart period oscillations as indices of the sympathetic input to the myocardium, (b) verify the specificity of high-frequency heart period fluctuations associated with respiratory sinus arrhythmia (RSA) as an estimate of the parasympathetic control of the heart, (c) quantify the sympathetic and parasympathetic influences on these noninvasive indices and the potential systematic biases in these estimates associated with pharmacological blockades, and (d) quantify basal autonomic control of the heart as a function of postural state and investigate the sensitivity of noninvasive indices to changes in the autonomic control across postures (sitting vs. standing).

Measures based on heart period variability and systolic time intervals were selected because they represent two of the most widely used classes of psychophysiological measures for assessing autonomic contributions to cardiac function. The sinoatrial node of the heart is innervated by both sympathetic and parasympathetic fibers, although notable differences exist in the temporal dynamics and frequency dependencies of these autonomic innervations. Given these temporal dynamics, indices based on the magnitude of specific periodicities in cardiac interval changes have been used to gauge the relative vagal or sympathetic contributions to cardiac chronotropy. Berntson, Cacioppo, and Quigley (1993b) and Grossman and Kollai (1993) recently summarized neurophysiological evidence regarding the origins of respiratory frequency variability in heart period and their impli-

cations for psychophysiological measures of vagal chronotropic control.

Respiratory-frequency rhythms are clearly apparent in cardiac nerves of both autonomic branches (e.g., Koizumi, Terui, & Kollai, 1985; Richter & Spyer, 1990). These rhythms arise in large part from the conjoint action of a central respiratory generator and afferent pulmonary stretch receptors that impose or gate phasic excitatory and inhibitory influences on both vagal and sympathetic motor neurons (Berntson et al., 1993b). Despite the presence of respiratory rhythms, sympathetic contributions to RSA are minimal (e.g., Akselrod, Gordon, Shannon, Barger, & Cohen, 1981; Anrep, Pascual, & Rossler, 1936; McCabe, Yongue, Ackles, & Porges, 1985). The strong predominance of vagal contributions to high-frequency variability is attributable in large part to the low-pass frequency characteristics of the sympathetic cardioeffector synapses, which greatly attenuate sympathetically mediated heart period variations in the high-frequency ($>0.12\,\text{Hz}$) range (Berger, Saul, & Cohen, 1989; Berntson et al., 1993b).

There is a growing body of evidence concerning the vagal contributions to RSA. Grossman and Kollai (1993) recently reported, however, that resting RSA may not predict interindividual variation in cardiac vagal tone, as defined by the change in heart period after atropine. These authors further argued that intraindividual variations in respiration can confound the association between RSA and cardiac vagal tone. These findings raise important issues regarding the sensitivity and range of validity of RSA as an index of cardiac vagal control.[1] Pharmacological blockades, however, can yield biased autonomic estimates (e.g., due to interactions among the autonomic branches at the level of the organ, indirect or reflexive alterations in the unblocked branch, or nonselective actions of the blocker agents) and may obscure relationships between cardiac vagal control and RSA. Therefore, we reexamined this relationship using a measure of cardiac vagal control that permits assessment of potential biases (Berntson, Cacioppo, & Quigley, 1994b).

Although sympathetic contributions to high-frequency heart period variability are minimal, both sympathetic and vagal cardioeffector synapses pass low-frequency rhythms to the sinoatrial node. Accordingly, low-frequency heart period variability has been linked to sympathetic outflows, parasympathetic outflows, or both (e.g., Akselrod et al., 1981; Grossman, Karemaker, & Wieling, 1991; Láng & Szilágyi, 1991; Pagani, Rimoldi, & Malliani, 1992). The ratio of low-frequency to high-frequency variability has also occasionally been used to index the relative contributions of the autonomic branches to cardiac control (see review by Láng & Szilágyi, 1991). Although the computation of a ratio based on low-frequency and high-frequency heart period variability has had some predictive success in clinical populations (e.g., Lombardi et al., 1987), its efficacy as an index of sympathovagal balance over basal cardiac control in a healthy young adult population has not been established.

The interpretation of the systolic time intervals has been thought to be less ambiguous with respect to their autonomic

origins because the ventricular myocardium is innervated primarily, though not entirely, by the sympathetic nervous system (Randall, Randall, & Ardell, 1991). The systolic time intervals include the PEP, LVET, and EMS. Of these intervals, PEP has received the most attention in psychophysiology (e.g., Allen, Obrist, Sherwood, & Crowell, 1987; Cacioppo, Uchino, & Berntson, 1994; Light & Obrist, 1983; Sherwood, Allen, Obrist, & Langer, 1986). Abbreviations in PEP accompany increases in heart rate (HR) that occur as a result of adrenergic cardiostimulation but not increases in HR that occur as a result of vagal blockade or atrial pacing (Harris, Schoenfeld, & Weissler, 1967). Studies further suggest that HR per se does not influence the PEP unless changes in HR are associated with inotropic changes or are accompanied by changes in preload or afterload (Lewis, Leighton, Forester, & Weissler, 1974). PEP also decreases following the infusion of sympathetic agonists and shows strong correlations with noninvasive indices of contractility (Ahmed, Levinson, Schwartz, & Ettinger, 1972; Walsh, Crawford, & O'Rourke, 1982) and circulating norepinephrine (Cousineau, LaPointe, & de Champlain, 1978). Harris et al. (1967) reported that infusions of isoproterenol, epinephrine, and norepinephrine each produced a shortening of PEP, although the effects of catecholamines on PEP can be complex because of their alpha- and beta-adrenergic stimulating properties (Lewis et al., 1974). Thus, sympathetic (β-adrenergic) activation of the myocardium shortens PEP, but increases in preload (ventricular filling) and decreases in afterload (aortic diastolic pressure) can also shorten PEP (Lewis, Rittgers, Forester, & Boudoulas, 1977). Left ventricular ejection time and EMS are abbreviated by epinephrine infusion (Salzman, Wolfson, Jackson, & Schechter, 1971), but these measures are also affected by preload and afterload (see reviews by Binkley & Boudoulas, 1986; Láng & Szilágyi, 1991). We therefore examined each of the systolic time intervals and five derived measures that have been used in studies of left ventricular function in cardiology (i.e., rate-adjusted PEP, LVET, and EMS indices and the ratio PEP/LVET; see Binkley & Boudoulas, 1986). Furthermore, we examined these cardiac indices during a postinfusion baseline and in response to an orthostatic stressor to insure that a wide range of autonomic influences would be produced during the experiment (Berntson et al., 1994b; Berntson, Cacioppo, Quigley, & Fabro, 1994d).

Although pharmacological blockades can help illuminate the underlying autonomic origins of cardiac indices, systematic biases in estimates of the contributions of the autonomic branches can arise both from methodological and physiological factors (e.g., due to interactions among the autonomic branches, indirect or reflexive alterations in the unblocked branch, or nonselective actions of the blocker agents). In a previous paper, we developed autonomic estimates based on data from four drug conditions (saline, parasympathetic blockade, sympathetic blockade, and dual blockade), which allow quantification of systematic biases (Berntson et al., 1994b). In the present paper, we apply these blockade analyses to derive criterion estimates of autonomic control against which noninvasive measures can be evaluated.

Methods

Subjects

Thirteen healthy (white) female undergraduate students (age [$M \pm SEM$]: 22.5 ± 0.8 years; height: 164.9 ± 1.8 cm; weight:

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[1] The concept of cardiac vagal tone has been used variously in the literature. Some authors have considered RSA a relatively direct index of vagal tone. Others (e.g., Grossman & Kollai, 1993) have considered vagal tone as the mean DC level of vagal influence, as derived from vagal blockade. The criterion autonomic estimates of the present study (derived from blockades) are in keeping with the latter view. To avoid ambiguity with the concept of vagal tone, however, we use the more general phrase "cardiac vagal control" to refer to both tonic and phasic influences.

59.3 ± 2.1 kg) qualified for participation in the study, provided informed consent, and were each paid $75 for their participation. Subjects were screened for health conditions using a health history questionnaire. The inclusion criteria were (a) no acute illness nor history of chronic illness, (b) moderate weekly exercise, (c) nonsmoker, (d) no current nor chronic use of any over-the-counter, recreational, or prescription drug, except as noted below, (e) low to moderate weekly alcohol intake (i.e., ≤5 drinks/week), (f) within 20% of ideal weight for height, (g) offspring of normotensive parents, (h) no needle or speech phobia, (i) English as the first language, and (j) no academic exam scheduled during the 3 testing days. Because pharmacological blockades may adversely affect a fetus, a final inclusion criteria was the regular use of a prescription birth control agent and a negative pregnancy test given the morning of the first session.

Subjects were scheduled using a forward tracking procedure to participate in the study during the follicular phase of the menstrual cycle. Each subject participated in the study on three consecutive afternoons during the week. Subjects were asked to refrain from drinking alcohol for 48 hr prior to the study, to use neither excessive nor limited caffeine relative to their normal daily intake,[2] and to get a normal night of sleep prior to each of the three sessions. On the morning of the first session, each subject was given a pregnancy test and a brief physical examination to verify that the subject was normotensive, in good health, and not pregnant. All subjects were informed of possible side effects of the pharmacological agents and of their right to withdraw from the study at any time and then signed an informed consent document. Two of the 13 subjects who qualified for participation complained of adverse side effects from the atropine sulfate and discontinued their participation in the study prior to its completion. These subjects were paid for their participation, but their data were not reduced or analyzed. In addition, technical difficulties precluded completion of the protocol for one subject. Thus, 10 subjects completed the experiment.

Procedure

Subjects who qualified for the study were tested on 3 separate days prior to and following infusion of either saline (control) vehicle, a fixed 14-mg dose of metoprolol (approximately 0.24 mg/kg), or a fixed 2-mg dose of atropine sulfate (approximately 0.034 mg/kg). Metoprolol, a β_1 antagonist, served to block sympathetic control of the heart, whereas atropine sulfate, a muscarinic antagonist, was used to block the parasympathetic control of the heart. The dosages were selected from the literature to achieve relatively complete autonomic blockade while minimizing nonselective actions and side effects of the drugs. Although competitive autonomic blockades can never be absolute, the dosages employed were sufficient to block the heart period effects of agonist administrations and potent autonomic reflexes (see review by Berntson, Cacioppo, & Quigley, 1993a).

Double-blind procedures were used in the administration of these agents, and the order of drug administration was counterbalanced across days and subjects. On the day subjects received metoprolol, they received an additional 2 mg of atropine sulfate following the completion of the normal protocol (dual blockade condition).[3]

Following venipuncture at each session, subjects sat quietly for 30 min to allow adaptation to the laboratory, and initial baseline recordings were made during the final 3 min of this adaptation period. Saline, metoprolol, or atropine sulfate was then infused for 15 min (using a double-blind procedure) while heart period and blood pressure were monitored. Recordings were then taken for 3 min to obtain a postinfusion baseline. Recordings were then obtained during 3-min periods while subjects were standing and while they were seated. The order of postural testing was counterbalanced across subjects, and 30 s were allowed after the assumption of a given posture before baseline measures were initiated. Subjects were next exposed to three psychological stressors (over approximately 48 min), the results of which are reported in Berntson et al. (1994a). Following completion of all tasks on the day subjects received metoprolol, subjects received an additional 2 mg of atropine sulfate to achieve dual autonomic blockade (at this point, the experimenter became aware of the drug condition). The dual blockade condition allowed determination of each individual's intrinsic heart period (in the absence of autonomic control) and the efficacy of the autonomic blockades. The subject sat quietly for 15 min while heart rate and blood pressure were monitored. Recordings were then taken for 3 min to obtain a postinfusion (dual-blockade) baseline and for 3-min periods while subjects were standing and seated. Again, the order of postural testing was counterbalanced across subjects, and 30 s were allowed after the assumption of a given posture before physiological measures were initiated.

Noninvasive Measures

A Minnesota Impedance Cardiograph (Model 304B) was used to measure electrocardiogram (EKG), basal thoracic impedance (Z_0), and the first derivative of the impedance signal (dZ/dt). Disposable EKG spot electrodes were placed in the tetrapolar configuration (Qu, Zhang, Webster & Tomkins, 1986; Sherwood, Royal, Hutcheson, & Turner, 1992). Although band electrodes provide more accurate magnitude measures of cardiac output values, the systolic time intervals can be measured with approximately equal accuracy using band or spot electrodes (Sherwood et al., 1992). The two outer (current) electrodes were placed over the fourth cervical vertebra and the ninth thoracic vertebra, and the two inner (recording) electrodes were placed 4 cm above the clavicle and over the sternum at the fourth rib. A 4 mA AC current at 100 kHz was passed through the two outer electrodes, and Z_0 and dZ/dt were recorded from the two inner electrodes. The EKG, Z_0, and dZ/dt signals were digitized at 500 Hz, and interbeat intervals were derived from a custom software package.[4]

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[2]Caffeine intake for the 24-hr period prior to testing was obtained for each session. Only three subjects reported any caffeine use, and only in minimal quantities (one or two cups). Caffeine intake probably did not influence the results, because the preliminary setup phases of the study (establishment of an intravenous line, electrode placements, adaptation period, instructions) required over 1 hr. The effects of caffeine, even if ingested immediately prior to the session, would be expected to dissipate over this period of time.

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[3]The dual blockade was tested at the end of the day in which subjects were infused with metoprolol because the time course of metoprolol is longer than that of atropine.

[4]We thank Robert Kelsey and William Guethlein for providing us with copies of their data acquisition and reduction software for impedance cardiography and for their helpful advice.

The interbeat intervals were checked and edited for artifacts using the detection algorithm of Berntson, Quigley, Jang, and Boysen (1990) and were subsequently verified by visual inspection. The metric of heart period is preferable to the metric of heart rate on biometric grounds (Berntson, Cacioppo, & Quigley, 1994c; Berntson et al., 1994d). For purposes of comparison with the literature, however, mean heart rate as well as heart period (HP) were calculated for the 3-min periods of the preinfusion baseline, postinfusion baseline, standing posture, and sitting posture.

The impedance data were ensemble averaged within 1 min epochs, and each EKG and *dZ/dt* waveform was verified or edited prior to analyses. Because spot electrodes do not give optimal vascular measures, we limited analysis to systolic time intervals: (a) PEP was quantified as the time interval in milliseconds from the onset of the EKG Q-wave to the B-point of the *dZ/dt* wave, (b) LVET was quantified as the interval in milliseconds from the B-point to the X-point of the *dZ/dt* wave, and (c) EMS was quantified as the sum of PEP and LVET. In addition, analyses were performed on a set of rate-corrected systolic time interval measures used in cardiology to index left ventricular function (see review by Binkley & Boudoulas, 1986): (a) the ratio PEP/LVET, (b) $PEPI = 0.4*HR + PEP$, (c) $PEP/HP^{0.5}$, (d) $LVET/HP^{0.5}$, and (e) $EMS/HP^{0.5}$. The mean for each of these measures was calculated for each minute for each subject. These minute-by-minute means were then averaged over the 3-min periods of the preinfusion and postinfusion baselines, standing posture, and sitting posture to increase reliability.

Because heart period oscillations within different frequency bands may differentially index the autonomic branches, heart period variance in the respiratory band and two lower frequency bands was analyzed for each minute using the methods of Porges and Bohrer (1990) by a PC-based software package (MXedit 2.01, Delta-Biometrics, Bethesda, MD). The 60-s heart period series were converted to 500-ms time series and were detrended with a 21-point cubic polynomial filter moved stepwise through the data to remove low-frequency trends. The data were further filtered by a (25 pt) digital band-pass filter to remove variance outside the respiratory frequency band (0.12–0.04 Hz). The natural logarithm of the variance was then calculated on the residual data, within the frequency range associated with respiration, and this value was taken as the index of high-frequency variability (HF).

Low-frequency oscillations in heart period are thought to have two separable components. The Mayer waves, which typically peak at about 0.1 Hz, correspond to slow (three to nine cycles/min) oscillations in mean arterial blood pressure, whereas a second component that typically peaks from 0.04–0.08 Hz is thought to reflect thermoregulatory fluctuations in vasomotor tone and adjustments of vascular resistance to local metabolic demands (Láng & Szilágyi, 1991). Both components can affect sympathetic and parasympathetic outflows to the heart through the baroreceptor heart period reflex. In view of these considerations, two partially overlapping band widths of lower frequency heart period oscillations were derived by the MXedit program (51-pt cubic detrend, with 0.04–0.08-Hz and 0.04–0.12-Hz band pass filters). The first low-frequency oscillation ($LF_{0.04-0.08Hz}$) was selected to sample very low-frequency rhythms but minimize the likelihood that the measure would be confounded by RSA. The second ($LF_{0.04-0.12Hz}$) was selected to ensure a broad-band index of possible sympathetic influence on low-frequency heart

period variability.[5] Finally, the ratio of the variance in the low-frequency (0.04–0.08 Hz) to that in the high-frequency (0.12–0.40 Hz) band widths was calculated. In all cases, minute-by-minute means were then averaged (within subjects) over the 3-min periods of the preinfusion baseline, postinfusion baseline, standing posture, and sitting posture to increase the reliability of the measures.[6]

Systolic and diastolic blood pressure were recorded via the auscultatory method using a Cortronics 7000 blood pressure monitor. Systolic, diastolic, and mean arterial blood pressure readings were averaged over each 3-min recording period. Given our focus on cardiac activity and the similarity in the results for systolic, diastolic, and mean arterial blood pressure, only the results for mean arterial blood pressure (MAP) are reported below.

Respiration was recorded using an EPM Systems strain gauge respirometer placed below the lowest current electrode. The analog signal was quantified at 250 Hz, and the digital data were smoothed by a 10-pt boxcar filter. Although we did not specifically calibrate the respirometer to a known tidal volume, our primary concern was the identification of within-session respiratory changes that might contribute to HF variance across experimental conditions. The respiration data were verified or edited to eliminate artifacts, and the mean respiration amplitude and period were calculated for each minute for each subject. These minute-by-minute means were averaged within each 3-min recording period to increase reliability. Technical problems resulted in the loss of data from one subject.

Blockade Estimates of Autonomic Control

The change in a measure of cardiac activity after blockade of a single autonomic branch reflects the subtractive loss of that branch and provides an index of the normal contribution of the blocked branch (e.g., cardiac vagal $control_{estimate} = HP_{saline} - HP_{atropine}$), whereas the residual autonomic control of a cardiac measure after the same blockade provides an index of the functional contribution of the unblocked branch (e.g., cardiac vagal $control_{estimate} = HP_{metoprolol} - HP_{dual\ blockade}$). We have shown that the alternative subtractive and residual estimates, derived from selective autonomic blockades, are inversely corrupted by systematic biases that can arise in blockade studies. These biases tend to be minimized by averaging the subtractive and residual estimates of the contributions of a given autonomic branch. Furthermore, the discrepancy between the subtractive and residual estimates provides a measure of the bias in these estimates (Berntson et al., 1994b). In the present study, estimates of the sympathetic (*s*) and parasympathetic (*p*) contributions to each measure, as well as an estimate of the bias in the blockade data

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[5] In addition, spectral analyses were run on the respiratory data from the four subjects with the most extreme respiratory frequencies to insure that the band width selected for the HF index would capture the full range of respiratory frequencies and thus RSA.

[6] The detrending and bandpass filters of the MXedit program require priming by a number of points (depending on the filter settings) at the beginning and end of the analysis epoch prior to generating valid data. Consequently, the HF analyses were based (approximately) on the middle 35 s of the 1-min sample epochs, and the LF values were derived from the middle 20 s. The three separate (minute-by-minute) values for each experimental condition were then averaged to yield the score for a given subject.

(ϵ_{blk}), were determined for each subject using the formulae developed by Berntson et al. (1994b):

$$s = [(F_{atropine} - F_{dual\ blockade}) + (F_{saline} - F_{metoprolol})]/2 \tag{1}$$

$$p = [(F_{metoprolol} - F_{dual\ blockade}) + (F_{saline} - F_{atropine})]/2 \tag{2}$$

$$\epsilon_{blk} = [(F_{atropine} - F_{dual\ blockade}) - (F_{saline} - F_{metoprolol})]/2$$
$$= [(F_{metoprolol} - F_{dual\ blockade}) - (F_{saline} - F_{atropine})]/2, \tag{3}$$

where F represents the mean value of a cardiac measure during a recording epoch. A t-test comparing the mean estimates of the sympathetic and parasympathetic contributions to each cardiac measure provides information about differences in the contribution of each autonomic branch, and the estimate of error bias provides a validity range around the mean, within which the estimate of sympathetic or parasympathetic contributions to a cardiac measure may be due to one or more forms of bias in blockade studies (Berntson et al., 1994b).

Results[7]

Preinfusion Baseline
Preinfusion basal measures were subjected to a one-way ANOVA to determine whether any measure differed as a function of drug condition (saline, atropine, metoprolol) prior to infusion. As expected, no test of any cardiovascular index approached statistical significance.

Postinfusion Baseline
The analysis of postinfusion basal measures revealed statistically significant main effects for drug condition (saline, atropine, metoprolol, dual blockade) for the following measures: heart period: $F(3,27) = 88.87$, $p < .0001$, $\epsilon = 0.47$; heart rate, $F(3,27) = 147.46$, $p < .0001$, $\epsilon = 0.72$; PEP: $F(3,27) = 4.19$, $p < .03$, $\epsilon = 0.78$; PEP1: $F(3,27) = 17.95$, $p < .0001$, $\epsilon = 0.91$; PEP/HP$^{0.5}$: $F(3,27) = 26.97$, $p < .001$, $\epsilon = 0.80$; LVET: $F(3,27) = 6.88$, $p < .01$, $\epsilon = 0.58$; EMS: $F(3,27) = 7.45$, $p <$

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[7] Several related measures were included in the analysis to foster comparisons of the present results with prior results in the psychophysiology and cardiology literatures. These measures included heart period and heart rate, the systolic time intervals (e.g., PEP, PEP1, PEP/HP$^{0.5}$, LVET, LVET/HP$^{0.5}$, PEP/LVET, EMS, and EMS/HP$^{0.5}$); and heart rate variability (HF$_{0.12-0.40Hz}$, LF$_{0.04-0.08Hz}$, LF$_{0.04-0.12Hz}$, and LF$_{0.04-0.08Hz}$/HF$_{0.12-0.40Hz}$). Analyses of variance (ANOVAs) with repeated measures were corrected using the Hunyh-Feldt epsilon values specified in the text; when Hunyh-Feldt epsilon values exceeded 1, the degrees of freedom were not adjusted (i.e., epsilon was specified as 1). The results reported in the text are unchanged by use of the Greenhouse-Geisser rather than the Hunyh-Feldt correction. The dual blockade was performed at the end of the session in which metoprolol was infused; therefore, there was no preinfusion baseline in the dual blockade condition, and period (pre-infusion and postinfusion baselines) could not serve as a within-subjects variable in a factorial design. Rather, dual blockade was used to derive estimates of each subject's intrinsic heart period (β).

$.004$, $\epsilon = 0.69$; EMS/HP$^{0.5}$: $F(3,27) = 11.40$, $p < .001$, $\epsilon = 0.52$; HF: $F(3,27) = 244.01$, $p < .0001$, $\epsilon = 0.99$; LF$_{0.04-0.12Hz}$: $F(3,27) = 43.72$, $p < .0001$, $\epsilon = 1.00$; and LF$_{0.04-0.08Hz}$: $F(3,27) = 40.88$, $p < .0001$, $\epsilon = 1.00$. Cell means and pairwise comparisons for the cardiac indices are summarized in Table 1. No drug effect on respiratory activity or blood pressure approached statistical significance.

The estimate of the sympathetic and parasympathetic contributions (s and p, respectively) to each cardiac index and the bias in these estimates (ϵ_{blk}) are summarized in the right three columns of Table 1. These analyses indicated that (a) the sympathetic branch exerts significantly less neural control over heart period than does the parasympathetic branch under basal conditions ($Ms = -119.39$ ms and 372.46 ms, respectively), and both of these estimates are well above the range of error in these estimates due to various biases such as reflexive alterations in the unblocked branch or nonselective actions of the blocker agents ($M = \pm 32.74$ ms); (b) the sympathetic branch exerts significantly more neural control over basal PEP than does the parasympathetic branch ($Ms = -9.62$ ms and -0.48 ms, respectively), and the parasympathetic estimate falls within the range of error due to biases resulting from the blockades; and (c) the parasympathetic branch exerts significantly more neural control over basal HF than the sympathetic branch ($Ms = 5.54$ and -0.46, respectively), although the sympathetic branch may account for a nominal portion of the variance. Inspection of the remaining columns in Table 1 supports these characterizations and further indicates that (a) the means for HF across drug conditions converge on its parasympathetic determinism, because the vagal contributions to this measure were much larger than the sympathetic contributions (5.54 vs. -0.46 log units); and (b) the basal measures of low-frequency heart rate variability do not provide satisfactory indices of relative sympathetic control of basal cardiac activity, at least in young healthy female subjects, as indicated by the large parasympathetic contributions to these indices.

Orthostatic Stressor
The effect of posture on indices of the neural control of tonic cardiac activity was evaluated by 2 (Posture) × 4 (Drug Condition) repeated measure ANOVAs. Cell means, autonomic estimates, and pairwise comparisons for cardiac indices are summarized in Table 2. Replicating the preceding results, significant main effects for drug condition were found for the same set of measures as outlined above ($6.02 \leq Fs(3,27) \leq 279.32$, all $ps < .005$) with two exceptions: PEP/LVET, $F(3,27) = 9.47$, $p < .001$, $\epsilon = 0.77$, and LVET/HP$^{0.5}$, $F(3,27) = 5.48$, $p < .01$, $\epsilon = 0.81$, also differed significantly as a function of autonomic blockade.

The expected significant main effects for posture were found for heart period, $F(1,9) = 76.61$, $p < .0001$; heart rate, $F(1,9) = 69.45$, $p < .0001$; PEP, $F(1,9) = 28.39$, $p < .0005$; PEP1, $F(1,9) = 62.28$, $p < .0001$; PEP/HP$^{0.5}$, $F(3,27) = 60.54$, $p < .001$; LVET, $F(1,9) = 14.50$, $p < .005$; PEP/LVET, $F(1,9) = 45.77$, $p < .0001$; HF$_{0.12-0.40Hz}$, $F(1,9) = 79.98$, $p < .0001$; and MAP, $F(1,8) = 16.38$, $p < .01$. Standing decreased the mean heart period, LVET, PEP/LVET, EMS, HF, and MAP, whereas it increased heart rate, PEP, and PEP/HP$^{0.5}$. Of these postural effects, four were qualified by Drug Condition × Posture Interactions: heart period, $F(3,27) = 21.38$, $p < .0001$, $\epsilon = 0.60$; heart rate, $F(3,27) = 13.03$, $p < .0001$, $\epsilon = 0.81$; EMS, $F(3,27) = 147.33$, $p < .0001$, $\epsilon = 0.70$;

Table 1. *Mean (SEM) Cardiovascular Measures and Autonomic Nervous System (ANS) Indices: Postinfusion Baseline*

	Drug condition				ANS indices[a]		
Measure	Saline	Atropine	Metoprolol	Dual block	s	p	ϵ_{blk}
HP (ms)	846.97^c (39.19)	507.25^a (9.98)	999.10^d (43.43)	593.90^b (15.87)	−119.39$_a$ (10.80) [.78]	372.46$_b$ (38.91) [.92]	±32.74
HR (bpm)	72.29^b (3.28)	118.70^d (2.32)	61.15^a (2.73)	101.76^c (3.05)	14.04$_a$ (1.54) [.83]	−43.51$_b$ (3.11) [.94]	±2.90
PEP (ms)	94.17^a (3.95)	95.40^a (5.56)	104.53^b (4.16)	104.27^b (4.00)	−9.62$_a$ (2.54) [.93]	−0.48$_b$ (2.78) [.39]	±0.75
PEP/HP$^{0.5}$	3.27^a (0.17)	4.24^b (0.25)	3.34^a (0.18)	4.31^b (0.70)	−0.07$_a$ (0.10) [.88]	−0.97$_b$ (0.12) [.99]	±0.01
PEPI (ms)	123.08^a (4.41)	142.88^b (5.50)	128.99^a (4.65)	144.97^b (4.95)	−4.00$_a$ (2.35) [.68]	−17.89$_b$ (2.65) [.90]	±1.91
LVET (ms)	266.60^b (11.47)	220.37^a (4.31)	265.57^b (15.38)	239.47^{ab} (5.48)	−9.03$_a$ (7.37) [.47]	36.17$_b$ (11.86) [.78]	±10.07
LVET/HP$^{0.5}$	9.22^{ab} (0.17)	9.79^b (0.25)	8.49^a (0.18)	9.83^b (0.70)	0.34$_a$ (0.27) [.47]	−0.95$_a$ (0.49) [.71]	±0.39
PEP/LVET	0.362^a (0.024)	0.436^b (0.031)	0.407^{ab} (0.028)	0.441^b (0.028)	−0.025$_a$ (0.021) [.56]	−0.054$_a$ (0.026) [.73]	±0.02
EMS (ms)	360.77^b (11.82)	315.77^a (3.86)	370.10^b (16.84)	343.73^b (2.58)	−18.65$_a$ (6.22) [.67]	35.69$_b$ (12.12) [.79]	±9.32
EMS/HP$^{0.5}$	12.49^a (0.48)	14.03^b (0.13)	11.83^a (0.66)	14.14^b (0.20)	0.27$_a$ (0.21) [.42]	−1.92$_b$ (0.52) [.83]	±0.38
HF	6.68^b (0.30)	1.08^a (0.27)	7.08^b (0.40)	1.61^a (0.24)	−0.46$_a$ (0.19) [.87]	5.54$_b$ (0.26) [.99]	±0.07
LF$_{0.04-0.08Hz}$	3.96^b (0.44)	0.35^a (0.20)	3.45^b (0.45)	0.17^a (0.07)	0.34$_a$ (0.22) [.67]	3.45$_b$ (0.39) [.95]	±0.17
LF$_{0.04-0.12Hz}$	5.69^b (0.46)	1.03^a (0.36)	5.11^b (0.48)	0.98^a (0.23)	0.32$_a$ (0.27) [.54]	4.40$_b$ (0.49) [.94]	±0.27
LF/HF	0.59^a (0.07)	0.26^a (0.13)	0.49^a (0.06)	0.39^a (0.31)	−0.02$_a$ (0.14) [.14]	0.22$_a$ (0.18) [.65]	±0.12

Note: Means within a row that do not share a superscript differ by paired contrasts at $p < .05$, as do autonomic estimates with different subscripts.
[a]The coefficient of validity (given in brackets) for experimental contrasts $v_\delta = |\text{effect size}|/(|\text{effect size}| + |\text{error bias}|)$ (Berntson et al., 1994b). When $v_\delta < 0.5$, the error bias equals or exceeds the magnitude of the experimental effect, and the contrast should not be considered valid. Mean ANS estimates (s, p) that fall within the associated SEM and/or within ϵ_{blk} should not be considered meaningful.

and HF$_{0.12-0.40Hz}$, $F(3,27) = 4.47$, $p < .03$, $\epsilon = 0.72$. No other test was statistically significant (see Table 2).

The mean estimate of the sympathetic and parasympathetic contributions to basal cardiac activity in each posture and the estimate of the bias in these estimates are summarized in the right three columns of Table 2. The most consistent finding is the expected parallel between the results observed during the postinfusion baseline and those observed during standing and sitting. These analyses confirmed that the parasympathetic branch exerts greater neural control over basal heart period (and heart rate) than does the sympathetic branch whether subjects were sitting or standing and that the sympathetic influence is enhanced and the parasympathetic influence is diminished when standing. Standing produced longer rather than shorter PEPs, consistent with prior research. The estimates of the sympathetic and parasympathetic contributions support the interpretation of these postural increases in PEP to preload or afterload: (a) the influence of the sympathetic branch on basal PEP was sig-

Table 2. *Cell Means (SEM) as a Function of Posture and Autonomic Blockade*

	Drug condition				ANS indices[a]		
Measure	Saline	Atropine	Metoprolol	Dual block	s	p	ϵ_{blk}
HP (ms) Sitting	849.67[c] (34.37)	516.27[a] (11.08)	978.20[d] (38.04)	604.82[b] (16.01)	-108.54_a (12.44) [.84]	353.39_b (32.18) [.95]	±19.99
Standing	702.50[c] (34.36)	455.73[a] (9.41)	852.63[d] (33.74)	560.97[b] (17.23)	-127.68_a (12.23) [.85]	269.22_b (26.42) [.92]	±22.45
HR (bpm) Sitting	71.71[b] (2.90)	116.73[d] (2.51)	62.26[a] (2.53)	99.92[c] (2.94)	13.13_b (1.60) [.78]	-41.34_a (2.70) [.92]	±3.68
Standing	87.21[b] (3.94)	132.26[d] (2.74)	71.41[a] (2.80)	107.98[c] (3.67)	20.04_b (1.91) [.83]	-40.81_a (2.39) [.91]	±4.24
PEP (ms) Sitting	96.07[a] (4.03)	98.77[ab] (3.39)	105.07[b] (4.11)	102.80[ab] (3.60)	-6.52_a (2.44) [.72]	-0.22_a (2.34) [.08]	±2.48
Standing	104.47[a] (2.98)	101.67[a] (5.00)	115.60[b] (4.05)	116.40[b] (2.39)	-12.94_a (2.55) [.88]	1.00_b (3.25) [.36]	±1.80
PEP/HP$^{0.5}$ Sitting	3.32[a] (0.17)	4.35[b] (0.15)	3.38[a] (0.16)	4.20[b] (0.20)	-0.04_a (0.09) [.27]	-0.93_b (0.08) [.89]	±0.11
Standing	3.98[a] (0.17)	4.77[b] (0.23)	3.99[a] (0.19)	4.94[b] (0.17)	-0.09_a (0.10) [.53]	-0.87_b (0.11) [.92]	±0.08
PEPI (ms) Sitting	124.75[a] (4.42)	145.46[b] (3.48)	129.97[a] (4.35)	142.77[b] (4.51)	-1.27_b (2.29) [.25]	-16.76_a (2.11) [.81]	±3.96
Standing	139.35[a] (3.70)	154.57[b] (4.95)	144.16[a] (4.61)	159.59[b] (3.52)	-4.92_a (2.12) [.98]	-15.32_a (2.76) [.99]	±0.10
LVET (ms) Sitting	257.87[b] (12.38)	219.32[a] (3.71)	277.83[c] (11.64)	243.40[b] (5.18)	-22.03_a (4.06) [.91]	36.49_b (10.80) [.95]	±2.06
Standing	231.37[b] (5.91)	202.17[a] (7.25)	261.10[c] (4.04)	219.27[ab] (3.95)	-23.42_a (4.29) [.79]	35.52_b (4.44) [.85]	±6.32
LVET/HP$^{0.5}$ Sitting	8.90[a] (0.46)	9.66[b] (0.11)	8.93[a] (0.40)	9.90[b] (0.16)	-0.14_a (0.15) [.56]	-0.87_a (0.41) [.89]	±0.11
Standing	8.76[a] (0.14)	9.47[b] (0.29)	8.97[ab] (0.09)	9.27[ab] (0.13)	-0.01_a (0.17) [.05]	-0.51_a (0.17) [.72]	±0.20
PEP/LVET Sitting	0.384[a] (0.03)	0.453[b] (0.02)	0.386[a] (0.03)	0.427[ab] (0.02)	0.012_a (0.01) [.46]	-0.055_a (0.03) [.80]	±0.014
Standing	0.456[a] (0.02)	0.515[b] (0.03)	0.446[a] (0.02)	0.535[b] (0.02)	0.012_b (0.01) [.44]	-0.074_a (0.02) [.83]	±.015
EMS (ms) Sitting	353.93[b] (11.94)	318.08[a] (4.30)	382.90[c] (12.45)	346.20[b] (3.25)	-35.67_a (5.78) [.84]	43.40_b (11.42) [.87]	±6.70
Standing	335.83[b] (5.12)	303.83[a] (7.09)	376.70[c] (3.67)	335.67[b] (2.17)	-36.35_a (5.81) [.89]	36.52_b (5.06) [.89]	±4.51

Table 2. *Continued*

Measure	Drug condition				ANS indices[a]		
	Saline	Atropine	Metoprolol	Dual block	s	p	ϵ_{blk}
$EMS/HP^{0.5}$ Sitting	12.22[a] (0.50)	14.01[b] (0.13)	12.31[a] (0.46)	14.11[b] (0.20)	−0.09[a] (0.16) [.41]	−1.79[b] (0.43) [.93]	±0.13
Standing	12.73[a] (0.19)	14.23[b] (0.25)	12.96[a] (0.22)	14.22[b] (0.21)	−0.10[a] (0.20) [.45]	−1.38[b] (0.13) [.92]	±0.12
HF Sitting	6.58[c] (0.35)	1.11[a] (0.23)	7.08[c] (0.34)	1.73[b] (0.20)	−0.56[a] (0.18) [.90]	5.41[b] (0.30) [.99]	±0.06
Standing	5.34[b] (0.29)	0.56[a] (0.16)	5.31[b] (0.50)	1.09[a] (0.15)	−0.26[a] (0.25) [.48]	4.50[b] (0.40) [.94]	±0.28
$LF_{0.04-0.08Hz}$ Sitting	3.66[b] (0.47)	0.43[a] (0.15)	3.54[b] (0.46)	0.26[a] (0.12)	0.14[a] (0.24) [.88]	3.25[b] (0.40) [.99]	±0.02
Standing	3.56[c] (0.45)	0.23[a] (0.14)	3.01[b] (0.54)	0.52[a] (0.17)	0.13[a] (0.29) [.24]	2.91[b] (0.46) [.87]	±0.42
$LF_{0.04-0.12Hz}$ Sitting	5.39[b] (0.51)	1.45[a] (0.28)	5.23[b] (0.48)	1.06[a] (0.25)	0.28[a] (0.33) [.72]	4.05[b] (0.48) [.97]	±0.11
Standing	5.22[b] (0.57)	1.04[a] (0.27)	4.70[b] (0.56)	1.27[a] (0.33)	0.15[a] (0.36) [.28]	3.81[b] (0.59) [.91]	±0.38
LF/HF Sitting	0.563[b] (0.08)	0.383[ab] (0.11)	0.500[b] (0.06)	0.255[a] (0.14)	0.095[a] (0.04) [.76]	0.212[a] (0.10) [.88]	±0.03
Standing	0.658[b] (0.08)	0.257[a] (0.14)	0.592[b] (0.09)	0.478[b] (0.13)	−0.077[a] (0.10) [.35]	0.257[a] (0.11) [.65]	±0.14

Note: Means within a row that do not share a superscript differ by paired contrasts at $p < .05$, and autonomic estimates with different subscripts differ at $p < .05$.
[a]The coefficient of validity (given in brackets) for experimental contrasts $v_\delta = |\text{effect size}|/(|\text{effect size}| + |\text{error bias}|)$ (Berntson et al., 1994b). When $v_\delta < 0.5$, the error bias equals or exceeds the magnitude of the experimental effect, and the contrast should not be considered valid. Mean ANS estimates (s, p) that fall within the associated SEM and/or within ϵ_{blk} should not be considered meaningful.

nificantly greater than the influence of the parasympathetic branch, which did not differ from zero; and (b) despite the fact that PEP was longer when standing than sitting, the effect of standing was essentially to double the sympathetic (shortening) effect on basal PEP.

Simple reactivity (standing − sitting) change scores (Δs and Δp) were also calculated for each cardiac index to supplement the analyses of absolute levels (s and p) as outlined above. Cell means and pairwise comparisons for reactivity scores of the cardiac indices are summarized in Table 3. ANOVAs on mean change scores for cardiac indices across the four drug conditions revealed that the autonomic blockades affected only three cardiac indices: heart period, $F(3,27) = 21.38$, $p < .0001$, $\epsilon = 0.60$; heart rate, $F(3,27) = 13.03$, $p < .0001$, $\epsilon = 0.81$; and HF, $F(3,27) = 4.47$, $p < .03$, $\epsilon = 0.72$. No increase was apparent in either LF index, despite significant sympathetic activation as revealed by autonomic blockades. This lack may be attributable to the parasympathetic withdrawal that accompanies standing, which would decrease the vagal component of the LH indices. Despite sympathetic activation, analysis of PEP reactivity also failed to yield a significant change on the assumption of an upright posture, probably because of the documented effects of variations in preload/afterload, which can lead to a nonautonomic (mechanical) effect on contractility and thus PEP. This result raises an important caveat in the application of PEP measures across postures. Again, analyses of respiration amplitude and period did not approach statistical significance.

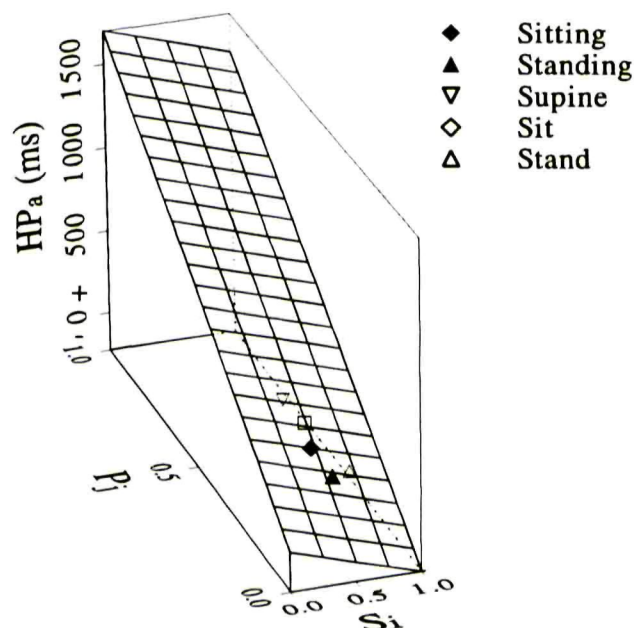### Indices of Individual Differences in Cardiac Autonomic Control

PEP and HF emerged as the most specific and sensitive cardiac index of the sympathetic and vagal activation of the heart, respectively. Therefore, we next investigated (a) whether resting PEP predicted interindividual variation in cardiac sympathetic tone, defined as the estimate of the sympathetic contributions to baseline heart period (Equation 1); and (b) whether resting HF predicted interindividual variation in cardiac vagal tone, defined as the estimate of the parasympathetic contributions to baseline heart period (Equation 2). The postinfusion baseline was selected because this period was the most comparable

across days (e.g., the period always immediately followed infusion) and the blockades were most potent. Postinfusion PEP under saline predicted individual differences in postinfusion cardiac sympathetic activation ($r = 0.82$, $p < .01$). Consistent with the finding of Grossman and Kollai (1993), however, postinfusion HP under saline, but not HF, predicted individual differences in postinfusion cardiac vagal activation ($r = 0.96$, $p < .01$; and $r = 0.24$, n.s., respectively).[8] No other correlation among these measures was statistically significant. Thus, although HF was the best index of cardiac vagal activation at the group level, it was a less effective index of individual differences in resting cardiac vagal tone in a small homogeneous sample of young healthy women.[9] This low correlation may well be due to session to session variance in HF, given that the HF estimate was derived from the single saline session, whereas the parasympathetic estimate was derived across the three drug conditions.

Discussion

The basal autonomic control of the heart and postural effects on autonomic control can be depicted in a bivariate (Sympathetic Activation × Parasympathetic Activation) autonomic space (Berntson, Cacioppo, & Quigley, 1991; Berntson et al., 1993a, 1994d; see also Stemmler, Grossman, Schmid, & Foerster, 1991). The cardiac effector surface in Figure 1 represents the chronotropic state of the heart associated with all physiological loci in autonomic space (Berntson et al., 1993a). Because the same basal heart period may be achieved by various combinations of sympathetic and parasympathetic activation, basal heart period alone does not identify a specific autonomic origin, even though knowledge of the location on the autonomic plane uniquely defines a basal chronotropic state. However, the

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[8] When hierarchical regression was performed to extract the contributions to HF of interindividual variation in respiration period and respiration amplitude, the correlation between cardiac vagal tone and HF was .27 (n.s.). We also calculated this correlation using the same procedures as Grossman and Kollai (1993) (i.e., $HP_{\text{postinfusion with atropine}} - HP_{\text{preinfusion with atropine}}$) and related that to $HF_{\text{preinfusion with atropine}}$. The correlation between these two variables was $-.34$ (n.s.), which is similar to the correlation observed by Grossman and Kollai (1993). Finally, using different algorithms for extracting RSA from the heart period time series (e.g., determining the peak respiratory frequency for each subject minute by minute or across a 3-min baseline using spectral analysis and quantifying in the corresponding period of heart period variability data the total power ±0.06 Hz of this peak respiratory frequency; determining the peak respiratory frequency for each subject minute by minute or across a 3-min baseline using spectral analysis and quantifying in the corresponding period of heart period variability data the total power within a 90% confidence interval about this peak respiratory frequency) did not change any of the results reported in the text and altered the correlation between HF and cardiac vagal tone only nominally (e.g., from .27 to .36, n.s.).

[9] The homogeneous population and baseline conditions examined in this study may have contributed to the modest correlation between RSA and cardiac vagal tone. Alternatively, Porges and Maiti (1992) argued that the right nucleus ambiguous, which is linked to the limbic system and is responsive to processes associated with motion, emotion, and communication, contains the source nuclei for the right vagal input to the sinoatrial node. The left vagus, Porges and Maiti (1992) proposed, is governed primarily by the dorsal motor nucleus and is associated with vegetative functions. Finally, the effects of the right vagus on the heart is the stronger determinant of RSA in this scheme. In the Porges–Maiti scheme, therefore, RSA predicts psychophysiologically significant interindividual variation even though it may be only weakly related to interindividual variation in total tonic cardiac vagal control of the heart.

Figure 1. Basal (resting) loci of chronotropic control for human subjects, as represented on the cardiac effector surface. The estimates of sympathetic and parasympathetic activation were scaled by the dynamic ranges of the autonomic branches derived by Berntson et al. (1993a) to transform these data into the proportional (0 to 1) units of the autonomic axes. Mean values under all postural conditions are depicted on the effector surface. ANS = autonomic nervous system; HPa = autonomic contribution to heart period as a change from the intrinsic period, estimated under double blockade; and Si annd Pj are the independent activities of the sympathetic and parasympathetic innervations at point ij within the autonomic plane. For the present study: ◆ = mean basal locus for sitting; ▲ = mean basal locus for standing. From Berntson et al. (1993a, Figure 3): ▽ = mean basal locus for supine; ◇ = mean basal locus for sitting; △ = mean basal locus for standing. The alignment of these points indicates that orthostatic stressors produce a reciprocal activation on the heart.

tonic heart periods associated with the autonomic blockades provide the information needed to determine these locations on the autonomic plane (Berntson et al., 1993a, 1994b). Basal cardiac states for various postures and studies are illustrated in Figure 1. There are considerable differences in basal autonomic tone, but the distribution of basal loci is nevertheless systematic: (a) all loci lie generally within the lower one third of the vagal dynamic range and within the middle two thirds of the sympathetic range; (b) the data points within studies contrasting postural effects on autonomic influences indicate reciprocal changes in sympathetic and parasympathetic activation; and (c) vagal control tends to be highest and sympathetic control lowest during the supine position, the reverse tends to occur during standing, and the autonomic loci during sitting fall between these extremes. These data suggest that the postural effects on basal heart period are implemented primarily by reciprocal activation of the sympathetic and parasympathetic branches (see also Porter et al., 1990). A likely contributor to this reciprocal control is the baroreflex. Although the present subjects were women on birth control medication, the present results are in close accord with those of previous studies on men (for review, see Berntson et al., 1993a), suggesting considerable generality of the present findings.

Table 3. *Mean (SEM) Reactivity to Orthostatic Stressor*

Measure	Drug condition				ANS indices[a]		
	Saline	Atropine	Metoprolol	Dual Block	Δs	Δp	ϵ_{blk}
HP (ms)	-147.17^a (22.05)	-60.54^b (7.30)	-125.57^a (15.75)	-43.85^b (5.05)	-19.14_b (7.42) [.89]	-84.18_a (14.59) [.97]	±2.46
HR (bpm)	15.51^b (2.47)	15.53^b (1.91)	9.15^a (1.04)	8.06^a (1.14)	6.92_b (1.17) [.93]	0.53_a (0.98) [.49]	±0.56
PEP (ms)	8.40^{ab} (1.86)	2.90^a (5.00)	10.53^{ab} (1.37)	13.60^b (2.48)	-6.42_a (2.51) [.60]	1.22_a (2.59) [.22]	±4.28
PEP/HP$^{0.5}$	0.66^a (0.06)	0.41^a (0.24)	0.61^a (0.07)	0.74^a (0.09)	-0.14_a (0.12) [.42]	0.06_a (0.11) [.24]	±0.19
PEPI (ms)	14.60^a (1.62)	9.11^a (5.22)	14.19^a (1.60)	16.82^a (2.30)	-3.65_a (2.60) [.47]	1.43_a (2.49) [.26]	±4.06
LVET (ms)	-26.50^a (11.90)	-17.15^a (6.73)	-16.73^a (10.94)	-24.13^a (2.91)	-1.39_a (4.76) [.14]	-0.97_a (12.13) [.10]	±8.37
LVET/HP$^{0.5}$	-0.14^a (0.46)	-0.19^a (0.29)	0.04^a (0.36)	-0.63^a (0.12)	0.13_a (0.18) [.30]	0.36_a (0.47) [.54]	±0.31
PEP/LVET	0.072^a (0.02)	0.062^a (0.03)	0.060^a (0.02)	0.108^a (0.02)	-0.017_a (0.02) [.36]	-0.019_a (0.03) [.39]	±0.03
EMS (ms)	-18.10^b (12.33)	-0.01^a (0.01)	-6.20^b (11.02)	-10.53^b (2.54)	-0.69_a (2.85) [.06]	-6.88_a (10.96) [.38]	±11.21
EMS/HP$^{0.5}$	0.51^a (0.49)	0.22^a (0.20)	0.65^a (0.38)	0.11^a (0.09)	-0.01_a (0.14) [.07]	0.42_a (0.44) [.76]	±0.13
HF	-1.24^{ab} (0.20)	-0.55^a (0.25)	-1.76^b (0.38)	-0.64^a (0.16)	0.31_b (0.30) [.58]	-0.90_a (0.29) [.80]	±0.22
LF$_{0.04-0.08Hz}$	-0.10^{ab} (0.26)	-0.20^{ab} (0.18)	-0.52^a (0.34)	0.26^b (0.18)	-0.02_a (0.25) [.04]	-0.34_a (0.28) [.44]	±0.44
LF$_{0.04-0.12Hz}$	-0.17^a (0.26)	-0.42^a (0.30)	-0.53^a (0.32)	0.21^a (0.26)	-0.13_a (0.32) [.21]	-0.25_a (0.36) [.34]	±0.49
LF/HF	0.095^{ab} (0.05)	-0.126^a (0.11)	0.092^{ab} (0.05)	0.223^b (0.15)	-0.016_a (0.14) [.12]	0.217_a (0.18) [.64]	±0.12

Note: Means within a row that do not share a superscript differ by paired contrasts at $p < .05$, and autonomic estimates with different subscripts differ at $p < .05$.
[a]The coefficient of validity (given in brackets) for experimental contrasts $v_\delta = |\text{effect size}|/(|\text{effect size}| + |\text{error bias}|)$ (Berntson et al., 1994b). When $v_\delta < 0.5$, the error bias equals or exceeds the magnitude of the experimental effect, and the contrast should not be considered valid. Mean ANS estimates (s, p) that fall within the associated SEM and/or within ϵ_{blk} should not be considered meaningful.

In addition, our findings on heart period variability are consistent with those of prior experimental research. In the classic study of Anrep et al. (1936), (a) high-frequency variability in cardiac chronotropy (i.e., RSA) was largely abolished by vagotomy and was not generally attenuated by sympathectomy and (b) sympathetic contributions to RSA were minimal or null and were seen only under conditions of depressed vagal control. The present results similarly demonstrated that heart period variability in the respiratory frequency band (HF) is much more strongly influenced by vagal than by sympathetic outflows. Under sympathetic blockade, HF did not differ from the saline condition, for either sitting or standing conditions. Further, the sympathetic blockade reduced basal heart rate by an average of 12.1 bpm but did not significantly alter HF variance (Tables 1 and 2). When vagal control was depressed by the parasympathetic blockade, HF was sharply attenuated and was minimally altered

by postural manipulation. The autonomic estimates (s and p) confirmed that HF was determined strongly by vagal control, with only minimal sympathetic influences evident.

High- and low-frequency variability have been proposed as indices of the parasympathetic and sympathetic contributions, respectively, to tonic cardiac control. In addition, the ratio of low-frequency/high-frequency heart period variability has been used to index the relative contributions of the autonomic branches to cardiac control. Our results are in general agreement with the use of RSA to index vagal contributions to tonic cardiac control, at least at the group level. In concert with previous findings, however, the present data also demonstrate that the vagal contributions to low-frequency heart period variability can be substantial and that these low-frequency indices do not provide viable selective metrics of sympathetic cardiac control (cf. Akselrod et al., 1981). This interpretation arises from the demonstrated contribution of vagal control to low frequency rhythms rather than from a failure to document sympathetic influences. Consequently, this interpretation could not be attributable to inadequate power of the statistical analyses.

This conclusion is also probably not dependent on the specific low-frequency band widths employed because we included both narrow-band (0.04–0.08 Hz) and wide-band (0.04–0.12 Hz) analyses, the latter to minimize potential contamination from HF variance. In addition, we performed spectral analyses of the heart period time series from a sample of the subjects to verify that the band widths we used were appropriate for these subjects and testing conditions. The results were uniform in not supporting the use of low-frequency variability to index sympathetic cardiac activation or the use of the ratio as an index of the sympathovagal balance of cardiac autonomic control when testing healthy young individuals (see also Ahmed, Goldberger, Singer, & Kadish, 1986).

Our data on systolic time intervals, however, are consistent with the use of PEP to index tonic cardiac sympathetic activation under certain measurement conditions. Postinfusion baseline recordings revealed that PEP varied as a function of the sympathetic but not the parasympathetic activation on the heart. Correlational analyses based on postinfusion values further indicated that resting PEP predicted interindividual variation in cardiac sympathetic tone even though PEP reflects an inotropic state and our measure of cardiac sympathetic tone was derived from the effects of autonomic blockade on heart period (chronotropic state). PEP is complexly determined, however, as indicated by the effects of postural manipulations. Despite sympathetic activation being higher while standing than sitting (see Table 2, top four rows), the PEP (and the heart-rate adjusted PEP1 measure) was longer. The estimates of the autonomic origins of PEP (summarized in Table 2) revealed that (a) PEP was influenced by sympathetic activity but not by parasympathetic activity, (b) the sympathetic influence on basal PEP was approximately doubled while standing, and (c) the sympathetic contributions determined only a fraction (approximately 7–12%) of the PEP. The PEP may serve as a useful marker of β-adrenergic activation, therefore, as long as variations in preload and afterload are small. Unlike PEP, the remaining systolic time intervals and metrics based on these time intervals were influenced by both sympathetic and parasympathetic activity (see Tables 1–3) and do not provide selective autonomic cardiac indices.

The present results favoring heart period as a metric of the autonomic activation of the heart are consistent with those of recent animal studies (e.g., Berntson, Quigley, Fabro, &

Cacioppo, 1992) and human studies (Carlson et al., 1992) in which the activity of the sympathetic and vagal motor nerves have been measured or manipulated. These studies have demonstrated that the transfer functions between autonomic outflows were essentially linear when cardiac chronotropy was measured in terms of heart period but not when measured in terms of heart rate. In some instances, the choice of a metric for cardiac chronotropy (i.e., heart period, heart rate) does not alter the interpretation of the effect of a manipulation. In the present study, however, (a) the effects of posture when expressed in terms of heart rate reactivity implied that the functional effects of standing were due entirely to changes in sympathetic activation, whereas (b) when expressed in terms of heart period reactivity, the effects of standing were appropriately revealed to be due to both sympathetic activation and reciprocal parasympathetic withdrawal (see the first two rows, right three columns in Table 3). Indeed, the correlation between the estimates of posture-induced changes in sympathetic and parasympathetic activation based on the heart period data was +.71, $p < .02$, reflecting an increase in sympathetic control of the heart (shortening heart period) and a decrease in vagal control (also shortening heart period) with standing. Prior literature on postural effects (e.g., Spodick, Meyer, & St. Pierre, 1972; see review by Berntson et al.,1993a) as well as the analyses summarized in Table 2 support the validity of the interpretation derived from the heart period reactivity metric. The indirect indices of the parasympathetic and sympathetic activation of the heart provided by HF and PEP, respectively, further indicated the reciprocal actions of the autonomic branches during postural manipulations.

To summarize, prior blockade studies of noninvasive indices have tended to use single blockades, populations with cardiovascular disease, or a subset of the measures evaluated here. Moreover, none have provided quantitative information regarding the sympathetic and parasympathetic influences on each cardiac index or the magnitude of potential biases in these indices introduced by pharmacological blockades. Three general findings regarding the tonic control of the heart were especially noteworthy. First, measures based on low-frequency heart period variability that have been related to sympathetic activation in cardiac patients were found to be strongly influenced by parasympathetic activity in a healthy female undergraduate population at rest. Second, heart rate and LVET were affected by parasympathetic as well as sympathetic blockades, suggesting mixed autonomic influences on these measures. Accordingly, systolic time intervals that involve heart rate (i.e., PEP1) or LVET (i.e., EMS, PEP/LVET) were poor markers of tonic sympathetic chronotropic control. Third, the high (respiratory)-frequency heart period variability (HF) provided the best noninvasive index of parasympathetic control of the heart, whereas PEP provided the best noninvasive index of sympathetic control. Although both fared well in this study, neither HF nor PEP appear to be singular in their determinants. The autonomic estimates revealed nominal sympathetic contributions to HF, and prior research has demonstrated that HF can be influenced by large changes in tidal volume or respiratory rate. Resting HF proved to be a poor predictor of vagal control of the heart, as indexed by pharmacological blockades, on an individual basis in our small sample of healthy females at rest. Resting heart period was a better predictor of vagal control, but the basal conditions involved minimal sympathetic activation of the heart in these subjects. Because heart period is influenced by sympathetic

and parasympathetic activity (e.g., see the right three columns of Tables 1–3), inferring cardiac vagal activation from heart period may be misleading (Cacioppo & Tassinary, 1990). PEP, although not influenced by parasympathetic activity, may vary with changes in preload or afterload. Thus, the results of this blockade study argue strongly against interpreting basal PEPI,

LVET, PEP/LVET, EMS, LF, or LF/HF as selective indices of sympathetic or parasympathetic influences in a normal population. The present findings also argue for attention to potential confounding factors when interpreting PEP or HF as indices of the autonomic control of the resting heart.

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