Nonlinear properties of vagal and sympathetic modulations of heart rate variability in ovine fetus near term

Martin G. Frasch,1,2 Thomas Müller,3 Dirk Hoyer,4 Christian Weiss,2 Harald Schubert,3 and Matthias Schwab2

1Department of Obstetrics and Gynecology, Lawson Health Research Institute, The University of Western Ontario, London, Ontario, Canada; 2Department of Neurology, 3Institute of Laboratory Animal Science, 4Biomagnetic Center, Department of Neurology, Friedrich Schiller University, Jena, Germany

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Fetal heart rate (FHR) monitoring is commonly used although clinical studies questioned its diagnostic value. Sophisticated FHR variability (fHRV) measures such as fHRV complexity may improve the sensitivity and specificity of FHR monitoring. A more detailed understanding of the physiology underlying fHRV complexity is essential to harness its use for monitoring fetal health. To examine the specific effects of vagal and sympathetic modulations on fHRV complexity, we blocked vagal activity with atropine and sympathetic activity with propranolol in near-term fetal sheep (n = 7, 0.85 gestation). Under these conditions, we analyzed the linear and nonlinear parts of fHRV complexity from autonomic information flow. Overall fHRV complexity decreased with both drugs compared with nonrapid eye movement sleep baseline (P < 0.05). With atropine, this was because of a decrease of the linear part of fHRV complexity on the long-term time scale (P < 0.05), suggesting that vagal modulation of fHRV is adequately described by linear fHRV measures. With propranolol, the nonlinear part of fHRV complexity decreased on the short-term time scale (P < 0.05), suggesting that sympathetic influences on fHRV can be detected by the nonlinear part of fHRV complexity. Thus the complex interplay of vagal and sympathetic modulations of fHRV is reflected differently and specifically in the linear and nonlinear properties of fHRV complexity, and on different time scales. Analysis of linear and nonlinear properties of fHRV may improve sensitivity and specificity of FHR monitoring.

fetal sheep; autonomic nervous system; autonomic information flow; complexity

FETAL HEART RATE (FHR) monitoring is used to monitor fetal well-being noninvasively in utero. Some studies questioned its diagnostic value (24). Recent studies in human fetuses suggested potential of FHR variability (fHRV) complexity estimation to improve clinical monitoring of fetal health (18, 31). This requires a deeper understanding of the physiological meaning of fHRV complexity, including how its linear and nonlinear properties are modulated by sympathetic and vagal activity of the autonomic nervous system (ANS). Understanding fHRV dynamics during physiological (e.g., sleep states dependent changes) and pathophysiological (e.g., asphyxia) conditions has evolved over the past two decades, propelled by analyses of the linear aspects of fHRV in human and ovine fetuses (4, 11, 16, 20, 29, 32). However, characterization of the linear fHRV properties is fundamentally limited in its power to describe the nonlinear structure of the underlying sympathovagal interactions (12, 14).

The origin of the nonlinearity of sympathovagal interactions lies in their intrinsic complexity. This complexity emerges from interaction of neuronal brain stem networks as weakly coupled nonlinear oscillators that are influenced by various afferent signals (3, 17, 28, 30), suggesting that sympathetic and vagal influences are superimposed nonlinearly on fHRV and can act synergistically. The autonomic information flow (AIF) function was introduced and validated in adult rats and human neonates (9, 15). In human neonates, AIF depends on sleep states (9). Auto-autonomic information flow (aAIF) estimates complexity of a signal based on predictability. A higher predictability of fHRV is the result of more information in the signal and, therefore, renders a lower complexity. As such, aAIF of heart rate variability (HRV) reflects linear and nonlinear properties of the ANS. We derived from aAIF of HRV the measures aAIFshort (estimating the complexity on the short-term time scale of a single heart beat by assessing the predictability of the next heart beat) and aAIFint (estimating the complexity on the long-term time scale by assessing the predictability of a given heart beat until the 10th heart beat in the future, i.e., as integral over all physiologically relevant time scales of HRV) (9). These measures describe HRV complexity on different time scales of physiologically relevant oscillations of the vagal and sympathetic modulations of HRV, thus relating the complexity properties of HRV to the underlying physiology for the first time (15).

Groome et al. (12) demonstrated the presence of the nonlinear properties of fHRV in human low-risk fetuses in late gestation, showing a higher vagal tone to be associated with more efficient regulation of homeostasis. These findings suggest that a deeper understanding and delineation of the nonlinear vs. linear fHRV properties influenced by modulations of vagal and sympathetic activities of the developing ANS in utero has the potential to improve the sensitivity and specificity of FHR monitoring. We, therefore, assessed the contribution of vagal and sympathetic modulation to the linear and nonlinear properties of fHRV by pharmacological blockade of vagal and β-receptor-mediated sympathetic activities in the near-term fetal sheep, an important model of human pregnancy (34).
METHODS

Experimental procedures were approved by the animal welfare commission of Thuringia. Thirteen Long-Wool Merino × German Blackheaded Mutton cross-bred ewes of known gestational age were acclimated to the animal facilities for at least 5 days before surgery. After food withdrawal for 24 h, surgery was performed under halothane general anesthesia. Following 1 gram of ketamine (Ketamin 10; Atarost), intramuscular anesthesia was induced by 4% halothane (Halothane Liquid 250 ml; Rhodia Organique Fine) using a face mask. Ewes were intubated, and anesthesia was maintained with 1.0–1.5% halothane in 100% oxygen. Ewes were instrumented with catheters inserted in the common carotid artery for blood sampling and in the external jugular vein for postoperative administration of drugs.

Following hysterotomy, fetuses were instrumented with polyvinyl catheters (Rüschelit, Rüsch, Germany) inserted in the left common carotid artery for arterial blood pressure recordings and blood sampling and in the left external jugular vein for drug administration. An additional catheter was placed in the amniotic cavity to record the amniotic pressure to permit correction of fetal mean arterial blood pressure (FBP) for hydrostatic pressure. Wire electrodes (LIFYY; Metrofunk Kabel-Union) were implanted in the left suprascal muscles, muscles of the right shoulder, and in the cartilage of the sternum for electrocardiogram (ECG) recording, in the uterine wall to record myometrial activity, and in the skull to record electrocorticogram (ECOG) as bihemispherical leads from frontal and parietal regions and fixed with dental cement on the skull bone.

All ewes and fetuses received 0.5 gram ampicillin (ampicillin; Ratiopharm) intravenously and in the amniotic sac two times a day during the first three postoperative days. Metamizol (Arthripur; Atarost) was administered intravenously to the ewe (30–50 mg/kg) as an analgesic for at least 3 days. All catheters were maintained patent via a continuous infusion of heparin at 15 IU/ml in 0.9% saline delivered at 0.5 ml/h.

After at least 3 days of postoperative recovery, experimental protocol was started at 0900. In seven sheep, at 127 ± 3 days gestational age (term 150 days) ECG, ECoG, FBP, and uterine electromyogram (EMG) were recorded continuously for the duration of the whole experiment. Arterial blood samples were taken daily at 0900. The samples were analyzed for fetal blood gases, hemoglobin concentration, and oxygen saturation using a blood gas analyzer (ABL600; Radiometer; measurements corrected to 39°C).

Five-minute ECG epochs were selected in high-voltage (nonrapid eye movement, NREM) and low-voltage (rapid eye movement, REM) sleep, since at this gestational age sleep cycling is developed (4). Sleep states were determined from ECoG visually and confirmed quantitatively by means of spectral edge frequency analysis of the bifrontal ECoG. Consequently, this group of fetuses received 2.5 mg atropine sulfate (atropinsulfat; B. Braun, Melsungen, Germany) intravenously as a 5-ml bolus to induce vagal blockade and, 24 h later, 2 mg propanolol (Obsidan; Alpharma-Isis, Langenfeld, Germany) as a 2-ml bolus over 60 s to induce a β-receptor-mediated sympathetic blockade according to Yu et al. (34). Starting 5 min after the injections, ECG was analyzed over 5 min.

FBP and amniotic pressure were recorded continuously using calibrated pressure transducers (B. Braun). Myometrial activity was monitored to recognize pressure artifacts during contractions. All biophysical parameters were amplified (amplifier model 5900 and 6600; Gould) and digitized using a 16-channel analog-to-digital board (DI-400-PGH; DATAQ Instruments) at a sample rate of 1,000 s⁻¹ for ECG and 100 s⁻¹ for blood pressures and uterine EMG and continuously stored on a hard disc of a personal computer.

For calculation of fHR and fHRV, the individual R peaks were sequentially detected and triggered with a precision of ± 0.49 ms. The fHRV was further analyzed as described earlier (8). Briefly, the artifacts were visually controlled for and removed manually (Watisa Signal Processing Software, Institute for Medical Statistics and Documentation, University of Jena). The resulting instantaneous R-R interval sequence was linearly interpolated at 1,000 Hz equidistant sample rate and resampled at 20 Hz for further signal analysis. We chose linear interpolation because it avoids any nonlinear transformation and hence minimizes bias. A nonlinear interpolation method such as spline interpolation would produce a less clear nonlinear transformation. The high sampling rate of the ECG that was also used in the R-R interval identification and the equidistant resampling provided the possibility of an antialiasing filter before downsampling the R-R interval series.

The software package Matlab 6.1, R13, was used to calculate all fHRV measures (The MathWorks, Natick, MA). RMSSD is the square root of the mean of the sum of the squares of differences between adjacent R-R intervals of ECG. RMSSD is a linear short-term measure of fHRV that reflects modulations of fHRV by vagal activity and was calculated as described earlier (6, 7, 8). To assess the contribution of sympathetic and vagal modulations to linear and nonlinear fHRV properties, we also studied the complexity of the fHRV with its linear and nonlinear parts.

The concept of complexity is as follows: in a linear system, the superposition principle is valid. It means that, if two stimuli are applied to a system, two independent responses are generated and simply added. In any case of difference from that superposition principle, the system is defined as nonlinear. A stochastic signal can be predicted only in part. The lower its predictability, the higher its complexity. This holds both for linear and nonlinear signals. The aAIF describes the predictability of a signal in terms of information flow, which is able to predict the signal based on all stochastic properties, linear and nonlinear. If we want to consider only the linear properties, we calculate surrogate data by phase randomization, a procedure that distorts the nonlinear properties. After that procedure, the aAIF function predicts the signal based on its linear part only. Because aAIF (predictability) and complexity (nonpredictability) are inversely correlated, we calculated fHRV complexity index aAIFshort as [2 – aAIF] from the aAIF function normalized to 2 (Fig. 1). The fHRV complexity index aAIFint was derived from aAIF function as an estimate of the fHRV complexity on the long-term time scale by assessing the predictability from a given heart beat until the 10th heart beat in the future, i.e., as integral over all physiologically relevant time scales of HRV (Fig. 1) (9). These aAIF measures were assessed with and without nonlinear parts using the Theiler test of surrogate

![Fig. 1](http://ajpregu.physiology.org/)

Fig. 1. Symbolic representation of the application of auto-automonic information flow (aAIF) to estimate complexity of fetal heart rate variability (fHRV) by predicting its change over the time scale ω. This allows the derivation of complexity measures from the aAIF function (aAIFoverall) for the short-term time scale (aAIFshort, one heart beat, i.e., beat-to-beat variability) and the long-term time scale (aAIFint, 10 heart beats). Hatched area under the aAIF curve is determined from the surrogate data (aAIFsur, the linear part of aAIF). The difference between the aAIFoverall and aAIFint curves renders the nonlinear part of the aAIF. From the aAIFint, the estimates of the linear part, and from the nonlinear part of aAIF, the estimates of the nonlinear part of fHRV complexity are derived on short-term (one heart beat) and long-term (10 heart beats) time scales. aAIFshort and aAIFint increase with increasing information flow, improving predictability and decreasing complexity.
data to determine the linear parts of fHRV complexity. Because the embedding dimensions (ED) necessary and sufficient to describe the complexity properties of fHRV are not known a priori, all aAIF-derived measures of fHRV were calculated for ED = 1 and ED = 2. That is, to predict future heart beats in fHRV, one (ED = 1) or two (ED = 2) preceding heart beats from the past were taken as the known information. In other words, ED represents the number of coordinates of the phase space in which the aAIF of fHRV is analyzed. If a higher ED is required to detect changes in fHRV complexity properties, this suggests higher complexity of the signal.

With the use of this approach, the following aAIF-derived measures were analyzed, as shown in Fig. 1: Overall aAIF: 1) aAIFshort assesses the predictability of the next heart beat, measured as bit and was determined for ED = 1 and ED = 2 as $aAIF_{short(1)}$ and $aAIF_{short(2)}$; higher values of $aAIF_{short}$ mean a better predictability and therefore a lower complexity of fHRV; 2) $aAIF_{int}$ is defined as an integral over all aAIF values from a given heart beat until the 10th heart beat in future, measured as bit-s and was determined for ED = 1 and ED = 2 as $aAIF_{int(1)}$ and $aAIF_{int(2)}$; higher values of $aAIF_{int}$ mean an increase of predictability of aAIF and therefore a lower complexity of fHRV; Linear part of the fHRV was calculated as surrogate data with identical power spectra but distorted phase dependencies. Hence, aAIF of the surrogate data reflect the linear part of the fHRV complexity for $aAIF_{short}(1)$ and $aAIF_{short(2)}$ and $aAIF_{int}$ for ED = 1 and ED = 2 as $aAIF_{short(sur1)}$ and $aAIF_{short(sur2)}$; $aAIF_{int(sur1)}$ and $aAIF_{int(sur2)}$; Nonlinear part of aAIF: nonlinear properties were tested for by comparing AIF of original and surrogate data (22): [Overall AIF - linear part of AIF] for $aAIF_{short(sur1)}$ and $aAIF_{short(sur2)}$; and $aAIF_{int(sur2)}$; and Table 1. Nonlinear part of the aAIF describes the complexity of nonlinear fHRV properties.

Correlations between the fHRV measures and FHR were determined using Spearman correlation coefficients. Because no interaction between fHRV and FHR was detected, FHR measures were compared between REM/NREM baselines and after drug administrations using the Wilcoxon test. The $P$ values were adjusted for multiple comparisons with the Bonferroni-Holm method (5–8). All results are given as means ± SE. $P$ values <0.05 were considered to be significant.

RESULTS

Baseline arterial blood gases, arterial oxygen oxygen saturation, and pH were within the physiological range for the gestational age (PO$_2$ 24.9 ± 0.4 mmHg; PO$_2$ 47.2 ± 0.7 mmHg; SaO$_2$ 68.2 ± 0.9%; pH 7.36 ± 0.02). No differences in fHRV measures were found between REM and NREM baselines (Table 1). Administration of atropine resulted in a FHR increase from 175 ± 7 (average of FHR values during REM and NREM baselines) to 227 ± 17 ($P < 0.05$) beats/min. RMSSD decreased to 3.2 ± 0.6 from 5.5 ± 0.9 ms during NREM baseline ($P < 0.05$) but not vs. RMSSD during REM baseline (5.6 ± 0.7 ms). Administration of propranolol led to a FHR decrease to 153 ± 4 beats/min ($P < 0.05$) and no change in RMSSD (5.9 ± 1.4 ms).

Both atropine and propranolol administrations led to an increase of $aAIF_{int(2)}$ compared with NREM sleep, reflecting a decreased overall complexity on the long-term time scale ($P < 0.05$, Table 1, Fig. 1).

Following the administration of atropine, $aAIF_{int(sur2)}$ was increased compared with NREM sleep, indicating a reduced complexity of the linear part of fHRV on the long-term time scale ($P < 0.05$, Table 1, Fig. 1). The nonlinear part of fHRV complexity was not changed.

Following the administration of propranolol, the nonlinear part of $aAIF_{short(1)}$ was increased compared with NREM sleep,

![Table 1. Effects of atropine and propranolol administration on aAIF-derived measures of fHRV complexity](http://ajpregu.physiology.org/DownloadedFrom/R704/VAGAL_AND_SYMPATHETIC_MODULATIONS_OF_NONLINEAR_FETAL_HRV)
i.e., the nonlinear part of fHRV complexity on the short-term time scale decreased ($P < 0.05$, Table 1, Fig. 1). Changes of the linear and nonlinear parts of fHRV complexity after atropine or propranolol administrations could not be statistically proven if they were compared with REM sleep.

To test whether changes in fHRV complexity measures were the result of changes in FHR itself as FHR changed due to atropine or propranolol administrations, we tested for their correlation under atropine and propranolol and found no correlations ($r < 0.2$, not significant [NS]).

Because our findings suggested that fHRV changes under vagal blockade are described sufficiently using the linear fHRV measure RMSSD, we tested for its possible correlation to the aAIF measures following atropine administration and found no correlation ($r < 0.5$, NS). However, when considering the relation between all values of RMSSD and aAIF-derived fHRV measures ($n = 28$), we found that RMSSD correlated to the overall and linear part aAIF measures of fHRV complexity ($r = -0.54 \pm 0.04$, all $P < 0.02$), but not to any of the “nonlinear part” aAIF measures of fHRV ($r < 0.1$, NS).

**DISCUSSION**

Analysis of fHRV allowed us to assess the contributions of linear and nonlinear parts of aAIF to the overall fHRV complexity properties on different physiologically relevant time scales (9, 15). We show that changes in fHRV complexity reveal patterns specific to the vagal and sympathetic modulation of either linear or nonlinear properties and cannot be detected studying the overall complexity properties of fHRV. Sole estimation of overall fHRV complexity does not fully reflect the complex interplay of linear and nonlinear modulations of fHRV through ANS activity.

The decreases of overall fHRV complexity following vagal and sympathetic blockades suggest that both vagal and $\beta$-receptor-mediated sympathetic modulations contribute to the complexity of fHRV. In agreement with this, the concept of reciprocal vagal or sympathetic activation has been challenged by the evidence of a nonreciprocal autonomic modulation of HRV (13), suggesting concomitant vagal and sympathetic activation as in a complex network. This notion could be extended to the possibility that an inhibition of either one of the branches of the ANS may also result in a coinhibition of the other branch. The consideration of linear and nonlinear parts of AIF-derived fHRV complexity seems to detect a “branch-specific fHRV modulation signature,” i.e., decrease of the linear part of fHRV complexity following vagal blockade and of the nonlinear part following sympathetic blockade, thus reflecting a complex interplay of vagal and sympathetic activities in modulation of fHRV.

We previously reported changes of the fHRV under vagal and $\beta$-receptor-mediated sympathetic blockades using conventional methods of linear fHRV analysis in time and frequency domains (5–7). Here we compared the effects of vagal blockade on linear and aAIF-derived fHRV complexity measures. Vagal blockade led to a twofold RMSSD decrease, i.e., a decrease of the linear fHRV properties in the time domain on the short-term time scale. Here we report that it also resulted in a decrease of the linear part of fHRV complexity. Moreover, RMSSD correlated to the overall and linear part aAIF measures of fHRV complexity, but not to any of the nonlinear part aAIF measures of fHRV. These findings suggest that RMSSD is an adequate measure to detect changes in fHRV properties resulting from vagal modulations (5–7).

The absence of change of the nonlinear part of fHRV complexity after vagal blockade is in agreement with former studies in sheep fetuses of similar gestational age (28). Although respiratory activity in fetus near-term reflects maturation of central nervous system’s (CNS) respiratory generators and exhibits fractal, i.e., nonlinear, properties (28), vagally mediated linear fHRV properties are not related to respiratory activity in sheep fetuses at the gestational age when we conducted our study (19), and the relation of vagally mediated nonlinear fHRV properties and fetal respiratory activity has not been studied. Unlike in antenatal life, studies on conscious adult rats suggest that vagal activity contributes to nonlinear HRV properties (2, 10). In healthy adult awake humans, respiratory activity mediates the nonlinear HRV properties (25). However, overall HRV complexity that reflects vagal modulation of HRV decreases when respiratory activity decreases (26), suggesting that HRV complexity depends on behavioral states (e.g., awake, REM, NREM) as these states influence respiratory activity (27). Overall, we believe that the seemingly contradictory findings in respect to the interaction between vagal activity and nonlinear HRV properties are rooted in differences between prenatal and postnatal life, in the behavioral state during the study, and in physiological species differences. ANS activity in human and sheep fetuses (4) as well as in adult rats and humans (33) is variably dependent on behavioral states. In contrast to near-term ovine fetuses we studied and that are known to spend >90% of the time in REM or NREM sleep states (27), both adult rats and humans in the studies discussed above were awake (2, 10). In adult sheep, rats, and humans, electrocortical activity changes similarly during low-voltage/REM and high-voltage/NREM sleep states (27). However, the dynamics of sleep-state-dependent ANS changes are species specific: while near-term sheep and human fetuses behave reciprocally regarding sympathetic and vagal modulations of fHRV, with sheep fetuses having higher vagal activity during REM sleep and human fetuses showing lower vagal activity during REM sleep (4), vagal modulation of fHRV in adult rats seems to be sleep state independent (33).

The sleep-state-dependent changes in vagal and sympathetic modulation of fHRV in fetal sheep near-term are likely the reason why we could detect the changes in linear and nonlinear parts of fHRV complexity only when compared with NREM but not REM sleep state baseline. Why could we find no difference between NREM and REM sleep state baselines themselves? This may be because 1) the influence of vagal tone on fHRV was much more pronounced during pharmacological interventions than at baseline, thus making it difficult to detect differences between REM and NREM baselines, i.e., as discussed above, even if REM periods studied contained some respiratory activity, it would not contribute to the vagally mediated fHRV properties; 2) of the relatively low group sample size; and 3) of limitations of the fHRV analysis method itself. The sample size was sufficient to detect the more pronounced changes induced by pharmacological blockades, but it might have been too small to find the more subtle differences between sleep states at baseline with this method.

The selective decrease of the nonlinear part of fHRV complexity following $\beta$-receptor-mediated sympathetic blockade
Perspectives and Significance

To our knowledge, this is the first pharmacological study of fHRV properties where sleep state dynamics were considered. Our results suggest overall that such sleep-state-dependent changes in fHRV have to be accounted for as varying baseline conditions when studies altering vagal and sympathetic modulation of fHRV are conducted. Our finding demonstrates a distinct influence of β-receptor-mediated sympathetic modulation on the nonlinear part of fHRV complexity that cannot be detected with linear methods of fHRV analysis. Thus aAIF-derived complexity of fHRV presented here may become a useful measure of the changes of sympathetic modulation of fHRV to detect compromised fetuses. In contrast to the sympathetic modulations of fHRV, changes in the vagal modulation of fHRV near term appear to be adequately described by the linear part of fHRV complexity or by the less sophisticated linear fHRV measure, RMSSD.

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