HEART RATE VARIABILITY FOR ASSESSING STRESS IN COWS

Alina Anton, Gheorghe Solcan

University of Agricultural Sciences and Veterinary Medicine, Iassy, Romania, antonclaraalina@yahoo.com

Abstract

Measurement of heart rate variability (HRV) is a non-invasive technique that can be used to investigate the functioning of autonomic nervous system, especially the balance between sympathetic and vagal activity. HRV is measured by determining the constantly changing temporal distance between succeeding heartbeats (R-R intervals). Five lacting cows, well trained to blood sampling were challenged with an intramuscular single dose (0.5 µg/kg) of ACTH (Synacthen Depot). HRV was measured for each cow for 5 min, at 0 h (before treatment) and every 30 min for 2 h. HRV parameters were analysed in the time domain, frequency domain and nonlinear components. Blood samples (10 mL) were collected from the coccygeal vein of all cows at 0, 30, 60, 90 and 120 min, after the measurement of HRV, for serum cortisol. The heart rate of cows increased significantly (P<0.05) under the influence of Synacthen administration. All computed time domain parameters declined significantly after ACTH administration. The decline of root mean square of successive interbeat interval differences (RMSSD) was more pronounced than that for standard deviation of all interbeat interval (SDNN), after ACTH administration. The power of low-frequency component divided by power of the high-frequency band (LF/HF) increased also within 30 min of administration of ACTH. All nonlinear parameters (%DET and %REC) exhibited a significant rise 30 min after ACTH administration. Serum cortisol concentration also increased (P < 0.05) within 30 min of administration in cows. The nonlinear parameters were most important to indicate the level of stress in cows. HRV is a valuable physiological indicator for stress in cows.

Key words: frequency domain, heart rate variability, nonlinearity, time domain, stress.

INTRODUCTION

An increase in hypothalamic pituitary-adrenocortical activity, causing the rise of blood cortisol, indicates a physiological response to different stressors; consequently a measurement of serum cortisol is frequently used to study stress response (Sapolsky et al., 2000). Measures of cardiovascular parameters including heart rate (HR) have a long tradition as indicators of health and welfare in many species. The heart is under sympathetic and parasympathetic control, and HR is the effect of the non-additive regulatory functions of the interacting antagonistic branches of the autonomic nervous system (Berntson et al., 1991). As a consequence of the ongoing regulatory

mechanisms, HR is never constant but varies from beat to beat even in the absence of physical or psychological stress. This beat-to beat variability is referred to as HR variability (HRV) or heart period variability (Hagen et al., 2005).

The aim of the study was to evaluate the usefulness of heart rate variability (HRV) and its specific parameters as a new approach to assess stress load in cow.

MATERIALS AND METHODS

Five lacting cows, well trained to blood sampling were challenged with an intramuscular single dose (0.5 µg/kg) of ACTH (Synacthen Depot, 1mg/mL). The ECG with a Poly-Spectrum software was used for measurement of heartbeat activity in cows (R-R interval). The green electrode was placed on the sternum or better 2 or 3cm right from the sternum. The red electrode was placed on the left side of the cow thorax about 30 cm below the top of the thorax. The black electrode was placed approximately 10 cm above the red electrode. The yellow electrode was placed on the right side of the cow's thorax in a position similar to the red one on the left side. All electrodes need to be positioned in a way so that they can be tightly fixed. The jelly electrode liquid in the sponge of the adhesive electrode is able to penetrate the hair coat even if the cow is not clipped.

HRV was analyzed with Kubios HRV software, version 2.0 (Biomedical Signal Analysis Group, Department of Applied Physics, University of Kuopio, Finland) from 5 min interval of each ECG recording: at 0 h (before treatment) and every 30 min for 2h. The HRV analyzed parameters were: standard deviation of all interbeat interval (SDNN); root mean square of successive interbeat interval differences (RMSSD); normalised power of the low-frequency band, boundaries 0.04– 0.25 Hz (LFnorm); normalised power of the high-frequency band, boundaries 0.25–0.58 Hz (HFnorm); LF/HF; percentage of recurrent points in the recurrence plot, i.e., vector-repetition in the multi-dimensional space (REC); percentage of recurrent points that appear in sequence, forming diagonal lines in the recurrence plot (DET).

Blood samples (10 mL) were collected from the coccygeal vein of all cows at 0 (T0), 30 (T1), 60 (T2), 90 (T3) and 120 min (T4), after the measurement of HRV, for serum cortisol.

Statistical analysis was performed with the SPSS statistics for Windows. All data were normally distributed (Kolmogorov-Smirnov test). ACTH effect was examined using ANOVA. Significance was set at $P<0.05$, and all values are given as the mean ± one standard error of the mean (SEM). The relationships between cortisol measured in serum and heart rate and HRV parameters samples were evaluated by Pearson correlation coefficients.

RESULTS AND DISCUSSIONS

Determination of hypothalamus-pituitary-adrenal (HPA) axis activity is the standard procedure to evaluate stress conditions in farm animals (Mormede et al., 2007). A well-known stimulus of HPA resulting in an increase of circulating cortisol is stress. In our study cortisol levels measured before and after ACTH injections in cows are shown in Figure 1. Administration of ACTH (0.5 µg/kg) increased ($P<0.05$) serum cortisol concentrations in cows within 30 min of administration, and concentrations remained increased ($P<0.05$) throughout the blood sampling period (120 min). Doses of ACTH have varied from 0.125, 0.25, 0.50, or 1 IU of ACTH per kilogram of BW (body weight) in pregnant Brahman heifers (Lay et al., 1996) to 1 mg/kg of B W for dairy cows (Bertoni et al., 2005). In our study, the peak plasma cortisol concentrations after 0.25 mg ACTH /500 kg BW (equivalent to 25 IU/500 kg BW) injection in the dairy cows were well comparable with the values in dairy cows reported by Bertoni et al. (2005). Also, Verkerk et al. (1994) that used very low (0.0125 mg) and relatively high doses (0.4 mg) of $ACTH_{1-24}$ determine almost the same peak level of cortisol.

Nevertheless the utilization of cortisol as an indicator of stress requires some caution for some type of stress, for the effect of circadian rhythms as well as blood sampling itself that can cause stress effects (Negrão et al., 2004). It has been suggested that the extent of corticosteroid raise may be more related to the capacity of the animal to learn about the situation than to the real aversion to it; in fact the stress input can decline very much during a prolonged stress situation due to habituation (Smith and Dobson, 2002).
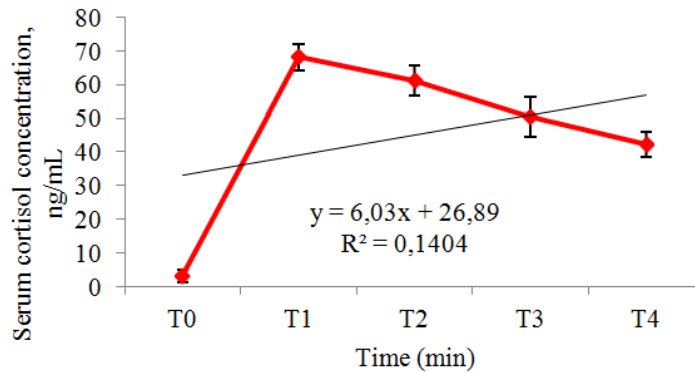
Figure 1. Mean serum cortisol concentrations for cows administered with 0.5 µg ACTH/kg BW

The heart rate (Figure 2) of cows increased significantly ($P < 0.05$) under the influence of Synacthen administration. Study revealed a direct correlation ($P=0.05$, $r=0.798$) between serum cortisol and heart frequency.

An increase in heart rate indicates increased sympathetic activity, decreased parasympathetic (vagal) activity or a combination of both (Borell et al., 2007). Rapid changes in heart rate are mostly caused by shifts in vagal regulation and occur within less than 5s while heart rate responses to sympathetic regulation occur more slowly (Hainsworth, 1995).

In our study heart rate reached the maximal levels at 30 min after ACTH administration (T1) followed by a decrease till the end of study.
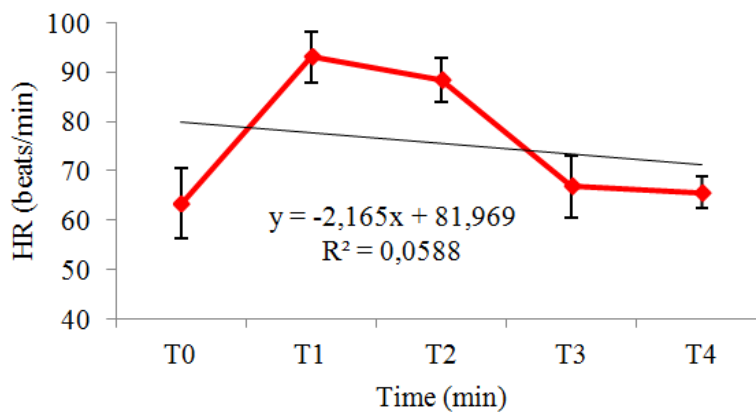

Figure 2. Heart rate at cows during the study

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The R-R intervals (Figure 3) of cows increased significantly (P < 0.05) under the influence of Synacthen administration. Study revealed no correlation between serum cortisol and R-R intervals.

Normal R-R interval values of cows are 819±114.6 ms (Hagen et al., 2005). R-R intervals reached the maximal levels at 60 min after ACTH administration (T2) followed by a decrease till the end of study.
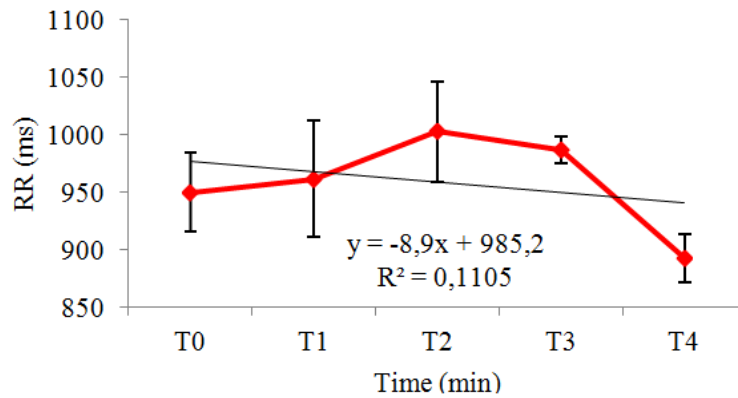

Figure 3. R-wave to R-wave interval at cows

All HRV parameters in time domain, frequency domain and nonlinear components are presented in Table 1. The time domain parameters declined significantly after ACTH administration.

RMSSD takes into account short-term, high-frequency components of HRV, strongly reflects vagal tone, and is thus highly correlated to other heart-rate variability measures such as HFnorm, LF/HF, recurrence and determinism (Hagen et al., 2005).

Table 1. Changes in HRV parameters at cows during the study

Time	Time domain		Frequency domain			Nonlinear parameters	
	SDNN (ms)	RMSSD (ms)	LFnorm	HFnorm	LF/HF	REC (%)	DET (%)
T0	39.15±8.1	14.55±3.4	83.3±4.3	16.7±5.2	5.17±4.2	45.20±4.7	93.4±0.3
T1	27.5±4.1	4.8±3.4	99.09±8.6	0.95±0.5	104.30±9.7	49.70±5.8	98.97±0.1
T2	16.2±2.1	17.4±3.1	66.9±12.1	33.1±2.5	2.02±0.7	43.23±4.4	95.2±0.6
T3	27.0±4.1	8.8±4.0	56.65±5.6	43.35±1.9	1.30±0.5	30±3.8	94.5±0.2
T4	37.1±2.2	5.4±3.2	81.85±7.3	18.15±6.1	4.50±2.1	35.52±4.1	96.1±0.3
Reference (Borell et al., 2007)	36±10.8	15±8.8	162±87	9.9±6.2	2.78±1.78	3.4±2.4	84±6.0

The decline of root mean square of successive interbeat interval differences (RMSSD) was more pronounced than that for standard deviation of all interbeat interval (SDNN), after ACTH administration. Pearson test revealed an indirect correlation (P=0.04, r=-0.842) between serum cortisol and RMSSD and no correlation with SDNN.

The higher HR after the treatment and lower RMSSD values suggest that cows were subjected to an increased level of stress. SDNN, the measure of total variability in the time domain, was not correlated with mean HR and sympatho-vagal balance LF/HF, and moderately correlated with RMSSD (P=0.048, r=0.735).

Like RMSSD in time domain, the HF band of HRV is regarded to be a good indicator for vagal activity (Friedman and Thayer, 1998). Simultaneously, we found higher values of LF, 30 min after ACTH administration. The LF oscillation of HRV has often been regarded as an accurate reflection of sympathetic activity (Malliani, 1995).

The power of low-frequency component divided by power of the high-frequency band (LF/HF) increased also within 30 min of administration of ACTH.

All nonlinear parameters (%DET and %REC) exhibited a significant rise 30 min after ACTH administration. Pearson test revealed a direct correlation (P=0.05, r=0.712) between serum cortisol concentration and %DET. Mohr et al. (2002) suggest that %DET indicates quantitative changes in the level of stress load.

The results of this study indicate that HRV is an indicator for the assessment of welfare at cows. Further studies are required and should consider intraindividual differences in the HRV exposed to various procedures, and large population of cows recordings to better determine the predictive value of HRV in the identification of individual vulnerability to stress.

CONCLUSIONS

The higher HR and the lower HRV after ACTH treatment, suggest a clear shift of the sympatho-vagal balance towards the sympathetic tone.

Pearson test revealed a direct correlation (P=0.05, r=0.798) between serum cortisol concentration and heart frequency.

The decline of root mean square of successive interbeat interval differences (RMSSD) was more pronounced than that for standard deviation of all interbeat interval (SDNN), after ACTH administration. The power of low-

frequency component divided by power of the high-frequency band (LF/HF) increased also within 30 min of administration of ACTH.

All nonlinear parameters (%DET and %REC) exhibited a significant rise 30 min after ACTH administration.

Pearson test revealed an indirect correlation (P=0.04, r=-0.842) between serum cortisol concentration and RMSSD, and direct correlation (P=0.05, r=0.712) with %DET.

HRV is a valuable physiological indicator for stress in cows.

AKNOWLEDGEMENTS

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