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Effect of Autonomic Blockers on Heart Period Variability in Calves: Evaluation of the Sympatho-Vagal Balance

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Summary

This study was designed to validate the measures of heart period variability for assessing of autonomic nervous system control in calves. Eight calves received an injection of either 0.5 mg/kg atenolol (sympathetic tone blockade), 0.2 mg/kg atropine sulfate (parasympathetic tone blockade), 0.5 mg/kg atenolol + 0.2 mg/kg atropine sulfate (double autonomic blockade) or saline. In the time-domain, we calculated the mean instantaneous heart rate (HR), mean of RR intervals (MeanRR), standard deviation of RR intervals (SDRR) and that of the difference between adjacent intervals (RMSSD). In the frequency-domain, the power of the spectral band 0-1 Hz (TPW), the power of the 0-0.15 Hz band (LF), that of the 0.15-1 Hz band (HF), and the LF/HF ratio were considered. The net vago-sympathetic effect (VSE) was calculated as the ratio of MeanRR in a defined situation to MeanRR during the double blockade. Atenolol injection had no effect on cardiac activity, whereas atropine induced large modifications which were moderated when atenolol was administered at the same time. VSE, HR, MeanRR and RMSSD were found to be valid indicators of the parasympathetic tone of calves because of large variations due to the drug and low individual variations. No measure reflected the sympathetic tone.

Key words

Autonomic nervous system • Heart period • Cholinergic antagonists • Adrenergic antagonists • Calves

Introduction

Average heart rate is very often used as an index of animal emotional reactivity (e.g. Wolf 1970, Obrist 1981, Cabanac 1999). However, it provides little information on the underlying physiological mechanisms that govern its modification in many behavioral situations (Sayers 1973). As a matter of fact, a high heart rate can result from an increase in sympathetic tone, a withdrawal of parasympathetic tone, or both. Heart rate variability (HRV), which corresponds to variations in instantaneous

heart rate, is considered to provide a better assessment of the autonomic modulation of the heart activity. HRV has been extensively analyzed in humans (reviewed by Malik 1998, Stein and Kleiger 1999) and in some mammals including dogs (reviewed by Calvert 1998), hamster (Giudice *et al.* 2000), rats and voles (Ishii *et al.* 1996), rabbits (Moguilevski *et al.* 1996), and horses (Kuwahara 1996, Physick-Sheard *et al.* 2000). For the most part, variations in the duration of successive beat to beat intervals (RR intervals) are caused by fluctuation in the input from the autonomic nervous system to the sinoatrial

node, some components being controlled by the parasympathetic branch and some others by the sympathetic branch.

Several methods have been proposed for the assessment of HRV (reviewed by Task Force 1996). Briefly, the time domain measures represent statistical calculations of RR intervals or numerical estimation of the geometrical shape of the distribution of RR intervals. The frequency domain measures use spectral methods for expressing variations along the RR interval series as the sum of sinusoids, each sinusoid being characterized by its frequency. The relative powers of contributing frequencies are usually calculated using Fast Fourier Transformation or autoregressive algorithm.

Although there are still controversies on how to interpret the data, some measures are interpreted as indices of autonomic modulation (for review see Task Force 1996). In the time domain, the standard deviation of RR intervals (SDRR) gives the overall HRV, whereas the standard deviation of the differences between adjacent RR intervals (Root of the Mean Squares of Successive Differences, RMSSD) is considered to reflect the influence of the vagal input. In the frequency domain, the spectrum is usually divided into two frequency bands: the high frequency band (HF) and the low frequency band (LF). The power of the high frequency band is considered to reflect the vagal control to the heart modulated by breathing, whereas the power of the low frequency band depends, to some extent, on the baroreceptor modulation of both vagal and sympathetic tones.

Some HRV indexes have been proposed to evaluate the sympathovagal balance, a concept reflecting the dual opposing effect of the two autonomic systems on heart activity. Among them, the ratio between the power of the low frequency band and that of the high frequency band (LF/HF) has been proposed for humans (see Task Force 1996). More recently, Goldberger (1999) proposed to assess this balance with a new method based on the following reasoning: in the absence of sympathetic and vagal inputs to the heart, the sinus node fires at its intrinsic rate, leading to an intrinsic RR interval designated as RR₀. When the vagal tone predominates, the RR is longer than the RR₀; when sympathetic tone predominates, then RR is lower than RR₀. Thus Goldberger (1999) introduced an index of the net vago-sympathetic effect (VSE) as the ratio of the mean RR interval, in a defined situation, to the RR₀ interval observed during complete autonomic blockade. When the sympathetic and the vagal tones are completely balanced, VSE equals one. VSE is greater than one when the

parasympathetic tone predominates, and lower than one when the sympathetic tone predominates.

The present study was designed to assess the sympathetic and parasympathetic tones in conscious calves and to check the validity of HRV measures for the evaluation of heart autonomic modulation and the sympatho-vagal balance. This was achieved by using autonomic pharmacological blockades.

Methods

Animal preparation

Eight male Prim'Holstein calves were used. They were bucket-fed a milk replacer (Cremunic Sanders) twice a day according to a feeding regimen used for producing veal calves (Toullec 1988). In compliance with the European legislation for the protection of calves (Directive 97/2), they were accommodated in group and received some solid foods. They were housed indoors in a straw bedded pen, 54 m² in size. They were provided with 100 to 200 g pelleted food (fibers 6 %, proteins 17 % Croustivo, Centraliment, France) per day. A food supplement containing vitamins and minerals (Appevo, Celtic NA, 560 ppm iron) was added to the solid food (dose per day and calf: 10 g for 10 days then 2 g). These ingredients were given immediately after the morning milk meal. During feeding, the calves were caught individually in a self blocking feeding barrier. The hematocrite was measured when the calves were 6 weeks old. Iron (Imferon[®], Sanofi, France) was injected to calves if their packed cell volume was too low.

During the two weeks preceding the start of the experimental procedure (see below), the calves were maintained at the feeding barrier for two hours after every morning meal. They were subjected to a simulated experimental procedure. The experimenter approached each calf from behind, fixed the electrode belt on its chest and touched the jugular area with his hand as for drug injection. No drug or saline was injected. During the week preceding the experimental procedure, the places for fixing the electrodes and the jugular area were shaved, and the calves were weighed.

Experimental procedure

The experimental procedure was started when the calves were 9±1 weeks old. The animals were tested in two batches of four calves each, one batch being tested on odd days and the other batch on even days. This allowed a two days interval between injections on the same animal, in order to ensure that the previously

administered drug had been completely metabolized (half-life of elimination for intravenous atropine or atenolol in humans are around 4 h and 7 h respectively, according to Kirch *et al.* (1980) and Aaltonen *et al.* (1984).

Within each batch, the drugs were injected according to a Latin square design. The following drugs (all from Sigma) and doses were administered: atenolol, a beta-1 selective adrenergic receptor antagonist (0.5 mg/kg body weight), atropine sulfate, a muscarinic receptor competitive antagonist (0.2 mg/kg), a mixture of atenolol and atropine for double autonomic blockade (0.5 and 0.2 mg/kg, respectively) and physiological saline (NaCl, 0.9 %).

Drug efficiency and correct dosage had been checked during pre-experimental tests: complete beta-blockade with atenolol had been verified with beta-1 adrenoreceptor agonist isoproterenol (0.1 to 0.2 µg/kg) injections, the dose of atropine and its effect was adjusted by monitoring the electrocardiogram (ECG) in order to avoid dizziness or agitation which are generally observed with higher doses of this drug.

Observations were conducted between 09:00 and 11:00 h, that is at least one hour after feeding. The four calves of a batch to be tested were maintained at the feeding barrier. Two adhesive electrodes (HP 40489 E, Hewlett Packard, USA) were glued and fixed on the shaved skin of each animal chest. One electrode was placed along the sternum and the other above the right scapula. An elastic belt was passed around the electrodes. Electrodes were connected to a PowerLab system (A.D. Instruments, UK) associated with an Apple PowerPC computer. Twenty minutes after electrodes had been fixed, the appropriate drugs were slowly injected into the left jugular vein. The ECG was recorded from 20 min before the injections up to 20 min after. The rate of acquisition was 1000 points per second.

Data analyses - HR and HRV measures

The ECG were analyzed with the A.D. Instruments Chart software. A specialized HRV extension package of this software was used. This extension is designed to compute a table of RR intervals from ECG data and to generate a range of plots, spectral (by way of the Fast Fourier Transformation) and statistical measures according to the Task Force (1996) and Committee report (1997) recommendations. Since spectral analysis of HRV requires some level of stability, ECG recordings were visually inspected and two-minute segments where the

HR was seen as stationary, were chosen for HRV analysis. R waves were triggered from the derivative of the raw ECG signal in order to remove the trend. The following time domain measures were considered: mean instantaneous heart rate (HR), mean of RR intervals (MeanRR), standard deviation of RR intervals (SDRR), standard deviation of the differences between adjacent RR intervals (Root of the Mean Square of Successive Differences, RMSSD). The following frequency domain measures were extracted: the power of the spectral band 0-1 Hz (Total Power, TPW), the power of the low frequency band (0-0.15 Hz, LF), the power of the high frequency band (0.15-1 Hz, HF). The 1 Hz limit was chosen according to prior measurements of the respiratory frequency, which was between 0.6-0.9 Hz in our calves. The ratio of LF to HF (LF/HF) was calculated. In addition, VSE was obtained by dividing MeanRR (for each drug injected) by RR0 (MeanRR after injection of atenolol and atropine together).

Statistical analyses

The SAS package (version 6.12) was used for statistical analyses. Paired-t tests were run to compare values before and after drug injection in order to check for changes over time. The effects of drugs were then compared with each other by analysis of variance using the following model: measurement (after drug injection) = $\beta_0 + \beta_1 a_i + \beta_2 b_j + \beta_3 c_{ij} + \epsilon_{ij}$ where a_i represents differences due to the drug, b_j represents differences due to the calf itself, and c_{ij} represents the level of measurement before drug injection. We checked that we could not exclude the hypothesis of a Gaussian distribution and of homogeneous variances of the residues of these models. *Post-hoc* comparisons between drugs were made with the Least Squares Means procedure. To compare the validity of each index, the proportion of variability explained either by the drug effect or by the subject (calf) effect was calculated from the analysis of variance (r^2 values). A parameter was considered to be valid when r^2 was high for the drug effect and low for the subject effect (Goldberger 1999).

The result section will only focus on significant effects ($P < 0.05$).

Results

Changes over time

The comparison of HRV parameters before and after the injection of each drug are summarized in Table 1. Saline injection did not affect any HRV parameter. Atenolol injections were followed by a decrease in HR, an increase in MeanRR and VSE, and a decrease in LF/HF. Atropine caused a large increase in HR, decreases

in MeanRR, RMSSD, and VSE. It was also followed by a decrease in the power of the spectrum (TPW), which was more marked in the low frequency band (LF) than in the high one (HF), resulting in a higher LF/HF ratio. The injection of atenolol and atropine together led to a decrease in RMSSD, TPW and LF.

Table 1. Changes in HRV parameters following drug injections of either 0.5 mg atenolol/kg, 0.2 mg atropine/kg, 0.5 mg atenolol + 0.2 mg atropine/kg, or saline.

	Stenolol			Atropine			Atenolol + Atropine			Saline	
	Mean D	Paired-t	P	Mean D	Paired-t	P	Mean D	Paired-t	P	Mean D	Paired-t P
HR (bpm)	-13.43	-2.81	*	29.27	5.39	**	7.97	1.52		-6.90	-2.34
MeanRR (ms)	72.20	3.21	*	-128.97	-6.71	**	-44.76	-1.58		36.49	2.04
SDRR (ms)	2.59	0.43		-1.64	-0.59		4.45	1.31		-1.74	-0.41
RMSSD (ms)	4.65	1.57		-7.58	-3.18	*	-5.72	-2.46	*	2.71	1.49
TPW (ms ²)	-28.26	-0.30		-142.50	-3.33	*	-131.84	-4.82	**	11.48	0.31
LF (ms ²)	-73.12	-1.08		-122.68	-3.14	*	-117.48	-4.61	**	-1.91	-0.06
HF (ms ²)	44.14	1.45		-21.95	-2.41	*	-16.06	-1.99		14.55	1.54
LF/HF	-12.17	-3.62		6.64	0.43		3.41	0.23		-13.37	-3.09
VSE	0.13	2.81	*	-0.25	-5.86	**	-0.09	-1.62		0.08	1.99

The difference D between values (after – before) injection were calculated and compared to 0 by t test. * P<0.05; ** P<.01.

Table 2. Comparison of drug effects on HRV parameters after injections of either 0.5 mg atenolol/kg, 0.2 mg atropine/kg, 0.5 mg atenolol + 0.2 mg atropine/kg, or saline.

	Atenolol		Atropine		Atenolol + Atropine		Saline	SD	F drug	P
	Mean	Letter	Mean	Letter	Mean	Letter				
HR (bpm)	97.8	c	139.8	a	118.4	b	104.2	9.2	24.0	***
MeanRR(ms)	623	c	435	a	519	b	592	52	15.0	***
SDRR (ms)	21.00		16.74		22.17		18.84	7.78	0.7	
RMSSD (ms)	13.2	b	1.58	a	2.48	a	11.8	5.41	9.2	***
TPW (ms ²)	154.6	b	33.9	a	24.9	a	183.6	113.13	4.1	*
LF (ms ²)	92.81	ab	29.36	a	14.65	a	134.94	76.19	3.9	*
HF (ms ²)	64.3	b	1.0	a	4.6	a	42.0	44.3	3.5	*
LF/HF	5.39		30.1		23.6		8.1	21.6	2.3	
VSE	1.19	c	0.83	a	0.99	b	1.14	0.11	12.1	***

The drug effects were compared by analyses of variance, values before injections being taken as covariates; for each HRV parameter, values with no common letter (a, b, c) differ significantly (P<0.05). * P<0.05; ** P<0.01; *** P<0.001.

Comparison between drugs

The analyses of variance revealed significant drug effects on all parameters except SDRR and LF/HF

(Table 2). Atenolol injections were found to have similar effects to those of saline, since no significant differences were observed between these two treatments. The effect

of atropine injection – either alone or in combination with atenolol – differed largely from those of the other treatments. After atropine injection, HR was higher while MeanRR, VSE, RMSSD and TPW were lower than after saline or atenolol injections; furthermore, LF and HF were lower than with saline. The injection of atropine and atenolol together had effects similar to those of atropine alone, however, they were less marked. The difference between atropine alone and atropine plus atenolol was significant only for HR, MeanRR and VSE.

Validity of HRV parameters

Table 3 presents r^2 values for the drug effect and calf effect in the analyses of variance. The r^2 values for drug effects were the highest for VSE, HR, MeanRR and RMSSD (between 0.41 and 0.61). For these parameters, the corresponding r^2 values for calf effect were the lowest (between 0.03 and 0.11). The total power, the powers of the LF and of the HF bands, and the LF/HF ratio should be similarly explained by the calf and drug effect. Variations in SDNN were found to depend much more on the calf than on the drug.

Table 3. Values of r^2 for drug and calf effects.

	r^2 calf	r^2 drug
HR (bpm)	0.11	0.49
MeanRR (ms)	0.06	0.48
SDRR (ms)	0.34	0.06
RMSSD (ms)	0.10	0.41
TPW (ms^2)	0.29	0.23
LF (ms^2)	0.25	0.28
HF (ms^2)	0.13	0.18
LF/HF	0.14	0.23
VSE	0.03	0.61

Discussion

The main results of this study concerned the finding that atenolol injection had no effect on cardiac activity of calves, whereas atropine induced large modifications which were moderated when atenolol was administered at the same time. The drugs predominantly affected VSE, HR, MeanRR and RMSSD.

First of all, the heart rate of calves increased after the injection of atropine and their heart rate variability decreased. The decrease in HRV is observed in the time domain through shorter differences in the

duration of adjacent RR intervals (RMSSD) and in the frequency domain through a lower TPW. More specifically, the power of LF declined while that of HF was not significantly affected. Previous studies reported a similar increase of heart rate in subadult and adult cattle after the injection of atropine, together with a lower power spectrum (Gregory and Wotton 1981, Clabough and Swanson 1989). Hence in cattle as in other species, the vagal tone – which is suppressed by atropine injection – acts to reduce the heart rate and to increase the heart rate variability.

Second, we found no difference in HR or HRV after atenolol or saline injection. At first sight, it might seem that calves are not responsible to atenolol. However, this hypothesis is ruled out by the ineffectiveness of isoproterenol after atenolol blockade and by the fact that atenolol reduced the effects of atropine when both drugs were injected together. The most likely explanation is that the sympathetic tone of calves is very low or even absent when they are in a quiescent state. Our calves had been habituated to the handling procedure. In addition, we could not detect any behavioral reaction when the drugs were being injected: calves did not try to move away from the experimenter and moved calmly when they were released at the end of the experiment. Hence none of the tested HRV indexes could reflect sympathetic activity. Furthermore, the lack of HRV measures indicating sympathetic tone has already been put forward in dogs (Bailey *et al.* 1996, Houle and Billman 1999) and humans (Kingwell *et al.* 1994).

Third, the intrinsic firing rate of the sinus node was found to induce an interval between beats of 519 ms. As was hypothesized by Goldberger (1999), the interval between beats increased compared to this intrinsic value, when the parasympathetic tone only was active (with atenolol) and was decreased when the sympathetic tone only was active, leading to a VSE higher or lower than one, respectively. Again, we observed that the VSE after saline was not different from that after atenolol injection, which supports the idea that the heart rate of calves, in our experimental context, is mainly controlled by parasympathetic tone.

If we now discuss the relative validity of each HR or HRV measures, we can already conclude that SDRR and the ratio LF/HF cannot be used to assess the sympatho-vagal balance of calves since they do not vary during autonomic blockade. Despite this, the other frequency domain parameters (TPW, LF and HF) were affected by drug injection. However, their validity is

questionable because of the individual variations which were as large as the drug effects. In our calves, the best indicator of autonomic modulation of cardiac activity seemed to be the VSE index, since the individual variations for this measure, were low and the effects of drugs were large. Then HR or MeanRR, which are linked with each other, are also good indicators of autonomic modulation with effects of drugs which were about five times larger than the individual variations. Concerning HRV measures, only RMSSD is likely to be used successfully to assess vagal modulation of cardiac activity in calves. RMSSD is a time domain parameter which takes into account only the high frequency (rapid) variation of RR interval and then specifically quantifies the influence of the parasympathetic branch on the heart rate. Its reliability is well recognized in other species including man, dog and rat (Hull *et al.* 1990, Stein *et al.* 1994, Sgoifo *et al.* 1997). In addition, none of the measures of HR or HRV were found to reflect sympathetic tone in calves. As suggested above, this might be due to a lack of sympathetic tone in our calves under the conditions of this study. Calves were observed one hour after the morning milk meal. At this time, calves are usually very quiet: their heart rate and their behavioral activity are low. Under stressful conditions, such as during handling, branding or separation from their peers,

the heart rate of cattle is enhanced (Lay *et al.* 1992, Boissy and Le Neindre 1997, Lefcourt *et al.* 1999). An increase in heart rate can be due to a lower parasympathetic tone and/or to a higher sympathetic tone. Autonomic blockades should be performed under such stressful conditions in order to check that, whatever the situation, calves have a low sympathetic tone and consequently no measure can be used for assessing the sympathetic tone in calves.

In conclusion, in calves as in other animals, the parasympathetic tone acts on cardiac activity by moderating heart rate and increasing its variability. The sympathetic tone seems to be very low in calves, at least when they are not under stressful conditions. HR, MeanRR and RMSSD can be used to assess the parasympathetic tone of calves. Nevertheless, the best index appears to be the net vago-sympathetic effect (VSE) which is obtained by comparing the duration of beat-to-beat intervals to its intrinsic value measured under double autonomic blockade.

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