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Short communication

The magnitude of respiratory sinus arrhythmia of a large mammal (the horse) is like that of humans

Giuseppe Piccione[a], Elisabetta Giudice[a], Claudia Giannetto[a], Jacopo P. Mortola[b,*]

[a] Department of Veterinary Sciences, Faculty of Veterinary Medicine, Messina University, 98168 Messina, Italy
[b] Department of Physiology, McGill University, Montreal, QC, H3G 1Y6, Canada

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ABSTRACT

Heart rate (FH) accelerates in inspiration and decelerates in expiration, a phenomenon known as Respiratory Sinus Arrhythmia (RSA). Although the presence of RSA has been documented in many species, how its magnitude compares among species is unknown. We asked whether the magnitude of RSA in a large mammal, the horse, differed from that of previously measured humans. From electrocardiogram and pneumography, the peaks and troughs of FH were identified breath-by-breath in four horses (Italian Saddlebred geldings) during resting wakefulness. RSA was computed as the peak-trough FH difference, in percent of mean FH. Horses had lower FH and respiratory frequency (FR) than humans, but similar FH/FR. RSA ranged between 6% and 15%, with an average of 9 ± 2%, not statistically different from the mean value in humans (12 ± 1%). Like in humans, in horses the FH/FR values below the mean had correspondingly lower RSA, while values above the mean had correspondingly higher RSA. If confirmed in other species, these results suggest that RSA is body size-independent. The correlation with FH/FR, rather than FH or FR, supports the view that RSA optimizes the coupling between pulmonary blood flow and ventilation.

1. Introduction

Breathing-related changes in sinus rhythm, or respiratory sinus arrhythmia (RSA), have long been recognized in humans and in other species. They reflect the intermittent inhibition of the parasympathetic control of the cardiac rhythm. During the inspiratory phase of the breathing cycle the integration of peripheral and central mechanisms causes inhibition of the cardio-inhibitory nucleus ambiguus, which leads to cardiac acceleration. The peripheral mechanisms include changes in intrathoracic and blood pressure and the inputs of the pulmonary stretch receptors while central mechanisms consist in the interactions between the respiratory and the cardio-inhibitory centers of the brainstem (Daly, 1986). Experiments in dogs under vagal stimulation and artificially-controlled ventilation have indicated that RSA improves gas exchange, by fine tuning the matching between pulmonary blood flow and ventilation (Hayano et al., 1996).

The occurrence of RSA most commonly is demonstrated from the power spectrum of a sequence of inter-beat-intervals constructed by Fast Fourier Transformation of the series; RSA corresponds to the high-frequency range of the spectrum that overlaps with breathing frequency (FR) (Malik, 1996). By this approach, we now know that RSA is present in many species, possibly representing a general phenomenon of the mammalian cardio-respiratory coupling. However, power spectrum analysis cannot give quantitative data on heart rate (FH, beats/min, bpm) and its difference between inspiration and expiration. Therefore, despite the ubiquity of RSA, the quantitative question “is the magnitude of RSA similar, or does it differ systematically, among mammals?” does not have an answer. The only precise method to quantify the magnitude of RSA is through breath-by-breath analysis of the instantaneous FH. This cumbersome approach, from simultaneous measures of FH and FR, has been adopted occasionally in humans (Hirsch and Bishop, 1981; Mortola et al., 2018). In a large group of young men and women (142 subjects) the breath-by-breath inspiratory-expiratory ΔFH averaged 9 bpm, or ~12% (± 1) of mean FH (Mortola et al., 2018). Similar data from other species are rare and their numerical comparisons to humans are complicated by methodological issues (e.g., animal under restrain, sedation or anesthesia) or irresolvable differences in data analysis (e.g., ΔFH computed from the max and min instantaneous FH, from the average FH of the whole inspiration and expiration, or as ratio between inspiratory and expiratory FH).

In humans, increases in FR lowered RSA (Hirsch and Bishop, 1981; Mortola et al., 2016) and in dogs the higher FH the lower RSA (Hanton and Rabemampianina, 2006). Therefore, since FH and FR decrease systematically with body size (Peters, 1983), one may expect high

* Corresponding author at: Dept. Physiology, McGill Univ., Room 1121, 3655 Sir William Osler Promenade, Montreal, QC, H3G 1Y6, Canada.
E-mail address: jacopo.mortola@mcgill.ca (J.P. Mortola).

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values of RSA in large mammals. However, in humans, RSA correlated best with the ratio of these two variables (FH/FR, 'breathing specific heart rate'), rather than with FH or FR singly considered (Mortola et al., 2016, 2018). In such a case, because the FH/FR ratio among terrestrial mammals is body size-independent (Peters, 1983; Mortola, 2015), also RSA should be body size-independent. To test this possibility, we have measured RSA in resting horses. Being five-to-eight times heavier than humans, we figured that in this species differences in RSA with animal size, if existent, should be easily detectable.

2. Methods

2.1. Experimental animals

Measurements were obtained on four adult Italian Saddlebred gelding horses (estimated body weight 400–500 kg). Treatments, housing and animal care were carried out in agreement with the local Ethics Committee and in accordance with the standards recommended by the EU Directive 2010/63/EU for animal experiments. The specimens, enrolled in the study with the informed owner's consent, were carefully selected because of their most calm temperament and familiarity with human handling. A fifth horse was recorded but later excluded from the analysis because its FH was unusually high (Raekallio, 1992) and its FH and FR values (respectively, 69 bpm and 33 breath/min) were about three times the group average. Despite the familiarity to human handling, this horse probably was nervous about the instrumentation.

2.2. ECG and breathing recordings

The ECG was obtained from the classic limb method for standing quadrupeds, although there is no universally accepted lead system for large animals. We opted for three peripheral derivations, with cutaneous electrodes (10 mm in diameter) positioned on the caudal portion of the limbs, on skin areas shaved for better contact. Signals were sampled at 400 Hz, in a manner identical to the limb recordings on humans (Mortola et al., 2016, 2018), on an acquisition system (PowerLab ®, ADInstrument, Colorado Springs, CO) connected to a minicomputer. If the R wave was not easily detectable, the electrodes were repositioned. Two electrodes produced a peripheral lead of the ECG while the third one acted as ground. The ECG acquisition had hardware notch filter at 60 Hz, and low- and high-pass filters at 50 and 0.1 Hz.

Breathing was monitored from chest wall movements, with a Piezo Respiratory Belt Transducer (ADInstrument, Colorado Springs, CO) that responded linearly to changes in length. The acquisition rate was 100 Hz and no filtering was required. The belt was positioned around the lower portion of the chest wall, where the abdominal expansion during inspiration was most obvious.

Measurements were gathered between mid-morning and mid-afternoon hours. Ambient temperature, measured with a data logger (Hobo H8, Onset Corp, Bourne, MA), varied between 22 and 24 °C. With the animal standing, once a good signal was established, recordings continued for 2–3 min, or a period long enough to obtain at least thirty breaths free of artifacts. An ad hoc algorithm detected the peaks of the R waves of the ECG to compute the inter-beat interval (IBI, ms), converted into instantaneous FH [beats/min = 60/(IBI/1000)]. The accuracy of the electronic conversion of IBI into instantaneous FH had been checked in preliminary tests with an electrical stimulator (SD9, Grass, Warnick, RI) that delivered square pulses at variable frequencies; no discrepancy emerged over the whole range of FH. Data were analyzed breath-by-breath for a total of 30 breaths. Fig. 1 gives an example of the peak and trough detection. Differently from humans, in the horse each inspiration combines two phases, the relaxation of the previously contracted expiratory abdominal muscles followed by the contraction of the inspiratory muscles; in fact, horses characteristically breathe around, rather than from, the resting volume of the respiratory system

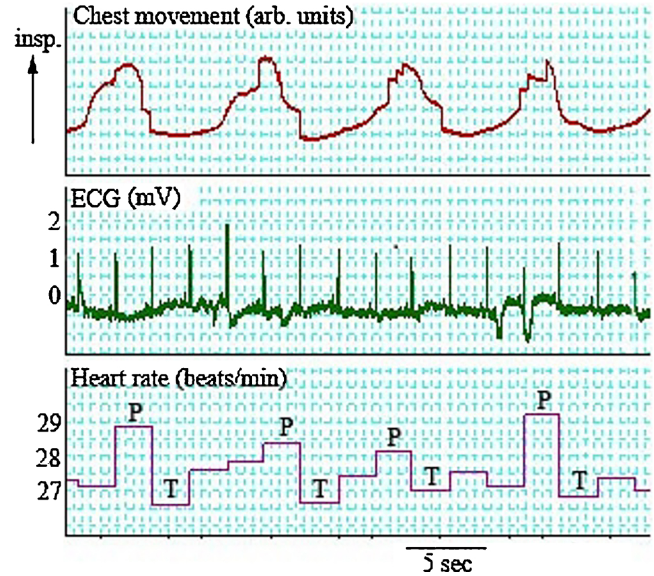

Fig. 1. Example of the breath-by-breath measurements of the peak (P) and trough (T) of instantaneous heart rate in a horse. From the top, chest movement (inspiration upward, arbitrary units), electrocardiogram (mV), beat-by-beat heart rate (FH, bpm). From the P-T difference (ΔFH), respiratory sinus arrhythmia was computed as ΔFH in percent of mean FH.

(Koterba et al., 1988). For each breath, the peak and trough FH were the highest and lowest values detected; then, RSA was the peak-trough difference in percent of mean FH. Mean FH (bpm) and mean FR (breaths/min) were computed over the total time of the 30 breaths.

Values in humans were the mean values of a large group of young subjects at rest (N = 142, 81♀ and 61 ♂, age 20 ± 1 year old, body weight 67 ± 1 kg; Mortola et al., 2018), obtained with an analytical approach identical to that of the current experiments.

2.3. Statistical analysis

Data are presented as means ± 1 SEM. Differences between the two sets of data (humans and horses) were evaluated statistically by two-tailed *t* test and considered significant at $P < 0.05$.

3. Results

Table 1 presents the individual values of the four horses. By comparison to humans (Mortola et al., 2018) the horses, as expected, had lower FH and FR. The average FH/FR and RSA did not differ statistically from the corresponding values in humans.

Table 1
Individual characteristics.

Horse #	mean FR breaths/min	mean FH bpm	FH / FR beats/breath	RSA %
1	5.6	31.3	5.6	14.7
2	6.7	31.3	4.8	9.8
3	7.1	27.7	3.9	5.6
4	12.3	27.4	2.2	7.3
average	*7.9 ± 1.5*	*29.4 ± 1.1*	*4.1 ± 0.7*	*9.3 ± 2.0*
Humans[a]	*13.6 ± 0.2*	*70.0 ± 0.7*	*5.8 ± 0.1*	*12.5 ± 0.3*
P	*< 0.01*	*< 0.001*	*ns*	*ns*

Values are means ± 1 SEM. FR, breathing frequency (breaths/min). FH, heart rate (bpm = beats/min). RSA, Respiratory Sinus Arrhythmia (ΔFH /mean FH, %).

[a] Average data of 142 subjects, from Mortola et al., 2018. P, P value of the statistical comparison between Humans and Horses (ns, no statistical difference).

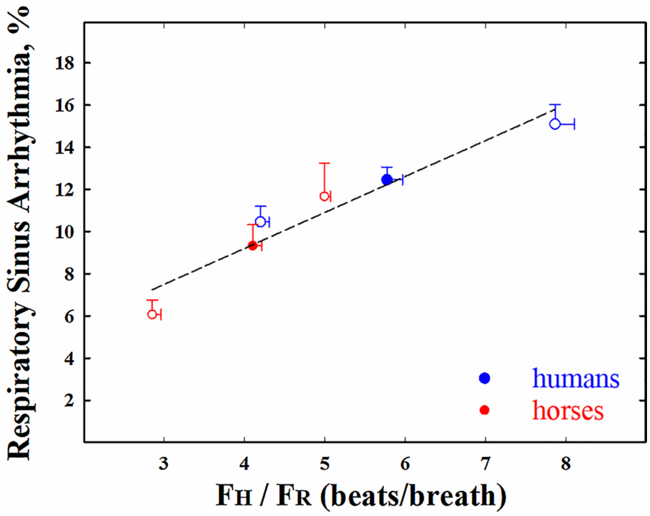

Fig. 2. Mean values (bars represent 1 SEM) of respiratory sinus arrhythmia (RSA) as function of the breathing-specific heart rate (FH/FR) in humans (blue filled circles) and horses (red filled circles). Open symbols are the averages of those data with FH/FR values, respectively, below or above the mean of the whole group.

In both humans and horses, RSA had a positive significant correlation with FH/FR; the set of FH/FR values below the mean had correspondingly lower RSA, while the set above the mean had correspondingly higher RSA (Fig. 2).

4. Discussion

The main finding was that in horses the magnitude of RSA was similar to that of humans. This result supports the view that RSA better correlates with the ratio between FH and FR, rather than FH or FR singly considered.

4.1. Methodology

Many conditions that modify the balance between sympathetic (acceleration) and parasympathetic (deceleration) tone on the sinus rhythm also affect RSA, most likely because they modify the relative importance of the breathing-related inhibition of the nucleus ambiguous. In addition to anesthesia, sedation or changes in the state of arousal, fear and strong emotions are all potential modifiers of RSA. We cannot exclude the possibility that emotional stimuli may have altered the data, other than the consideration that the resting FH was within the range observed in horses chronically instrumented and left undisturbed for hours (Raekallio, 1992).

For the breath-by-breath quantification of RSA we have chosen to measure the difference between the highest and lowest instantaneous FH. If, instead, ΔFH was computed as the difference between the mean inspiratory and expiratory FH, the resulting RSA value would have been lower. For example, the RSA of the breaths presented in Fig. 1 averaged 6.8%, but it would have averaged only 3.2% had ΔFH been computed as the difference between the inspiratory and expiratory means. Each analytical approach has its merit. However, analytical differences preclude the numerical comparison of RSA between the present study and data collected on other species.

4.2. Correlation with FH/FR

In humans, RSA has a strong negative correlation with FR (Hirsch and Bishop, 1981; Mortola et al., 2016). In Beagle dogs the ratio between maximum and minimum inter-beat-interval over a 10-s period decreased as FH increased (Hanton and Rabemampianina, 2006). If these correlations between FR or FH and RSA occurred inter-specifically, given that FR and FH decrease with species size (Peters, 1983), one could anticipate RSA to be very small or absent in small species and large in large mammals. The present data, however, showed that horses do not have RSA greater than humans, even though FR and FH were about half the human values.

In humans, experimental conditions designed to change either FH or FR revealed that RSA correlated best with the ratio between FH and FR ("breathing-specific heart rate", a proxy for cardio-respiratory coupling), rather than with FH or FR singly considered (Mortola et al., 2018). That result was interpreted as being in support of the view that RSA contributes to the efficiency of gas exchange, by fine-tuning the matching between pulmonary blood flow and ventilation (Hayano et al., 1996; Giardino et al., 2003). The directional changes between inspiration and expiration, required by the mammalian respiratory system, produce large air-flow oscillations. Equivalent oscillations in blood flow do not occur in the pulmonary circulation because, being the heart in series with the vasculature, cardiac output originates from high FH and small stroke volumes, by comparison to the FR and tidal volume of the pulmonary ventilation. RSA mitigates the differences in flow patterns by adjusting the number of heart beats according to the FH/FR. Among terrestrial mammals in resting conditions FH and FR decrease with body size, and their ratio is body size-independent (Peters, 1983; Mortola, 2015). Hence, the finding that RSA in horses is like in humans, and the positive correlation between FH/FR and RSA (Fig. 2), support the possibility that the FH-FR ratio is the relevant variable for the magnitude of RSA.

4.3. Conclusions

The magnitude of RSA of resting horses was like that of humans. The results agree with the views that RSA has a better correlation with the ratio between FH and FR than with either FH or FR. The existence of this correlation emphasizes that RSA may contribute to the coupling between pulmonary blood flow and ventilation in optimizing gas exchange.

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