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FORMS OF PHYSIOLOGICAL ALIASING WITHIN THE HEART RATE FLUCTUATIONS BY HIGHER FREQUENT RESPIRATORY MOVEMENTS

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In adult conscious rabbits, the respiratory frequency (RF) is almost always higher than half the heart rate (HR) Thus, no "classical" respiratory sinus arrhythmia occurred. But now, slow HR fluctuactions, which were not synchronous to the respiratory rhythm but effected by it, occurred systematically. By cholinergic blockade, these slow HR fluctuations could be essentially reduced but not completely abolished. Up to now, this phenomenon was not be taken into account in the analysis of HR fluctuations. Thus misinterpretations are inevitable. During general anaesthesia, the RF decrease up to 2 RF < HR, and now classical respiratory sinus arrhythmia occurred which also could be essentially reduced but not completely abolished by vagal blockade. The possibilities of calculation of such slow HR fluctuations were shown, as those during 1/2 HR < RF < 3/2 HR and RF > 3/2 HR.

K e y w o r d s: *Heart rate fluctuactions, respiratory sinus arrhythmia, power spectral analysis, coherence, physiological aliasing.*

INTRODUCTION

Respiratory sinus arrhythmia, i.e. fluctuations of heart rate (HR) synchronous with breathing, is caused by breathing phase related modulation of the HR. This direct modulation of HR by central nervous and peripheral cardiorespiratory coordination (1) is systematically possible up to a relation of respiratory frequency (RF) to the HR 1:2. If 2 RF > HR, the HR-sequence cannot any longer follow the modulating effects of the inspirium or expirium. Now, there are no breathing synchronous fluctuations of HR in the known sense of respiratory sinus arrhythmia. But then, like by the undersampling of time series, the relation 2 RF > HR effects slower, not breathing synchronous HR fluctuations as a form of physiological aliasing phenomenon. For the first time, such slow HR fluctuations were seen in human newborns and in examples in conscious dogs and rabbits (2—4), but not systematically described.

In the following, these HR fluctuations were systematically investigated in rabbits in awake, anaesthetized state and in vegetative blockades by spectral analysis. To confirm the physiologica state of such RF/HR relations and especially such high RF, at first the behaviour was determined (5) in comparison to the RF/HR relations. Then, these slow HR fluctuations are described.

During a time interval usually to be analysed, HR nd RF vary in a statistic manner. Thus, according to the sampling theorem and the aliasing effects (3, 6), the frequencies of such slower HR fluctuations do not show single discrete frequencies but more or less systematic frequency distributions (2—4). We try to evaluate the possible frequency bands and their power density maximum by taking into account the RF and HR distributions. Moreover, these slow HR fluctuations have frequency distributions within bands of HR fluctuations of other origin like socalled 10 sec-waves or blood pressure waves of HR. They must be separated from each other to avoid misinterpretations. By the absence of correlative relations between HR fluctuations and RF calculated by coherences, we can try to separate such slower HR fluctuations from those of different origins.

Possible haemodynamic effects of RF/HR = 0.5 and correspondence of one heart beat to inspiration and one to expiration causing very different praeload and stroke volume were investigated by arterial pressure registration.

MATERIAL AND METHOD

In animal stables to which the rabbits were accustomed, the behaviour of 25 adult rabbits (Weißes Großsilber) of both sexes (15 males; 10 females) were observed during 3 days according to (5) determining 3 behaviour types (regarding the criteria see results).

In a first experiment (18 rabbits), HR and the relation of HR to RF were determined within the behaviour types 1—3 during rest state of rabbits sitting or lying in experimental boxes to which the animals were accustomed, too. HR were continuously determined by ECG recorded by chronically applied small metal electrodes. The steepest part of ascending R-wave was used to trigger instantaneous HR determination. Respiratory movements (RM) and RF was determined by impedance respirography (details in 7).

In the second, i.e. the main experiment (7 rabbits), HR, RF were registered during 2 h in the stables and under experimental conditions. After recordings during rest, the animals were anaesthetized by Ketamine (Velonarcon^{R} 25 mg/kg) and Xylacine (Rompun^{R} 5 mg/kg) i.m. When the animals awoke from this general anaesthesia, the recordings were repeated before and after i.m. injection of 3 mg/kg Atropine (to get a permanent heart rate increase > 25 min^{-1}). In anaesthetized animals, the RF was additionaly determined by pneumotachography (Fleisch, Hugo Sachs); the body temperature was kept constant tween 37.5 and 38.5°C .

During another anaesthesia of the same type, a catheter was fixed within the A. femoralis for arterial pressure recording and taking blood samples to guarantee a normal acid-base-status and arterial oxygen pressure.

Then, before and after another 3 mg/kg Atropine injection (because of the high atropinase activity of rabbits), the same parameters were recorded. This was also done before and after the Nn. vagi were cutted, and in 3 cases the medulla oblongata was transsected 2 mm caudally from the

obex (with artificial ventilation). Additionally in 3 rabbits awaking from anasthesia 3 mg/kg atropine were injected. During the several phases of awake, anaesthetized, or transitional states the animals did not show any signs of pronounced excitements, anxiety or other reactions to stress (determined according to (8, 9)).

From stationary, artifact free 55-sec-intervals, (120—180 reciprocal RR-intervals) power spectra and coherence of HR fluctuations and RM were calculated (confidence areas according to (10); further details in 2, 3, 4)). In 3 cases, additionally corresponding power spectra of arterial pressure fluctuations were calculated.

The frequency regions of significant spectral power densities within the main and other spectral peaks were demarcated from the non significant ones by the local minima showing spectral amounts near basic noise. In cases of 2 RF > HR, the frequency ranges determined in this way were used together with that of HR for the calculation of the slower HR fluctuations which are not synchronous to respiratory rhythms but effected by them. To calculate circumscribed spectral peaks of such slow HR fluctuations, the difference of mainly existing HR during the recorded interval and the one or more peak frequencies of spectral power of RF were used (6).

Significant amounts of power spectral densities of such slower HR fluctuations without circumscribed spectral peaks were estimated by calculation of the dominant frequency range of relations of RF to HR, too.

In both cases, only frequency ranges without significant coherence between respiratory and HR rhythms were taken into account, because otherwise a direct influence between both rhythms in these lower frequencies can be the reason for such slower HR fluctuations. When RF > 3/2 HR, these HR fluctuations were calculated by 2 HR — RF according to (3, 6), and the possible existence of slow HR fluctuations was verified by th same procedure as in 1/2 HR < RF < 3/2 HR.

In all animals and experimental states (*Tab. 1*), two visually stationary 51.2 sec intervals without artifacts were taken into evaluation. Up to 3 exceptions, there were no different spectral results between these two examples. In these exceptions the most frequent types from at least 3 examples were chosen (*Tab. 1*). Other statistical examinations were done by parameter free tests (Wilcoxon-and H-test according to (11)).

Table 1. Distribution of forms of heart rate fluctuations (HRF) (< 0.1 Hz: significant power spectral densities of HRF < 0.1 Hz; ca. 0.1 Hz (0.07—0.2): HRF in the range of socalled 10 sec waves; RSA: respiratory sinus arrhythmia; Coh RM-HRF: significant coherences between respiratory movements and HR fluctuations, SHR = Slower HR fluctuations resulting from high frequency respiration ((2 RF > HR); significance level: $p < 0.05$)

Experimental state (n = 7)			
HRF:	awake	general anaesthesia	
		without blockade	with vagal
< 0.1 Hz	6	0	0
ca. 0.1 Hz (0.07—0.2)	7	4	4
RSA	0	7	(6)
sign. Cohn RM–HRF	0	7	(7)
SHR (2 RF > HR)	7	0(1)	0

= number of rabbits with significant power spectral peaks; in parenthesis; number of cases with strong reduction

RESULTS

Behavioural types, HR and HR/RF relations

The 3 different behavioural types of 18 rabbits which were the same in stables as under experimental conditions, showed the following significant differences (*Tab. 2*).

Table 2. Mean HR (per min) and HR/RF-relations and their SEM of the three types of behaviour (18 rabbits) in rest conditions and vagal blockade (H-test/Dunn: * signif. lower in comparison to Type 1 and 3; ** signif. lower than type 1 and 2; significance level: $P < 0.05$).

	Rest	Vagal blockade
Type 1		
HR	216.2 ± 19.1	302.4 ± 15.1
Type 2		
HR	211.5 ± 7.6	279.2 ± 12.8
HR/RF-rel.	0.79 ± 0.06 *	1.02 ± 0.10
Type 3		
HR	191.5 ± 13.9 **	252.5 ± 12.8
HR/RF-rel.	0.96 ± 0.09	1.51 ± 0.61

Type 3 (relaxed resting posture > 70% of time, few motor activities) has a significantly lower HR ($p < 0.05$) in comparison to the another one, and Type 2 (complex behavioural and more motor activities) showed significantly lower HR/RF relations ($p < 0.05$) in comparison to the other ones (type 1: tense resting posture > 70% of time, few motor activities). But the relation 2 RF > HR was always observed in all 3 behavioural types.

Slower HR fluctuations caused by respiration

Almost only in awake animals (*Tab. 1*), such slower not respiration synchronous, but respiratory caused HR fluctuations occur because of the relatively high respiratory frequency (mean of 7 animals 253 ± 18 min^{-1}) in comparison to the heart rate (mean of 7 animals 199 ± 8 min^{-1}). As can be seen in *Tab. 1 and 2,* they exist in conscious rabbits without exception, and often there are single or 2—3 spectral peaks of such slower HR fluctuations. There were no statistical differences between the RF and HR in the stables (196 ± 9 min^{-1} and RF: 230 ± 37 min^{-1}) and the above mentioned under experimental conditions. Thus, always the relation 2 RF > HR was found. Only in 6 of 35 spectra, the peak frequency of these slow HR fluctuations could be exactly calculated by the transformation rules quoted (3, 6). But, the demarcation of these slow HR fluctuations from other well known ("classical") rhythms is possible in almost all cases (*Tab. 1*).

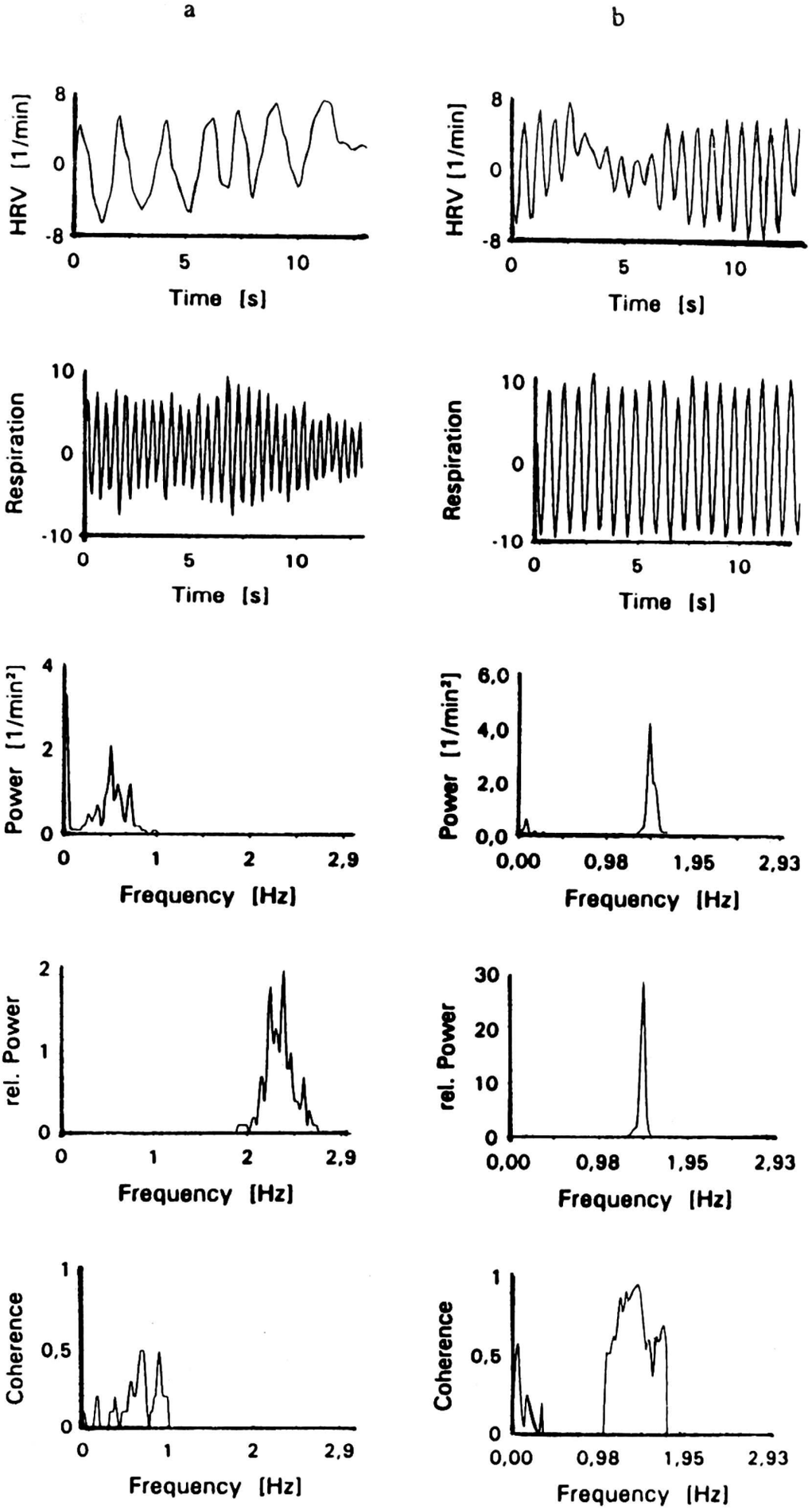

Fig. 1a. HR fluctuations (HRF), respiratory movements (RM), HRF spectrum, RM spectrum and RM-HRF coherence (from top to bottom) of a. a conscious rabbit and b. the same anasthetized rabbit. (a. HR: 2.95—3.02 Hz; significant amounts of RF spectrum ($p < 0.05$): 2.02—2.72 Hz. Calculated range of slower HR fluctuations by aliasing from 0.23 Hz (2.95—2.72) up to 1.00 Hz (3.02—2.02). Significant amounts of HR spectrum: 0.22—1.00 Hz excluding the initial 1/f-range (< 0.10 Hz). Coherence did not show significant amounts (confidence range of $p < 0.05$: > 0.70; b. similarities of spectral power distributions of RM and HRF including identical peak maxima in 1.49 Hz; significant coherence in the whole HRF and HR main peak range).

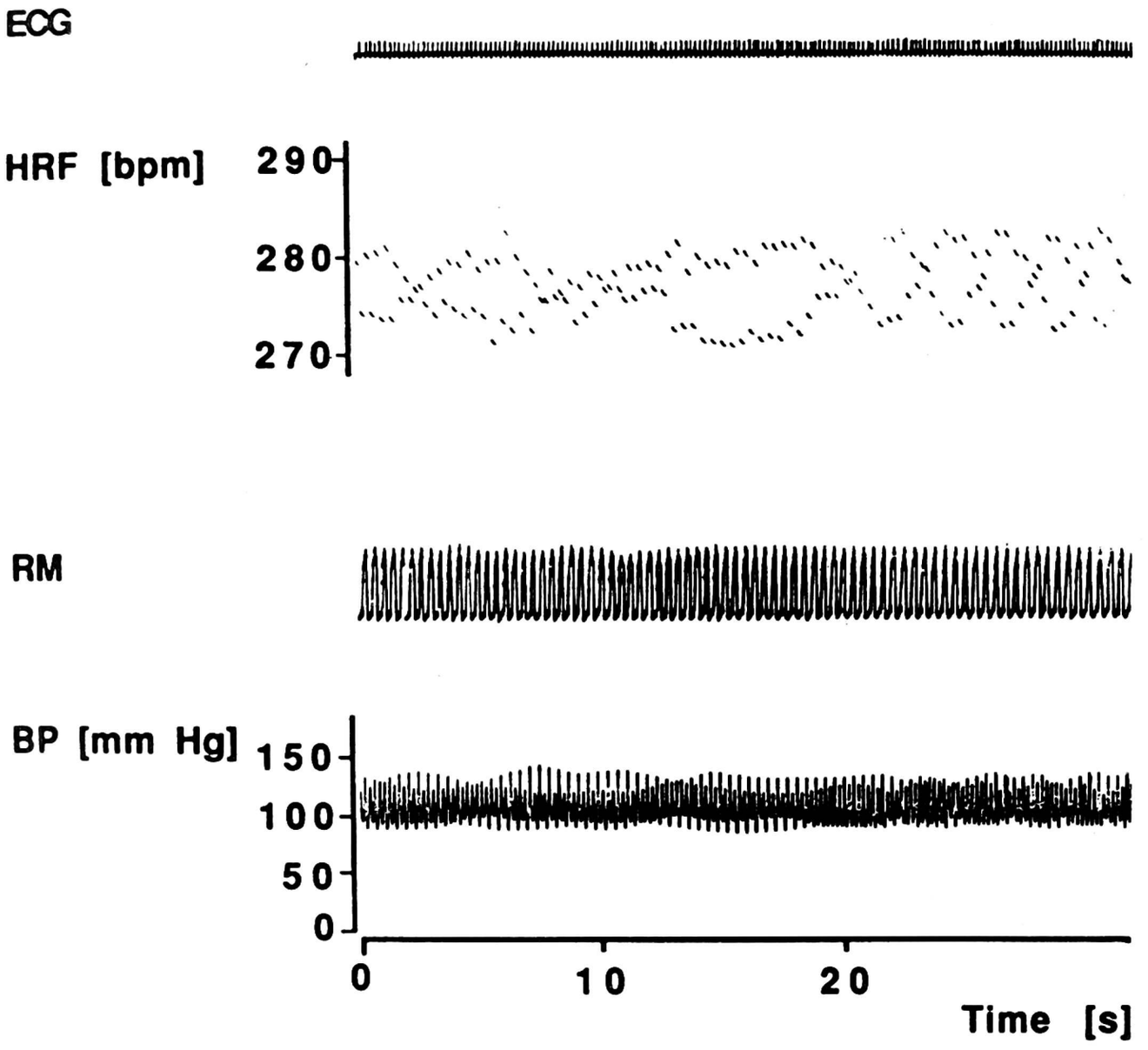

Fig. 2. Electrocardiogram (ECG), HR fluctuations (HRF), respiration movements (RM; 140 min^{-1}) and arterial pressure (BP) of an anaesthetized rabbit showing pulsus alternans during HR : RF = 2 : 1.

Fig. 1. gives a representative example of complete physiological aliasing in awake state (*Fig. 1a*) and a "classical" respiratory sinus arrhythmia in general anaesthesia (*Fig. 1b*). Vagal blockade in awake animals effected a heart rate increase of 43.83 ± 18 min^{-1}). The increase of HR is so strong that in our cases the relation is HR > 2RF. Then, these slow HR fluctuations are abolished.

Regarding the forms of such slower HR fluctuations effected by respiration rhythm, we observed 2 unimodal, 2 bimodal and 3 trimodal spectral peaks of these HR fluctuations in frequency regions without significant coherences. In 2 cases, also the higher harmonics of main peak of spectral power of respiratory movements caused slower HR fluctuations. 3 out of 7 rabbits showed the relation 3/2 HR < RF < 2 HR, the others showed HR < 2 RF < 3/2 HR. According to the transformation rules (3, 6) in the first case the slow HR fluctuations showed spectral peaks at 2 HR – RF, in the second case at

HR – RF. In the 3 rabbits with cholinergic blockades after awaking from anaesthesia, the main parts (> 60%) of such HR fluctuations were abolished.

The haemodynamic effects of such relatively high RF in comparison to the HF are important for seldom cases of exact 1 : 2 relations (*Fig. 2*). The resulting pulsus alternans can be explained by the correlation of single heart beat intervals to the whole inspirium or expirium, i.e. to phases of different cardiac preloads. In the 3 rabbits investigated correlating peaks of spectral power densities and significant coherences of arterial pressure fluctuations and HR fluctuations were observed mainly in anaesthetic state near 0.1 Hz, but never in the frequency region of calculated aliasings of HRF.

General anaesthesia reduces respiratory frequency considerably (in the mean by 61.29 ± 6 min^{-1}). Consequently, RF is almost always lower than 1/2 HR (6 out of 7 animals). The slower not respiratory synchronous, but respiratory caused HR fluctuations disappear and respiratory sinus arrhythmia appears (*Tab. 1*). Repeated vagal blockade during anaesthesia (tu guarante a sufficient block because of atropinase activity) depressed significantly, but did not completely abolish respiratory sinus arrhythmia (reduction to > 70% of spectral power density). The additional cutting of both Nn. vagi did not change the results. Brain stem transsection in 3 rabbits reduced respiratory sinus arrhythmia even more (to < 10%).

DISCUSSION

Such slower HR fluctuations which are not synchronous to the respiratory rhythm but effected by it exist in all 7 awake rabbits of the second (main) experiment showing all a high RF (*Tab. 1*). This RF level was also evaluated by observation of the 18 animals sitting in the stables or experimental boxes and showing the same behavioural types in both situations (*Tab. 2*). Murthy et al. (12) recorded a similar, partly higher HR in awake rabbits. Similar relations of 2 RF > HR were also recorded in resting human neonates and resting dogs (4, 13). Thus, we can presume physiological states in our recordings. Up to now, HR fluctuations in this frequency region of awake animals were interpreted not taking into account such critical relations as 2 RF > HR as possible reason of slow HR fluctuations.

The decision, whether HR fluctuations near O.1 Hz are really socalled „blood pressure waves" of HR or of other origin must exclude such physiological phenomena of „cardiac aliasing" (2, 3) by taking into account the instantaneous relations between RF and HR. Meanwhile we have observed such physiological aliasings in conscious dogs (13), human neonates (4) and rabbits (1). Moreover, evaluations of the respiratory sinus arrhythmia must also take into account the effect of such RF-HR relations. When always the relation 2 RF > HR can be found continuously than no respiratory sinus

arrhythmia exists. If such relations occur temporary, the RSA decreases, and false diagnostic evaluations as depressed short term variability of HR fluctuations are possible (4). But even single frequency components in the sense of harmonics of respiratory rhythms can provide components of slow HR fluctuations as seen in awake and rarely in anaesthetized rabbits. The evaluation of such slower HR fluctuations must take into account the frequency distributions of HR and RF and their maxima. An origin of such slower HR fluctuactions by direct coupling between such slower HR fluctuations and analogous components within the respiratory rhythms are excluded by the evidence of absent significant coherence between the two rhythms. From the differences of maxima of HR and RF distribution (or 2 HR – HR, if RF > 3/2 HR) the maximum of the slow HR fluctuations can be evaluated. From both distributions, also the whole frequency band of such slower HR fluctuations can be evaluated (*Fig. 1*).

To determine the possible contribution quantitatively, the real time sequence of relations of HR and RF must be taken into account as attempted in this study. Because of the changing RF–HR relations, often 2 or more partial peaks of such lower HR fluctuations occur.

The essential reduction of such slower HR fluctuations by atropine in 3 awaked animals proved a cholinergic mediation of this aliasing phenomenon.

By the decrease of RF in all rabbits during general anaesthesia, such relations as 2 RF > HR and also such slower HR fluctuations disappear which are not synchronous to the respiratory rhythm but effected by it. Now, the „classical" respiratory sinus arrhythmia appears. This is the evidence for such slower HR fluctuations by reasons of signal theory alone, i.e. by „cardiac aliasing" (2—4).

Because the respiratory sinus arrhythmia is strongly reduced during vagal blockade, the respiratory sinus arrtythmia in rabbits must be mediated mainly vagally. Recently Bernardi et al. (14) show persisting respiratory sinus arrhythmia in vagotomized, anaesthetized and mechanically ventilated rabbits. But they did not examine the effect of vagal blockade in spontaneously breathing animals, which is not comparable to ventilated states.

Because of the very strong and immediate vagally mediated effect of baroreceptor input, a strong correlation between arterial pressure variations and HR fluctuations is possible. But during such relations of 2 RF > RF, an indirect effect via respiratorely caused quick arterial pressure waves is excluded because of the completely absent quick HRF in the sense of „classical" RSA. Regarding a possible effect of arterial pressure waves on the slower, i.e. the aliasing parts of HR fluctuations, in not any of the 3 investigated cases a significant coherence between HRF and arterial pressure waves within the calculated aliasing frequency range exists. Thus, a respirocardial interaction only is assumed for the observed aliasing effects. The main reduction of these

effects observed in the three cases of cholinergic blockade suggests a mainly vagal mediation.

To exclude a possible inhibition of the atropine effects by atropinase activity of rabbits despite the relatively high doses, both Nn. vagi were cutted, too, and the recordings were repeated. There were no differences of the results with and without vagal cutting. Thus, the cholinergic blockades by atropine were complete.

Richter and Spyer (15) showed the origin of RSA as an interaction within the common cardiorespiratory brain stem system with the vagal cardiomotor output of Nucl. ambiguus. We can assume similar origins for these slower HR fluctuations. Probably, the origin of such slower HR fluctuations during 2 RF > HR relations must also include the intraction between vagal cardiomotor efferences and the sinus node rhythm. A direct respirocardial mediation of this physiological aliasing is also not excluded, but unlikely because of the small amplitudes and short duration of RM in cases of 2 RF > HR and the essential reduction of this aliasing phenomenon by cholinergic blockade.

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