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Autonomic control mechanism of maximal lower body negative pressure application

Nandakumar Selvaraj, Kirk H. Shelley, David G. Silverman, Nina Stachenfeld, and Ki H. Chon*,
Senior Member, IEEE

Abstract— **Autonomic control mechanisms during progressive hemorrhage in humans remain complex and unclear. The present study investigates the autonomic reflexes during maximal application of lower body negative pressure (LBNP) that mimics severe hemorrhage in conscious human subjects (n=10) using analyses of heart rate variability (HRV) and systolic blood pressure variability (BPV) and baroreflex sensitivity. Spectral analysis of HRV included linear power spectral density (PSD), and nonlinear principal dynamic modes (PDM) methods. The maximal LBNP application decreased (P<0.01) the systolic and pulse pressures (PP), root mean square successive differences, normalized high frequency (HF) power of HRV, and transfer function gains at low frequency (LF) and HF bands. Meanwhile, increases (P<0.05) in heart rate, diastolic blood pressure (DBP), LF_{HRV}, LF/HF_{HRV}, and sympathetic activity of HRV using PDM were observed during maximal LBNP tolerance. After the termination of LBNP, no significant changes (P>0.05) were found in all the parameters except DBP and PP between recovery and baseline conditions. Rapid application of maximal LBNP that simulated severe hemorrhage was found to be associated with unloading of baroreflex mediated increased sympathetic reflex.**

***Index Terms*—Heart rate variability, Blood pressure variability, baroreflex sensitivity, power spectral density, principal dynamic mode analysis**

I. Introduction

Autonomic control mechanisms that regulate the cardiovascular system during progressive hemorrhage in humans remain very complex and unclear [1]. The progressive hemorrhage and its associated autonomic reflexes can be investigated in humans with the application of lower body negative pressure (LBNP) as a model to study the central hypovolemia associated with severe hemorrhage [2]. The recording of muscle sympathetic nerve activity (MSNA) during higher levels of negative pressure is challenging [2-3] and not suitable for regular clinical use in large number of patients or outside the clinical laboratory [4].

On the other hand, power spectral density (PSD) analysis has been widely used to noninvasively infer the autonomic responses from heart rate variability (HRV) series. Experiments that involved application of LBNP ranged -15 mmHg to -60 mmHg have reported inconsistent findings using PSD analysis [5-7] that is shown to be inadequate for isolating the dynamics of sympathetic (SNS) and parasympathetic (PNS) nervous activities. Thus, the credibility of PSD analysis of HRV data has been criticized [8]. Hence, noninvasive approaches to reliably monitor the autonomic changes during progressive hemorrhage have been elusive.

We have developed a novel technique called principal dynamic mode (PDM) analysis that accurately isolates the dynamics of sympathetic and parasympathetic activities [9]. We validated the PDM method using pharmacological blockades of the ANS in humans and we showed that PDM method is more accurate than the PSD in assessing the true sympathetic and parasympathetic responses [10].

The present study was designed to investigate the autonomic control mechanisms involved during the application of LBNP up to individual's maximal tolerance before the subject develops syncope which would mimic severe hemorrhage. We assessed the autonomic neural responses by analyzing the HRV data using traditional time-domain, approximate entropy (ApEn), PSD and PDM methods. We also extended our analysis with the spectral measures of systolic blood pressure variability (BPV), coherence and baroreflex sensitivity (BRS).

II. Materials And Methods

A. Experimental protocol

The data analyzed in this investigation were obtained from our previous study [11]. The experimental protocol was approved by the Yale University School of Medicine IRB Committee and written informed consent was obtained from all the participants. Subjects were instructed to refrain from caffeine, alcohol, cigarettes or other vasoconstrictive compounds for a minimum of 4 hours prior to participation in the studies, but were otherwise allowed their normal amounts of food and fluid intake prior to enrollment.

Ten healthy male volunteers with no known cardiovascular or systemic disease and between the ages of 23 and 39 years participated in this study. The LBNP chamber was constructed of a sealed wood and acrylic box that is connected to a vacuum pump. Each subject was placed supine in the LBNP

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N. Selvaraj was with the Department of Biomedical Engineering, Worcester Polytechnic Institute, Worcester, MA 01609 USA and currently with Vital Connect Inc., Campbell, CA 95008 USA. (e-mail: snandaa@gmail.com)

K. H. Shelley, and D. G. Silverman are with the Department of Anesthesiology, Yale University, New Haven, CT 06520 USA. (e-mail: david.silverman@yale.edu; kirk.shelley@yale.edu).

N. Stachenfeld is with the John B. Pierce Laboratory, New Haven, CT 06519 USA. (e-mail: nstach@jbpierce.org).

*K. H. Chon is with the Department of Biomedical Engineering, Worcester Polytechnic Institute, Worcester, MA 01609 USA. (e-mail: kichon@wpi.edu).

chamber, which was sealed with a neoprene skirt just above the subject's pelvis. The LBNP protocol consisted of a baseline followed by the gradual decompression of the LBNP chamber until the subject showed presyncope symptoms or any concerns indicated by the subject. The time taken to reach the maximal LBNP application was 9 ± 2 min (mean ± SE) and the range of the maximal LBNP tolerance was -85 mmHg to -110 mmHg among 10 subjects. At the onset of presyncopal symptoms, the application of negative pressure was stopped and the data were continuously recorded during the subsequent recovery stage.

B. Data collection and feature extraction

Data collection involved the simultaneous recording of standard lead II ECG, multi-site photoplethysmograms, respiration and continuous noninvasive blood pressure (NIBP) (Finometer®PRO, Amsterdam, The Netherlands) signals. All data were recorded at 200 Hz using a microprocessor-based data acquisition system (PowerLab 16, ADInstruments, Colorado Springs, CO). In this study, we analyzed the ECG and NIBP recordings offline using Matlab® (Natick, MA).

Three minutes of data were obtained for analysis from baseline, maximal LBNP application and recovery conditions. The time duration of exposure to maximal LBNP before the onset of presyncope was 2.7±0.4 min among subjects. Hence, a 3-min window was applied before the onset of presyncopal symptoms and 3-min data were uniformly extracted for maximal LBNP condition which had partly included the penultimate LBNP stage in 7 subjects. Note that the data at maximal LBNP condition were completely free from the episodes of presyncope. The maximal LBNP application will be termed simply LBNP hereafter. The ECG and NIBP signals were up sampled to 1000 Hz to allow accurate detection of QRS complexes in the ECG, and the peak and valley events in NIBP waveforms using a custom algorithm. The features were visually verified for each beat and pruned if necessary.

C. Heart rate variability analysis

The RR interval series were analyzed in time domain that involved the calculation of the mean heart rate (HR), standard deviation of RR intervals (SDNN) and the root-mean square of the successive difference of RR intervals (RMSSD). ApEn, a widely used nonlinear statistical measure of complexity of data, was presently calculated from instantaneous R-R intervals based on m=2 and automatically selected threshold value r [12].

Power Spectral Density Analysis: HRV signal was generated from the instantaneous R-R interval series at a uniform sampling rate of 4 Hz using cubic spline interpolation technique. After the HRV signal was down sampled to 2 Hz, and the removal of mean and linear trends, PSDs of HRV data were obtained using the Welch periodogram method. A 512 point fast Fourier Transform (FFT) that correspond to the frequency resolution of 0.004 Hz was applied with 360 points Hamming window and no overlapping segments. The spectral power associated with the frequency bands (0.04-0.15 Hz) and (0.15-0.4 Hz) and their ratio were obtained (denoted as LF_{HRV}, HF_{HRV}, and LF/HF_{HRV}, respectively).

Principal Dynamic Mode analysis: The PDM analysis is a method based on extracting only the principal dynamic components of the signal via eigenvalue decomposition. The PDMs are calculated using Volterra-Wiener kernels based on expansion of Laguerre polynomials. The real symmetric square matrix of the estimated kernel values were subjected to Eigen decomposition. Significant eigenvalues that represent 90% HR dynamics were selected and the corresponding orthonormal eigenvectors represent the principle dynamic modes (PDMs) of the HRV signal. The set of selected eigenvectors were used to reconstruct the HRV signal which was subtracted from the actual HRV signal and thus the estimation error was obtained. The two most dominant PDMs were selected to represent the dynamics corresponding to the SNS and PNS activities. The more technical details of estimating the Volterra-Wiener kernel and extracting the PDMs from the kernel are described in [9-10]. Although the PDMs are in time-domain representation, we convert them to the frequency domain via the FFT and compute the power of the two ANS dynamics.

D. Blood pressure variability and baroreflex sensitivity analysis

From NIBP recordings, beat-to-beat values of systolic blood pressure (SBP), diastolic blood pressure (DBP), pulse pressure (PP), and mean blood pressure (MBP) were obtained and averaged for each level of the blood withdrawal and LBNP protocols. We also investigated the spectral changes in systolic BPV data obtained from the instantaneous SBP series, since BPV is also widely considered as a marker of sympatho-vagal interactions in humans [13]. The BPV data were preprocessed and analyzed using the Welch periodogram method (as previously described in PSD analysis of HRV data) to obtain the spectral powers associated with LF and HF bands and their ratio (denoted as LF_{BPV}, HF_{BPV} and LF/HF_{BPV}, respectively).

BRS was estimated using a frequency-domain approach [14] where the complex-valued transfer function was evaluated as the ratio of the cross-spectral density function between RR and SBP series to the power spectral density of SBP series. This approach increased the ability to measure BRS in all subjects compared with the conventional spectral technique based on classical coherence criterion (≥ 0.5). The cross-spectrum was estimated applying the Blackman-Tukey approach to the cross-correlation between RR and SBP series. The gain estimated as the transfer function modulus was averaged regardless of the value of coherence [14] within the LF, HF band to yield the indices $Gain_{LF}$ and $Gain_{HF}$, respectively. The average of the LF and HF gain values was also calculated as $Gain_{LFHF}$, the whole-band measure of BRS. The coherence, degree of linear coupling between RR and SBP series was also computed and averaged within the LF and HF bands as COH_{LF} and COH_{HF}, respectively.

E. Statistical analysis

Normality of each measure was assessed using D'Agostino & Pearson Omnibus normality test. The statistical significance among different conditions of experiment protocol was assessed using either parametric repeated measures ANOVA or repeated measures nonparametric Friedman test when appropriate. If statistical differences were found, either a Bonferroni post-test or Dunn's post-test was performed appropriately to determine the statistical significance between

TABLE I

HEART RATE VARIABILITY ANALYSIS

	Baseline	LBNP	Recovery	P value
HR (beats/min)	67.2 ± 3.5	114.3 ± 5.1 *	59.1 ± 2.6 †	<0.001
SDNN (ms)	52.89 ± 5.53	44.46 ± 5.57	56.07 ± 6.55	0.336
RMSSD (ms)	20.53 ± 2.44	7.62 ± 1.45 *	23.62 ± 3.01 †	0.001
ApEn	0.90 ± 0.02	0.64 ± 0.05 *	0.88 ± 0.05 †	<0.001
PSD analysis				
LF_{HRV} $(beats/min)^2Hz^{-1}$	8.87 ± 2.55	11.65 ± 3.72	5.16 ± 2.47	0.368
HF_{HRV} $(beats/min)^2$ Hz^{-1}	3.42 ± 1.38	1.69 ± 0.81	1.95 ± 0.60	0.135
LF_{HRV} (nu)	0.72±0.03	0.88±0.02 *	0.64±0.04†	<0.001
HF_{HRV} (nu)	0.27±0.03	0.12±0.02 *	0.34±0.05†	<0.001
LF/HF_{HRV}	3.42±0.76	11.24±2.23 *	2.45±0.54†	<0.001
PDM analysis				
SNS (au)	0.11±0.01	0.18±0.01 *	0.12±0.02†	0.001
PNS (au)	0.25±0.02	0.27±0.03	0.24±0.03	0.187
SNS/PNS	0.69±0.06	1.09±0.08 *	0.80±0.09†	<0.001

Data are given in Mean ± SE, The statistical significance of repeated measures ANOVA/Friedman's test are given as P values; * and † represent post-hoc (Bonferroni/Dunn's test) significance P<0.05 with respect to baseline and LBNP, respectively.

every possible pair of conditions. The statistical significance was set at P<0.05. The data were given in mean ± SE.

III. Results

The analysis of HRV data obtained from 10 subjects during LBNP application is shown in Table I. While the mean HR significantly increased from baseline to LBNP and later decreased from LBNP to recovery, RMSSD and ApEn both decreased from baseline to LBNP and later increased from LBNP to recovery. No significant changes were found between baseline and recovery for mean HR, RMSSD and ApEn. SDNN was not significantly altered (P=0.336) due to LBNP application.

While the absolute LF_{HRV} and HF_{HRV} measures showed no significant changes (Table I), the normalized LF_{HRV} was increased from baseline to LBNP and decreased from LBNP to recovery. SNS activity of PDM analysis also similarly increased from baseline to LBNP and decreased from LBNP to recovery. The normalized HF_{HRV} decreased from baseline to LBNP and later increased from LBNP to baseline. On the other hand, PNS activity showed no significant changes during LBNP and the recovery. Both the LF/HF_{HRV} and SNS/PNS increased from baseline to LBNP and later decreased after the termination of LBNP. The PSD and PDM measures were not significantly different between baseline and recovery conditions.

From the analysis of NIBP recordings obtained in 10 subjects during LBNP protocol (Table II), SBP and PP both decreased from baseline to LBNP and increased from LBNP to recovery. SBP was not significantly different between baseline and recovery, but PP was significantly lower during recovery compared to baseline. On the other hand, DBP increased from baseline to LBNP and later decreased from LBNP to recovery. A significant increase in DBP was also found during recovery with respect to baseline. No significant changes (P=0.081) were observed in MBP due to LBNP application. The maximal LBNP application induced non-hypotensive hypovolemia in healthy subjects as suggested by the normal levels of SBP and DBP (i.e. above the threshold of hypotension). PSD analysis of systolic BPV revealed that both the absolute LF_{BPV} and HF_{BPV} increased from baseline to LBNP and later decreased from LBNP to recovery. But the normalized LF_{BPV} and HF_{BPV}, and LF/HF_{BPV} measures did not show any significant changes (P>0.05) during LBNP protocol.

From the coherence analysis, the coherence at LF band did not show any significant changes (P=0.941) due to LBNP application. The coherence at HF band increased from baseline to LBNP (0.42±0.05 vs. 0.56±0.04, P<0.05) and later decreased from LBNP to recovery (0.56±0.04 vs. 0.41±0.05, P<0.05). The transfer function gains $Gain_{LF}$, $Gain_{HF}$ and $Gain_{LFHF}$ (for total frequency band) decreased from baseline to LBNP, and increased from LBNP to recovery conditions. The coherence and transfer function gain measures were not significantly different between baseline and recovery conditions.

IV. Discussion And Conclusion

A better understanding of the autonomic control mechanisms that regulate cardiovascular system during progressive hemorrhage could provide great benefits to effective patient management in emergency and critical care clinical settings. In the present study, we noninvasively assessed the autonomic reflexes during the application of LBNP up to an individual's maximal tolerance before syncope (that mimics severe hemorrhage) and the results indicated the autonomic control mechanism to be the baroreflex mediated sympathetic excitation characterized by the increase in HR and SNS activity.

Very few studies have investigated the autonomic responses to beyond -50 mmHg of LBNP application [3, 7, 15-16]. Our LBNP study is distinctive in such a way that we examined the autonomic effects of rapid application LBNP up to individual's maximal tolerance. The severe hemorrhage condition induced with maximal LBNP application caused a substantial increase in HR as shown in Table I. Progressive increases in LBNP have been previously shown to elicit linear increase in MSNA [3, 17] which is the direct evidence of increased sympathetic activity. In agreement with Cooke *et al* [15], we observed significant decrease in SBP and PP during maximal LBNP.

The significant decrease in RMSSD and normalized HF_{HRV} from baseline to LBNP reflect the vagal withdrawal during LBNP. A decrease in HF_{HRV} with the progressive increase in LBNP application has been previously reported [7, 16]. However, neither the absolute HF_{HRV} of PSD nor PNS activity of PDM presently showed significant changes during maximal LBNP application. No significant changes in absolute LF_{HRV} observed during LBNP (Table I) is in agreement with Cooke

TABLE II

BLOOD PRESSURE VARIABILITY AND BAROREFLEX SENSITIVITY ANALYSIS

	Baseline	LBNP	Recovery	P value
SBP (mmHg)	125.8 ± 5.5	113.4 ± 3.7 *	124.4 ± 3.7 †	**0.012**
DBP (mmHg)	61.0 ± 3.3	75.5 ± 3.3 *	68.5 ± 2.6 * †	**<0.001**
PP (mmHg)	64.9 ± 3.1	37.9 ± 2.2 *	55.9 ± 2.2 * †	**<0.001**
MBP (mmHg)	82.6 ± 3.9	88.1 ± 3.3	87.1 ± 2.8	0.081
PSD analysis				
LF_{BPV} $(mmHg)^2$ Hz^{-1}	11.26 ± 3.78	27.04 ± 6.11 *	5.81 ± 2.21 †	**<0.001**
HF_{BPV} $(mmHg)^2$ Hz^{-1}	1.00 ± 0.24	5.44 ± 1.40 *	0.58 ± 0.11 †	**<0.001**
LF_{BPV}(nu)	0.85 ± 0.04	0.82 ± 0.04	0.84 ± 0.03	0.709
HF_{BPV} (nu)	0.15 ± 0.03	0.18 ± 0.04	0.15 ± 0.03	0.682
LF/HF_{BPV}	15.21 ± 6.38	8.00 ± 2.42	9.17 ± 2.01	0.601
BRS analysis				
$Gain_{LF}$	8.59 ± 1.43	2.95 ± 0.58 *	10.67 ± 0.95 †	**<0.001**
$Gain_{HF}$	12.72 ± 1.44	2.90 ± 0.46 *	20.53 ± 3.48 †	**<0.001**
$Gain_{LFHF}$	10.65 ± 1.31	2.93 ± 0.47 *	15.60 ± 2.18 †	**<0.001**

Data are given in Mean ± SE, The statistical significance of repeated measures ANOVA/Friedman's test are given as P values; * and † represent post-hoc (Bonferroni/Dunn's test) significance P<0.05 with respect to baseline and LBNP, respectively.

et al [16]. But the normalized LF_{HRV} and LF/HF_{HRV} were found to be increased due to LBNP and later decreased to baseline value after the termination of LBNP. Similarly, SNS activity and SNS/PNS ratio of PDM analysis also increased from baseline to LBNP and later decreased from LBNP to recovery. Meanwhile, the normalized LF_{BPV} did not show significant changes due to LBNP application. An attenuated BRS as shown by the significant decrease in $Gain_{LF}$ and $Gain_{HF}$ due to the maximal LBNP application was found to be consistent with significant decrease in SBP and is in agreement with Cooke *et al* [7]. LBNP application of -60 mmHg has been shown to reduce SBP, stroke volume and cardiac output, and increase HR and peripheral resistance [3]. The present results suggest that the compensatory autonomic mechanism during severe hemorrhage induced with LBNP is predominantly the unloading of baroreflex mediated sympathetic activation that increases the peripheral vascular resistance and HR to counteract the reductions of stroke volume and defend the arterial pressure [7].

The maximal LBNP application resulted in loss of complexity of HRV as manifested by the significant decrease in ApEn. After the termination of LBNP application, the reduced complexity of HRV was reversed as shown by the significant increase in ApEn. This finding agrees with Batchinsky *et al* [18] who also demonstrated that hemorrhagic shock induced in anesthetized swine caused reversible decrease in complexity of HRV. Our results suggest that assessment of HRV complexity may permit identification of casualties with hemorrhagic shock and may be very useful in a clinical setting. One of the limitations of the present study is that the autonomic responses to low levels of LBNP could not be carried out, since the data length at lower levels of LBNP was not sufficient for HRV analysis in seven subjects.

In summary, the rapid application of maximal LBNP that simulated non-hypotensive severe hemorrhage was found to be associated with unloading of baroreflex mediated increased sympathetic reflex.

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