Time-Frequency Analysis of Heart Rate Variability in Neonatal Piglets Exposed to Hypoxia

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Abstract

To determine whether the sympathetic nervous system plays any role in the inter-individual variation of cardiovascular response to hypoxia in newborns, neonatal heart rate variability (HRV) analysis was used to assess autonomic response during hypoxia in piglets. Due to the nonstationary nature of HRV signals and the inaccuracy in pre-defined neonatal HRV frequency bands using standard spectral methods, we applied time-frequency distribution analysis to assess neonatal HRV.

Although sympathetic activity was initially enhanced to stimulate the cardiac compensation, it did not account for the variation of cardiovascular response since the power of low frequency component in HRV neither showed corresponding changes with the heart rate nor had any correlation with the brain injury. This may be attributed to immaturity of the neural pathway.

1. Introduction

Hypoxic-ischemic cerebral injury resulting from perinatal asphyxia is a significant contributor to poor neurodevelopment in newborns [1]. The cardiovascular function (CVF), which is vital in compensating the oxygen supply and perfusion during hypoxia in neonates, protects the essential organs such as brain and heart by the redistribution of cardiac output; and subsequently determines the cerebral blood flow (CBF) which enters a pressure-passive state as a result of continued hypoxia [1]. The failure of cardiovascular compensative response to hypoxia may cause irreversible damage to the neonatal nervous system.

The ability to keep the CVF plays a significant role in maintaining the CBF and, therefore, in preventing neural injuries in newborns. The authors in [2] have reported that there is an inter-individual variation in the response of heart rate (HR) and blood pressure (BP) to hypoxia. Also, it has been found that in the piglet model, the longer HR and BP remain above the baseline during hypoxia, the better the neurological outcome [2]. However, it is not clear whether this variation in CVF is intrinsic to the heart or mediated by its regulatory mechanisms such as the autonomic nervous system (ANS) [3].

ANS has two branches, namely sympathetic nervous system (SNS) and the parasympathetic nervous system (PNS). The SNS can rapidly increase HR and BP whereas the PNS functions in the opposite way [5]. The ANS regulate the HR and BP by their interaction. Thus, it can be inferred that the ANS activity mediates the cardiovascular response to hypoxia. Moreover, it is known that under hypoxic condition, the sympathetic branch of the ANS is exalted while the parasympathetic one is suppressed. Therefore, we hypothesize that the SNS activity results in the variation of the cardiovascular response; in other words, the response of HR and BP during hypoxia is expected to be accompanied by corresponding sympathetic activity [3]. In this study, we attempted to verify this hypothesis using heart rate variability (HRV) analysis to trace the evolution of autonomic activity during hypoxia in newborn piglets.

HRV is the heart rate variation resulting from the interaction of SNS and PNS. It has been widely used as a non-invasive tool to assess the ANS [5]. Spectral analysis of HRV provides specific information related to cardiac sympathetic and parasympathetic (vagal) activities. It is generally considered that the low frequency (LF) component of HRV is associated with both SNS and PNS functions in normal conditions [5]. In hypoxic condition, however, the LF component is mostly contributed by the sympathetic excitation. This led us to extract the spectral power of LF component to quantify the SNS activity.

The widely used LF band (0.04-0.15 Hz) has been established for human adults but not suitable for neonates (human and animals) due to the difference in the maturity of the ANS [6]. Also, the conventional spectral analysis approach based on the Fourier transform has two main limitations: the assumption of stationarity and the lack of temporal information [7].

Due to the above-mentioned drawbacks of the classical spectral analysis, the joint time-frequency distribution was introduced as an alternative approach. A time-

frequency distribution (TFD) can be thought of as the signal energy distribution over both time and frequency [8]. This enables the TFD analysis to not only provide the spectrum of HRV but also its evolution over time. Moreover, this approach allows the identification of the LF component without prior definition of frequency band.

In this study, we applied TFD analysis to characterize HRV of neonatal piglets exposed to hypoxia. The LF location was determined in terms of instantaneous frequency (IF). The power of LF component was used as a quantification of sympathetic activity. The evolution of the LF power over time was assessed to determine whether the SNS activity is the source of the variation of the cardiovascular response to hypoxia.

2. Methodology

2.1. Experiment and data acquisition

We used a neonatal piglet model of hypoxia-ischemia to simulate human perinatal asphyxia as the piglet has similar ontogenesis of the nervous and cardiovascular functions to that of a human baby [9].

Six full-term 1-day-old piglets were anaesthetized. The piglets were intubated and ventilated using a neonatal ventilator at a rate of 30 breaths per minute (BPM). HR and BP were monitored by standard neonatal intensive care techniques. Hypoxia was induced by reducing the ventilation rate to 10 BPM and the fraction of inspired oxygen (FiO_2) to 0.1 for 45 minutes. More details about the experiment can be found in [3].

The electrocardiogram (ECG) signals for HRV analysis was recorded by Powerlab (ADInstruments, Sydney, Australia) at a rate of 1 kHz. Ten-minute signal prior to hypoxia was used to compute the HRV baseline. The ECG was processed using MATLAB (Version R2009b, Mathworks Inc, Natick, MA).

The neurological outcome was determined by an integrated marker combining physiological measures and structural marker. This approach provides a reliable early assessment of neural injury by giving a neurological outcome score (NOS) between 0 (worst) and 20 (best) [2]. Another indicator of neural damage used is the time that BP remains below 70% baseline level (BP index) that is found to be inversely proportional to brain injury [2].

The pared *t*-test was used to analyze the difference between the LF location and its associated power before and during hypoxia. Correlations between LF component variation and hypoxic outcomes were studied using linear regression. A value with $p < 0.05$ was considered be statistically significant.

2.2. Computing HRV from ECG

The HRV signal was derived from the newborn piglet ECG as follows: Firstly, a method based on Hilbert transform and thresholding [11] was used to locate the R-peaks of the ECG. The instantaneous HR was computed as the inverse of the time difference of consecutive R-peaks. Outliers due to premature ventricular contractions and nonuniform sampling of the HR time series were handled by cubic spline interpolation. The resulting signal was uniformly resampled at 4Hz. The mean value and linear component were lastly subtracted from the resampled signal.

2.3. Time-frequency distribution

TFD is capable of characterizing nonstationary multicomponent signals such as HRV. A classic TFD, known as Wigner-Ville distribution (WVD), gives an ideal representation of monocomponent linear FM signals but introduces cross-terms in the cases of multicomponent or nonlinear FM signals. To overcome this limitation, a reduced-interference distribution (ρ_z) is usually used, which is defined as

$$\rho_z(t,f) = W_z(t,f) \overset{**}{_{(t,f)}} \gamma(t,f) \tag{1}$$

where $W_z(t,f)$ denotes the WVD and $\gamma(t,f)$ represents a two-dimensional (2D) smoothing kernel used to reduce the cross-terms but at the expense of a loss of time-frequency resolution [8]. The symbol $\overset{**}{_{(t,f)}}$ denotes the double convolution in time and frequency.

A large number of kernels have been proposed to reduce the cross-terms [8]. In a work [7] involving a comparison of four TFDs representing newborn HRV signals, modified B-distribution (MBD) is found to outperform the others in terms of cross-term reduction and time-frequency resolution. So we chose MBD to represent the HRV signals. Its kernel is defined by

$$g_\beta(t) = cosh^{-2\beta}(t) / \int cosh^{-2\beta}(\varsigma) d\varsigma \tag{2}$$

where the parameter β controls the trade-off between the cross-term suppression and the time-frequency resolution. The optimal value of β was obtained by minimizing a performance measure proposed in [12]. The optimal value of β was found to be 0.01.

2.4. IF estimation

45-min HR signals were divided into 3-min epochs with 50% overlap. Each epoch was mapped to the time-frequency domain using the MBD. The TFD over the 45 minutes was obtained by connecting 25-75% of the consecutive TFD segments in the time direction.

The HRV components were obtained by estimating the IFs of multicomponent signals according to a previously

proposed TF-based method [13]. This method was conducted as follows: The maximum peaks in the TFR were firstly found using the first and second derivatives with respect to frequency. The result was transformed into a 2D binary image by thresholding. The multiple IFs were finally obtained by a component linking process. Three components were identified and the middle one was considered as LF component. The peak coordinates provided the time and frequency location of the HRV components while the power (magnitude) was used to quantify the SNS activity. For each instantaneous component, the power was defined by the area between the mainlobe centered on the estimated IF and the horizontal axis. We selected a threshold value of $\mu = 0.001 \times$(maxima of peaks), which showed the best performance by comparison in the present application.

3. Results and discussions

3.1 Frequency location of LF component

Table 1 shows the mean frequency with its standard deviation of the LF component peaks for different piglets before and during hypoxia. The mean LF changed from 0.038(±0.002) Hz before hypoxia to 0.035(±0.003) Hz during hypoxia but these differences were not found to be statistically significant ($p = 0.095$). This result is in line with those in the literatures. Kuwahara *et al.* [14] defines the LF band to be 0.01-0.07 Hz when analyzing miniature swine HRV. Zwiener *et al.* [9] reports that the spectral power between 0.02-0.08 Hz in the piglet HRV is partly reduced by β-adrenergic blockade, which indicates the range of LF component. The frequency location exhibited in newborn piglets is lower than that seen in the adult (0.04-0.15 Hz with the center close to 0.1 Hz) [5]. Thus, this result confirms that adult frequency bands used in HRV analysis are not suitable for newborns.

Table 1. Frequency locations of LF component for piglets before and during hypoxia

Piglet No.	Pre-hypoxia Mean ± (STD) (Hz)	Hypoxia Mean ± (STD) (Hz)
1	0.038 ± (0.008)	0.032 ± (0.005)
2	0.038 ± (0.010)	0.037 ± (0.004)
3	0.038 ± (0.014)	0.031 ± (0.007)
4	0.043 ± (0.008)	0.037 ± (0.009)
5	0.036 ± (0.010)	0.037 ± (0.009)
6	0.037 ± (0.010)	0.037 ± (0.007)
Overall average	0.038 ± (0.002)	0.035 ± (0.003)

3.2. Power of LF component

The power of LF component in piglet HRV increased at the beginning of hypoxia and then declined to the baseline level within 5 minutes (R2B time). Table 2 shows the mean power at the initial stage of hypoxia (mean IS power) which significantly increases compared to baseline level ($p<0.05$). The power maintained its value around the baseline level afterward. For instances, Figure 1(a) and 1(b) respectively depict the HR of piglet No.1 and No.2 during hypoxia with the baselines (dashed line). LF power of the piglets HRV are shown in Figure 1(c) and 1(d) where the average power is 4.03 and 3.81 fold that of their baseline (dashed line) and returns to the baseline level after respectively 2.2 and 4.2 minutes of the hypoxic insult.

Table 2. The LF power in early stage of hypoxia and outcome after hypoxia in newborn piglets

No.	Baseline power (10^{-3} sec^{-2} /min)	R2B time (min)	mean IS power (10^{-3} sec^{-2} /min)	Elevation (fold)	BP index (min)	NOS
1	290.3	2.2	1168	4.03	27.33	13.47
2	389.5	4.2	1485	3.81	8.67	12.06
3	197.3	4.3	535.1	2.71	4.58	11.36
4	296.6	4.2	707.0	2.38	9.92	14.44
5	215.4	1.9	1166	5.41	8.66	11.25
6	376.4	2.8	1384	3.68	0.00	16.59

The initial increase in LF power can be interpreted as the sympathetic reaction to hypoxia. The enhanced SNS stimulation accelerates the HR and strengthens the cardiac contractility in order to maintain the cardiac output. It should be noted that the LF power reduction existed prior to the HR reaching maxima, as shown by the vertical dashed line in Figure 1. This may indicate that the sympathetic pathway is not fully functional or perhaps not the dominant component of the response.

In our study, the evolution of the LF power was similar in all piglets. It has been reported that neonatal piglets have better neurological outcome if their HR and BP are maintained above baseline level for a longer period during hypoxia [2]. In fact, the LF power presented here does not correspond with the response of the HR. In Figure 1, it took longer time to decrease the HR of the second piglet below the baseline level in contrast to the first one without any significant difference of the LF power tendency. In addition, linear regression analysis showed no correlation between the initial response of LF component to hypoxia and the severity of brain injury evaluated by NOS and BP index (Table 3). These findings tend to indicate that the sympathetic activity may not be the cause of the inter-individual variation of cardiovascular response to hypoxia.

Lee *et al.* [4] reports that HR increases in response to acute hypoxia even when the sympathetic ganglion is

blocked; in contrast, the HR response is eliminated by adrenalectomy. These results demonstrate that circulatory catecholamine released from adrenal plays a dominant role in regulating the neonatal cardiac response to hypoxia. Moreover, it has been found that the swine at birth is less sensitive to the norepinephrines and the maturation of chronotropic response continues in the first week of birth (under normoxic condition) [10]. Through the continuous HRV analysis, our results extend these findings by showing that sympathetic activity contributes to the initial cardiovascular response to hypoxia. The activity tends be disrupted possibly due to the immaturity of the SNS. Instead, the catecholamines derived from adrenal may mediate the continued response to hypoxia.

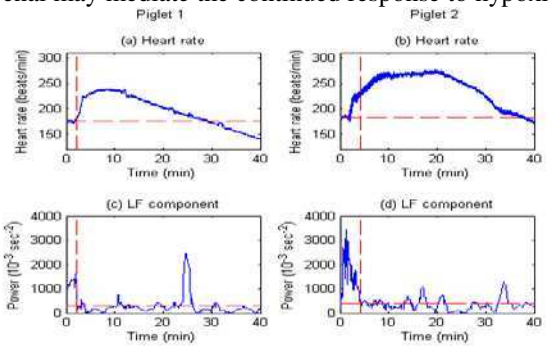

Figure 1. Newborn piglet HR and LF component behaviors during hypoxia.

Table 3. Correlation coefficients and significance between LF response and outcome after hypoxia

	R2B time		mean IS power		Elevation	
	R^2	p	R^2	p	R^2	p
BP index	0.137	0.471	0.002	0.930	0.032	0.735
NOS	0.008	0.867	0.061	0.637	0.085	0.575

4. Conclusion

In this study, we have used TFD to characterize the behavior of the HRV LF component of newborn piglets subjected to hypoxia. The findings tend to indicate that due to its immaturity, the newborn sympathetic nervous system may not be the dominant factor in mediating the cardiovascular response to hypoxia. Neuronal response has been found in the older piglets which have a more mature autonomic function. Thus, the timing of the SNS maturation should be investigated in a future study.

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