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Heart rate time series: decreased chaos after intravenous lactate and increased non-linearity after isoproterenol in normal subjects

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Abstract

In this study, we reanalyzed our previous heart rate time series data on the effects of intravenous sodium lactate ($n=9$) and intravenous isoproterenol ($n=11$) using non-linear techniques. Our prior findings of significantly higher baseline non-linear scores (NL: S_{netGS}) and significantly lower largest Lyapunov exponents in supine posture in patients with panic disorder compared to control subjects prompted this study. We obtained the largest Lyapunov exponent (LLE), and a measure of non-linearity (NL: S_{netGS}) of heart rate time series. LLE quantifies predictability and NL quantifies the deviation from linear processes. There was a significant increase in NL score, (S_{netGS}) after isoproterenol infusions and a significant decrease in LLE (an increase in predictability indicating decreased chaos), after intravenous lactate in supine posture in normal control subjects. Increased NL scores in supine posture after intravenous isoproterenol may be due to a relative increase in cardiac sympathetic activity or a decrease in vagal activity at least in certain circumstances, and an overall decrease in LLE may indicate an impaired cardiac autonomic flexibility after intravenous sodium lactate, as LLE is diminished by autonomic blockade by atropine. Band analysis of LLE (LF/HF) (LF: 0.04–0.15 Hz and HF: 0.15–0.5 Hz) showed an increase of these ratios during either condition with a higher sympathovagal interaction after the drug administration. These findings may throw new light on the association of anxiety and significant cardiovascular events.

Keywords: Anxiety; Normal control subjects; Non-linear; Chaos; Spectral analysis; Heart rate variability; Largest Lyapunov exponent

1. Introduction

We have been studying the pathophysiology of panic disorders, an anxiety disorder, for two particular reasons. Firstly, it is characterized by autonomic symptoms such as palpitations, shortness of breath and chest pain, in addition to a feeling of intense anxiety, and sometimes a fear of having a heart attack. Secondly, several studies suggest that these patients and other patients with anxiety

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disorders are at a higher risk for significant cardiovascular events and mortality (Coryell et al., 1986; Weissmann et al., 1990; Kawachi et al., 1994). Linear methods such as spectral analysis have been extensively employed in physiology, pharmacology and cardiology to study cardiac autonomic function (Malik and Camm, 1990; Malliani et al., 1991; Yeragani, 1995a). Decreased HRV (heart rate variability) is an important predictor of sudden cardiac death in patients with cardiac disease as well as normal subjects (Kleiger et al., 1987; Bigger et al., 1992; Molgaard et al., 1991).

Spectral analysis of HR usually shows a low frequency peck (LF) around 0.04–0.15 Hz, related to sympathetic as well as parasympathetic activity and a high frequency peak around 0.15–0.5 Hz, related to respiratory sinus arrhythmia and vagal function (Akselrod et al., 1981; Pomeranz et al., 1985; Pagani et al., 1986).

Recent literature has shown the utility of non-linear measures of heart rate (HR) variability to study cardiac autonomic function in health and disease (Goldberger and West, 1987; Katz, 1988; Glenny et al., 1991; Pincus et al., 1991; Yeragani et al., 1993b, 1997; Guzzetti et al., 1996; Lombardi et al., 1996; Voss et al., 1996; Ho et al., 1997; Braun et al., 1998; Storella et al., 1998; Kagiyama et al., 1999; Silipo et al., 1999; Yeragani et al., 2000). Analysis of time series using methods of non-linear dynamics can be performed by the estimation of Lyapunov exponents (LE) and degree of non-linearity (NL). The predictability is quantified by LE. The higher the predictability, the lower the degree of chaos. Interaction of the dynamical parameters is quantified by the degree of non-linearity. Several investigators have found that cardiovascular signals are non-linear and that non-linear techniques may be a valuable addition in this regard.

Some reports have suggested that patients with multiple sclerosis, brain stem lesions and other neurological conditions have decreased HR variability and also a significant decrease in the values of LLE (Ganz et al., 1993; Ganz and Faustamann, 1994; Faustamann and Ganz, 1994; Yotsukura et al., 1998; Monge-Argiles et al., 2000; Nordenbo et al., 1989; Monge-Argiles et al., 1998). This may reflect decreased cardiac flexibility due to a presumed decrease in vagal activity. In our previous study, we found that patients with panic disorders have a significantly higher baseline non-linearity (NL) score, S_{netGS} and a significantly lower LLE in supine posture compared to control subjects ($P=0.00001$) (Radhakrishna and Yeragani, 2001). We have also found that patients with major depression also have a diminished degree of chaos of time series of HR, and also an increase in sympathovagal balance as suggested by an increase in LLE (LF/HF), using band analysis of LLE, a new technique in this area (Yeragani et al., 2001a). This has prompted us to reanalyze our previous data on lactate and isoproterenol in normal control subjects using these new measures as both these substances induce panic anxiety in patients with panic disorder more frequently than in normal control subjects (Pitts and McClure 1967; Pohl et al., 1988). We hypothesized that lactate would significantly decrease LLE due to its vagolytic effects and isoproterenol would result in higher NL scores. Recently we also examined the measures of MED and LLE on VLF, LF and HF bands using filtering techniques of the raw time series and have obtained physiologically meaningful results (Yeragani et al., 2001a,b; submitted for publication). We have used the same technique in this study, especially examining the LF and HF LLE.

2. Methods

2.1. Subjects

Lactate: Nine normal subjects (seven men and two women) participated in the lactate study (age: 25 ± 1.7 years). We have used means and standard deviations (S.D.) throughout the text and tables of this article. The procedures for lactate and placebo infusions and the procedure for data acquisition have been described in detail in our previous reports (Yeragani et al., 1994, 1996). One molar sodium lactate was given at 6 ml/kg body weight over 20 min.

Isoproterenol: Eleven normal subjects (five men and six women) participated in this study (age 25.1 ± 1.3 years). The procedure for the adminis-

tration of isoproterenol was described in detail in our previous report (Yeragani et al., 1995b). The dosage of isoproterenol was 15 ng/kg/min over a 10-min period.

These studies were approved by the Institutional Review Boards at the Lafayette Clinic, Wayne State University School of Medicine, Detroit, MI, USA. All subjects were healthy and informed consent was obtained prior to their participation in these studies. The subjects were physically healthy with no history of hypertension, and their routine blood chemistries and electrocardiograms were within normal limits.

The intravenous infusions were administered in supine posture and an electrocardiogram (ECG) was recorded by a Hewlett-Packard 78173A ECG Monitor in lead II configuration using limb leads. The signal was recorded onto a PC using a 12-bit A/D board, at a sampling rate of 500 Hz in supine posture. We used 256 s of supine data for the analyses in this study. We used an peak detection algorithm to identify the R–R intervals (in ms) from the ECG. The HR (beats/min: 60 000/R–R interval in ms) time series were sampled at 4 Hz using the technique of Berger et al. (1986). We used an HR time series free of ventricular premature beats and noise. We used a tolerance level of 30% to determine ectopic beats and these were replaced by the average values in the immediate (preceding six beats) neighborhood.

2.2. Non-linear measures

The methods were described in great detail in our previous report (Radhakrishna et al., 2000; Radhakrishna and Yeragani, 2001). The reconstruction of the HR time series and the calculation of the MED, LLE and NL were all computed using a PC with custom designed software according to the following methods.

2.3. Time delay embedding and attractor reconstruction

The first step in non-linear dynamical analysis is the reconstruction of the attractor in phase space; since we do not know the a priori coordinates of the phase space, it is necessary to derive them from the observed time series. Takens' (1980) embedding theorem is used to derive them. This theorem ensures that the reconstructed attractor preserves all the properties of the original attractor.

If $x(n)$ for $n=1,2, \ldots, N$ is the time series, then time-delay vectors in phase-space are formed as:

$$X(i)=\left[x(i+\tau),x(i+2\tau)\ldots\ldots x\left(i+(m-1)\tau\right)\right] \quad (1)$$
$$i=1,2,\ldots\ldots,N-(m-1)\tau$$

where τ is the time delay and m is the embedding dimension. There are many methods to choose the time delay; we have used the autocorrelation method in our analysis. This time-delay value differs between different time series of the same length.

2.4. Estimation of minimum embedding dimension (*MED*)

Proper reconstruction of an attractor is guaranteed if the dimension of phase space is sufficient to unfold the attractor. It is shown that an embedding dimension of $m \geq 2d+1$ can achieve this, where d is the dimension of the attractor (Takens, 1980). In most cases of observed time-series analysis, we neither have knowledge of d or m. There are many different algorithms used in the estimation of these quantities (Grassberger and Procaccia, 1983; Theiler, 1987; Broomhead and King 1986; Mees et al., 1987; Kennel et al., 1992), but many of them have the disadvantage of either being too subjective and requiring a large number of data points or being computationally very intensive. The method proposed by Cao (1997) overcomes these difficulties and is suitable for short-term time series. Additionally, this method gives more reliable estimates of MED, even when the dimension is sufficiently large. The readers are referred to the above article for a detailed description of this technique.

We applied this method on time series of some of the standard maps and found their MED to tally with the literature.

2.5. Subjectivity of arriving at MED

Although subjectivity is a cause for some concern when MEDs are calculated by many people, it can be substantially reduced by training only a

few people to do this, and in this particular study, one of the authors calculated all the MEDs blind to the patients' condition. We chose the point of the beginning of saturation on the graph after plotting the *E*1 values. All personnel who were calculating were trained to use the same technique to find the point of saturation.

2.6. Largest Lyapunov exponent (LLE)

Lyapunov exponents are another invariant, which could be used to characterize the dynamical system. It quantifies sensitivity of the system to initial conditions. An *m*-dimensional dynamical system has *m* Lyapunov exponents. The presence of a positive Lyapunov exponent indicates chaos. It also quantifies the amount of instability or predictability of the system. A fully deterministic system will have a zero Lyapunov exponent since it is fully predictable, whereas a random system will have a large positive exponent, indicating no predictability. In most applications, it is sufficient to compute only the largest Lyapunov exponent (LLE) instead of all Lyapunov exponents. There are many algorithms available to estimate the LLE and Lyapunov spectrum (Sano and Sawada, 1985; Wolf et al., 1985; Sato et al., 1987; Zeng et al., 1991). Most of them are unreliable when operated on small data sets. In our present work, we used the method proposed by Rosenstien et al. (1993), which is robust against data length.

After reconstructing the attractor, this algorithm looks for the nearest neighbors of each phase point X_i on trajectory. The distance between two neighboring points at instant $n=0$ is defined by:

$$d_i(0) = \min_{X_j} \| X_j - X_i \| \tag{2}$$

where $\|..\|$ is the Euclidean norm. This algorithm imposes the constraint that nearest neighbors are temporally separated at least by the mean period of the time series. The LLE is then estimated as the mean rate of separation of nearest neighbors, i.e. we can write:

$$d_i(i) \approx C_j e^{\lambda_1 (i\Delta t)} \tag{3}$$

where C_j is the initial separation. Taking logarithm on both sides of Eq. (3), we obtain:

$$\ln\big(d_j(i)\big) = \ln C_j + \lambda_1 (i\Delta t) \tag{4}$$

Eq. (4) represents a set of approximately parallel lines (for $j=1, 2, \ldots, M$), where the slope is roughly proportional to the LLE. In practice, the Lyapunov exponent is easily and accurately estimated using a least-squares fit to the 'average' line defined by:

$$y(n) = \frac{1}{\Delta t} \big\langle \ln d_i(n) \big\rangle \tag{5}$$

where $\langle\ \rangle$ denotes the average over all values of *i*. This last averaging step is the main feature that allows an accurate evaluation of λ even when we have a short and noisy data set.

2.7. Tests for non-linearity

The erratic fluctuations that are observed in an experimental time series owe their dynamical variation to a mix of various influences: chaos, non-chaotic but still non-linear determinism, linear correlation, and noise, both in the dynamics and in the measuring setup. This emphasizes the need for estimating a non-linear structure in the time series. In our present work, we investigate a non-linear structure present with an HRV time series using two methods. We check whether non-linear time correlations are present among the time-series values. Both methods are based on the analysis of the extrema (local maxima or minima) as proposed by Di Garbo et al. (1998).

Though we have used only the values of S_{net}GS in this article, we are presenting a detailed description of the techniques that were used in the calculation of these measures.

2.7.1. Non-linearity test based on extrema of a time series

It has been shown that the dynamical behavior of the real time solution of an ordinary differential equation (ODE) is strongly connected to its analytical properties in the complex time plane, and in particular, to the distribution of the singularities nearest to the real axis (Ramani et al., 1989). The second consideration arises from a general property of a stochastic process, which states that given a mean square differentiable stochastic process $x(t)$,

the expected number of its extrema for unit time is contained in the joint density function of $x(t)$, $x^{\cdot}(t)$ and $x^{\cdot\cdot}(t)$ (Soong, 1973). These theoretical and numerical results suggest that the sequence of extrema of a time series contains dynamical information of the process generating them. Both methods statistically discriminate measures evaluated based on extremas for original and surrogate data sets.

Two types of surrogates are considered in our analysis, Fourier Shuffled (GS) and Phase Randomized (PR) surrogates. The following is the procedure used in generating Fourier shuffled surrogates:

1. Histogram Transformation;
2. Fast Fourier Transformation (FFT);
3. Phase Randomization;
4. Inverse FFT; and
5. Inverse Histogram Transformation.

In the case of PR surrogates, steps 1 and 5 are eliminated. We will give a brief description of two techniques used in quantifying non-linear structure in time series.

2.7.2. Pattern of singularities in the complex time plane (PSC) algorithm

The steps involved in quantifying non-linear correlations with the PSC method are:

1. Determine the couples $\{s_{t_j}, t_j \text{ for } j=1, 2, \ldots, n\}$ corresponding to local maxima and time, at which it occurred.
2. Determine the length of the broken line joining these extremas.

$$L=\sqrt{\sum_{j=1}^{n-1}\{(s_{t_{j+1}}-s_{t_j})^2+(t_{j+1}-t_j)^2\}}$$

3. n number of surrogates are generated and L for each surrogate is computed.
4. Determine the mean L_s and S.D. σ_s of these quantities.
5. Determine the measure of significance as proposed by Theiler et al. (1992):

$$S_{\mathrm{PSC}}=\frac{|L-L_s|}{\sigma_s}$$

2.7.3. Number of extrema for unit time (NET)

The following is the protocol of the NET method:

1. The number of extrema N_o for unit time, T_o, of the given time series is determined and used as discriminating statistics.
2. n numbers of surrogate data sets are generated and the number of extremas for each surrogate set N_i $(i=1, \ldots, n)$ is computed.
3. The average number of extrema for unit time N_s and their S.D. σ_s are determined and they are statistically discriminated by computing the significance:

$$S_{\mathrm{net}}=\frac{|N_o-N_s|}{\sigma_s}$$

Again, two types of surrogates are considered in our analysis: Fourier Shuffled (GS) and Phase Randomized (PR) surrogates. These are referred to as S_{netGS} and S_{netPR}, respectively.

2.8. Stability of the LLE and $\mathbf{S}_{netGS}$ values

We have used up to 1024 data points sampled at 4 Hz, which yielded 4096 points for the calculation of the MED and LLE from the Holter data sets available to us. We obtained results that significantly correlated with the ones we obtained with just 256 s of data sampled at 4 Hz (1024 data points). Thus, we inferred that Rosenstien et al.'s (1993) algorithm is robust for data sets with points up to 1000 points.

In our first report, where we used 256 s sampled at 4 Hz (1024 data points) (Radhakrishna et al., 2000), LLE in supine posture was 0.14 ± 0.03 vs. 0.19 ± 0.04 in standing posture, making the difference highly significant ($P<0.00001$). For the same data set, the MEDs were 12.6 ± 1.9 vs. 9.9 ± 2.7 ($P<0.00001$). These were paired tests and the number of subjects was 27.

In the second article, where we compared patients with panic disorder (Radhakrishna and Yeragani, 2001), there were highly significant differences with a great deal of statistical stability in supine posture [patients ($n=36$) vs. control subjects ($n=36$): MED: 12.1 ± 2 vs. 14.1 ± 2; $P<0.00001$; LLE: 0.14 ± 0.03 vs. 0.11 ± 0.02; $P<$

0.00001, respectively]. This finding again stresses the fact that, under certain laboratory conditions, even a series as short as 256 s might give valuable information. For S_{netGS}, the values for control subjects were 6.5 ± 3.2, and for patients, 10.2 ± 2.6 ($P < 0.00001$). In another recent article (Yeragani et al., 2001a), we were again able to show a high degree of consistency of results comparing normal control subjects and patients with depression, showing a diminished degree of chaos of HR time series in depression.

2.9. Reasons for using 256 s of data sampled at 4 Hz (1024 points)

First of all, in biological systems, it is usually not possible to obtain stationary data for long periods of time without non-stationarity and influences such as emotions and physiological influences. This becomes even more difficult when one uses an experimental paradigm wherein one uses intravenous substances to quantify the effect of certain drugs that provoke anxiety as well as increase in HR. Here, finding the most appropriate segment, which is stationary, becomes even more difficult. These experiments are also time-bound, and thus any algorithms of non-linear techniques that are robust for small data sets are of immense importance in this type of research.

We have also found a high degree of correlation between the values of LLE of two 5-min segments of HR time series, which were recorded in the laboratory under resting conditions, which also emphasizes the consistency of these results.

2.9.1. Sampling frequency and non-linear measures

When we used three typical subjects with 256 s of time-series data, the increase in sampling rate resulted in an increase in NL scores and the LLE with higher sampling rates of the signal. The same 256-s time series were sampled at 2, 4 and 8 Hz (512, 1024 and 2048 points) and there was an increase in NL scores and the Tau value with increasing sampling rate. This underscores the importance of the same sampling rate and the number of points to be used while comparing different populations. This partly explains the increase in the LLE, also with higher sampling rates. The calculation of MED did not change significantly with an increasing number of points even up to 8192, and the Tau values and MED values did not have much effect on LLEs in many circumstances.

2.9.2. Reliability of the NL measures

We took two 5-min segments recorded from another set of data that were recorded under resting conditions and compared the number of pairs from 15 to 30 and obtained very similar results for both epochs, in supine posture for 10 min, and compared them for the values of NL scores, MED and LLE for the consistency. The '*R*' values for $S_{net}GS$, MED and LLE were 0.92, 0.75 and 0.77, respectively (all $P < 0.01$). The respective means for the first and the second 5-min segments were 7.53 ± 2.0 vs. 7.45 ± 2.6, 11.4 ± 1.2 vs. 11.7 ± 0.95 and 0.138 ± 0.01 vs. 0.134 ± 0.01, respectively, for the above three NL measures. Now we also perform MED and LLE on a long-term series of 3600 beats and these results also yield essentially very similar results to what we obtained with 256-s segments (unpublished observations).

2.9.3. Filtering of the time series

The time-series data were filtered into VLF, LF and HF bands to include, predominantly, 0–0.04 Hz, 0.04–0.15 Hz and 0.15–0.5 Hz. We have previously used a similar technique to perform band analysis of the signal using FD and APEN (Yeragani et al., 1993b). We attempted to compare how each of these bands contributes toward the non-linearity and chaos of the UF time series. As we were particularly interested in possible measures of vagal and relative sympathetic activity, we calculated relative LLE-LF as (LLE-LF/LLE-UF) and also for the quantification of sympathovagal balance, we calculated (LLE-LF/LLE-HF) in supine posture before and after drug administration. Due to the nature of the filtering algorithm and the coefficients used for filtering, for the VLF, LF and HF time series, we used only 924 points, eliminating the first 50 and the last 50 points of data (12.5 s each).

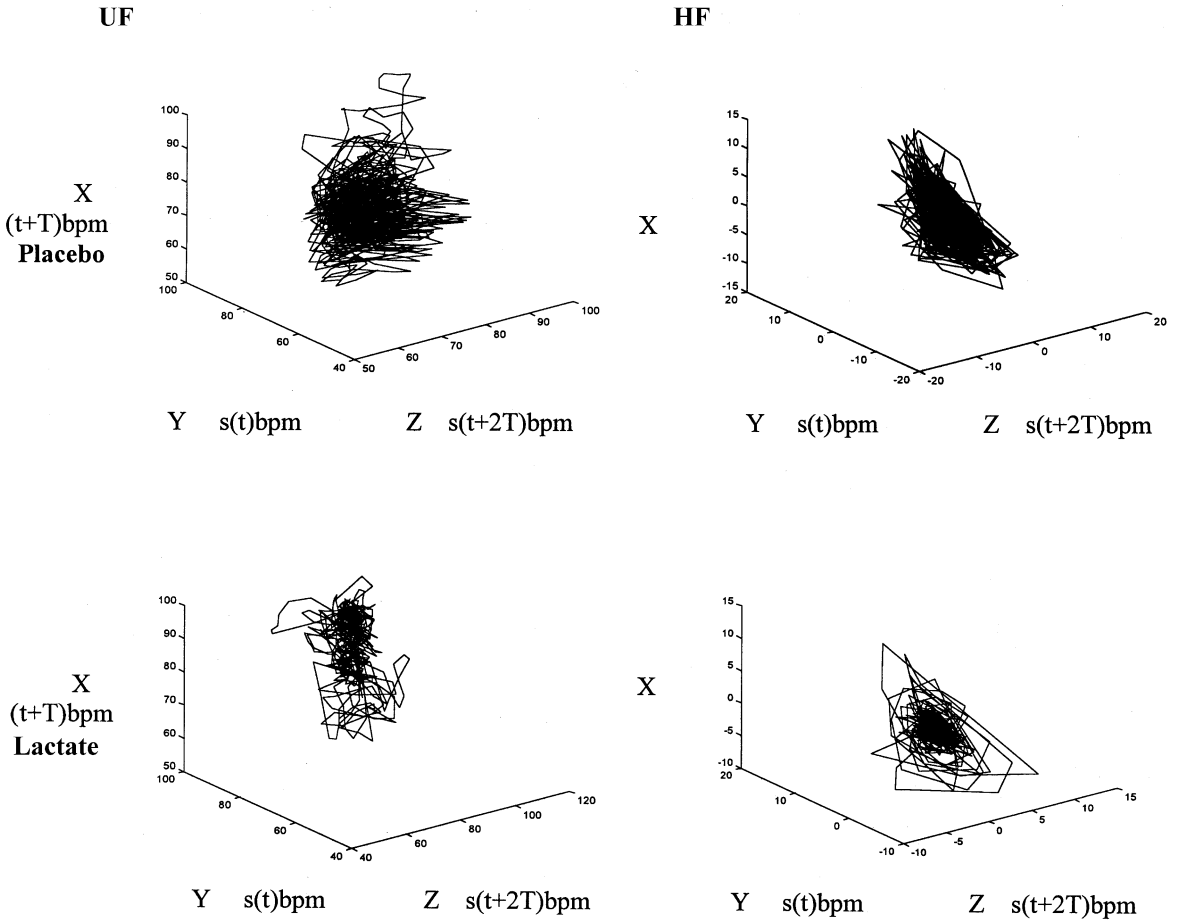

Fig. 1. The attractors of unfiltered and high-frequency filtered HR time series for placebo and lactate conditions.

2.9.4. Length of data series

As there was a significant difference between filtered and unfiltered time series in this study, we thought that this might have been due to a 1024-point time series, and hence, tested this algorithm to calculate MED on 8000 data points. Even in this instance, there is a consistent and significant difference between different MEDs of different bands.

Then we tried to take the VLF series (0–0.04 Hz) and filtered them again using a higher bandwidth so that all the frequencies up to 0.05 Hz would be allowed to pass. This did not result in any significant change of the attractor or the MEDs (unpublished observations). Thus, appropriate filtering of the data using the physiologically significant bands may yield important information.

2.9.5. Statistical analysis

We used paired '*t*' tests to compare LLE, MED and S_{netGS} for placebo and drug comparisons. All tests were two-tailed and a probability value of 0.05 was accepted as significant.

3. Results

Fig. 1 shows the attractors of unfiltered and high-frequency filtered time series of heart rate for placebo and lactate conditions. Table 1 shows the values of placebo and lactate infusion for HR non-

Table 1
Non-linear indices of HR of control subjects before and after sodium lactate (mean ± S.D.) ($n=9$)

Supine	Placebo	Lactate	t	P
HR	66.1 ± 13.6	102.4 ± 14.0	11.9	0.00001
UF-MED	11.0 ± 1.1	12.2 ± 1.7	5.12	0.0009
UF-LLE	0.11 ± 0.02	0.09 ± 0.02	4.7	0.002
LF-MED	14.9 ± 1.2	13.0 ± 2.1	3.2	0.01
LF-LLE	0.09 ± 0.01	0.10 ± 0.02	2.7	0.03
HF-MED	15.9 ± 2.1	17.4 ± 2.2	3.8	0.005
HF-LLE	0.07 ± 0.01	0.06 ± 01	4.0	0.004
LLE (LF/HF)	1.37 ± 0.25	2.0 ± 0.62	3.7	0.006
S_{netGS}	3.1 ± 2.6	4.2 ± 3.2	0.96	NS

d.f. = 8.

linear measures (mean ± S.D.). LLE-UF and LLE-HF were significantly lower in control subjects after lactate ($P=0.002$ and 0.004, respectively), and MED was significantly higher ($P=0.002$ and 0.005), whereas LLE-LF was significantly higher ($P=0.03$). This has resulted in a significant increase in LLE (LF/HF) ratios for the lactate condition ($P=0.006$). Table 2 shows the values of placebo and isoproterenol HR non-linear measures. There was a significant increase in S_{netGS} after isoproterenol infusions ($P=0.03$), as well as a significant increase of LLE-LF after isoproterenol infusions ($P=0.03$). Isoproterenol was also associated with a significant decrease of LLE-HF ($P=0.04$). This has also resulted in an increase in LF/HF ratios of LLE after isoproterenol infusions ($P=0.004$).

There were also no significant correlations between LLE and the HF power (0.15–0.5 Hz) of HR time series.

4. Discussion

These findings should be viewed in the context of our previous findings of decreased HR or HP (heart period) variability in patients with panic disorder compared to control subjects and also the effects of yohimbine, suggesting a relative increase in cardiac vagal function in anxiety (Yeragani et al., 1992, 1993a, 1998). The main findings of this study are that sodium lactate, a drug that induces symptoms of panic anxiety in patients with panic disorder and a minority of normal control subjects, has produced a substantial decrease in LLE of unfiltered time series and also that of HF filtered series, which strongly suggests a vagolytic effect. It also resulted in an increase in LLE (LF/HF) ratios, which might indicate sympathovagal balance. We and other investigators have previously shown that lactate infusions are associated with vagolytic effects on the heart (George et al., 1989; Yeragani et al., 1994, 1996). Thus, it is tempting to speculate that a decrease in HR HF power, and thus decreased RSA, is associated with decreased LLE, but this issue is complex. First, LLE increases very significantly in standing posture and also decreases during controlled breathing (Radhakrishna et al., 2000; Radhakrishna and Yeragani, 2001). However, as stated in the introduction, a decreased LLE is associated with different CNS disorders and appears to indicate a decrease in flexibility in cardiovascular control. This may be related to some aspect of vagal function and may further explain the higher incidence of significant cardiovascular events and sudden cardiac death (Voss et al., 1996; Ho et al., 1997). In this context, our previous findings of decreased LLE in patients compared to control subjects in supine as well as standing postures ($P<0.00001$) is of special interest. In that study, a higher HR alone in patients did not explain this finding (Radhakrishna and Yeragani, 2001). It should also be noted that LLE of HR time series is blocked by atropine (Hagerman et al., 1996; Zweimer et al., 1996).

Isoproterenol also induces panic attacks in patients with anxiety disorders, and thus its effect

Table 2
Non-linear indices of HR of control subjects before and after isoproterenol (mean ± S.D.) ($n=11$)

Supine	Placebo	Isoproterenol	t	P
HR	65.7 ± 9.5	86.6 ± 11.2	9.08	0.00001
UF-MED	14.3 ± 5.3	14.0 ± 3.5	0.12	NS
UF-LLE	0.13 ± 0.06	0.11 ± 0.05	0.60	NS
LF-MED	15.1 ± 3.0	13.2 ± 2.8	3.3	0.008
LF-LLE	0.09 ± 0.02	0.11 ± 0.03	2.6	0.03
HF-MED	15.1 ± 2.4	16.4 ± 2.3	1.7	0.12
HF-LLE	0.08 ± 0.03	0.06 ± 0.01	2.4	0.04
LLE (LF/HF)	1.20 ± 0.45	1.79 ± 0.53	5.3	0.0004
S_{netGS}	8.9 ± 2.4	12.3 ± 3.7	2.60	0.03

d.f. = 10.

in normal control subjects on cardiac autonomic function is of particular interest. It is a beta-adrenergic agonist that increases HR. The increase in LLE-LF and LLE (LF/HF) suggests a relative increase in cardiac function during isoproterenol infusions. The increase in S_{netGS} in this study after isoproterenol may indicate that some NL scores are also affected to some extent by sympathetic activity. This should be viewed in the context of our recent finding of a significantly higher S_{netGS} in patients with panic disorder compared to control subjects (Radhakrishna and Yeragani, 2001). It also correlated positively with the State Anxiety Inventory score (SAI, Spielberger et al., 1970) in that study. Thus, this measure may be somewhat related to anxiety and adrenergic effects. On the other hand, one cannot rule out the effect of vagal withdrawal in increasing the score of this non-linear measure. These new measures need further investigation.

The other unexpected finding from band analysis of LLE is that isoproterenol resulted in a small but significant decrease in LLE-HF, which suggests vagal withdrawal. We are unable to explain the finding at this time. However, it is tempting to speculate that vagal withdrawal might be one of the mechanisms that is associated with panic attacks in patients with panic disorder during isoproterenol infusions. However, before any definitive conclusions can be drawn, these measures should receive a comprehensive evaluation of the effects of age and the effect of parasympathomimetic and parasympatholytic substances, and adrenergic agents. It also needs to be seen how LLE is affected by various autonomic agents and the effects of various therapeutic agents.

The low values of S_{netGS} for the lactate condition cast some doubt on the normal range for this variable, but this is a small group of subjects and the values were obtained before an experimental procedure that was supposed to induce panic anxiety in patients with anxiety and that in some instances produces an increase in the state of anxiety in normal control subjects too. However, the increase in this measure during isoproterenol infusions may also be dependent upon vagal withdrawal in addition to cardiac sympathetic function as we found a significant decrease of LLE-HF in this study on band analysis, and future studies have to address this issue.

In our previous reports, we found that approximate entropy (APEN) and fractal dimension (FD), using the method of Katz (1988), correlated highly significantly with each other and also with the HF power on spectral analysis (Yeragani et al., 1993b, 1997). Unlike these measures, MED, LLE and NL measures had no substantial correlation with any of the time-domain measures (Radhakrishna et al., 2000). Though in this study HR was more significantly different before and after intravenous lactate or isoproterenol compared to the changes in LLE, one should realize that HR could increase due both to an increase in sympathetic activity and vagal withdrawal, and may also be related to other mechanisms. To separate these two main mechanisms is of immense importance in the understanding of the pathophysiology and treatment of various anxiety disorders.

Though these new measures appear promising, valuable additions to the linear measures, further studies are needed to elucidate the autonomic effects of various agents on these measures more clearly so that their effects in clinical populations such as anxiety disorders may be interpreted meaningfully.

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