Regulation of Heart Rate Variation by the Autonomic Nervous System in Neonatal Lambs

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ABSTRACT. We studied the role of the autonomic nervous system in the regulation of heart rate variation (HRV) in 12 chronically instrumented neonatal lambs. HRV was quantified from ECG tracings by computing periodic HRV distributions at frequencies of 0.02–1.00 Hz, using power spectral analysis of heart rate, and also by HRV indices. Heart rate declined more during the 1st than the 2nd mo after birth. Multiple regression analysis showed that the heart rate responses to vagal and to β -adrenergic blockade had an independent negative association both with age and with the initial mean heart rate, whereas the overall HRV response had a positive association with age. Vagal blockade led to a 70–80% decrease in the beat-to-beat HRV in all lambs ($p < 0.001$). The overall HRV indices decreased by 40–65% in lambs (< 30 d old ($p < 0.001$) and about 30% in those > 30 d old ($p < 0.05$). In the power spectrum the greatest decrease was seen in the high-frequency components of HRV. β -Blockade led to a decrease of about 50% in all HRV ($p < 0.001$) in the younger lambs, without frequency selection. In the older lambs, it had no effect on the beat-to-beat HRV, but the overall HRV (coefficient of variance) decreased maximally by 40% ($p < 0.01$), with a significant reduction in the low-frequency components of HRV. These results suggest that in the regulation of HRV after birth dual control via the autonomic nervous system is most important. In the older lambs, developmental changes result in precise regulation of the fast heart rate fluctuations mainly by the vagal division, whereas the slow fluctuations are partially regulated by the vagal and β -adrenergic divisions. (*Pediatr Res* 27: 383–391, 1990)

Abbreviations

HRV, heart rate variation
RMSM, root mean square of differences from the mean interval, overall HRV
CV, coefficient of variation for RMSM, relative overall HRV
RMSR, residual HRV (RMSM) after trend correction
CVR, coefficient of variation for RMSR, relative residual HRV
RMSSD, root mean square of successive interval differences, beat-to-beat HRV
CVS, coefficient of variation for RMSSD, relative beat-to-beat HRV

Reduced HRV indicating altered cardiac control has been detected in neonatal respiratory disorders, particularly in newborns with respiratory distress syndrome (1–3), and in brain death (4). Further, infants subjected to birth asphyxia, and those who died of sudden infant death syndrome have been found to have exhibited low respiratory-related HRV (5, 6). The mechanism of subnormal HRV in these cases is not known. HRV is also dependent on the maturity, age and sleep state of the neonate (7, 8). Inasmuch as HRV and particularly respiratory sinus arrhythmia are influenced by autonomic nervous system activity (9–12), we studied the HRV of lambs, because using spectral analysis no data are available on the relation of this activity to HRV during the neonatal period. We used spectral analysis and HRV indices and autonomic blocking agents to study the autonomic nervous control of HRV in lambs, because human neonates cannot for ethical reasons be experimentally manipulated.

MATERIALS AND METHODS

Experimental animals and surgical procedures. We studied 12 healthy full-term Finnish-breed newborn lambs. They were allowed a 3-d period of postnatal adaptation before surgery. They were housed with their mothers to allow normal lactation and not weaned before the end of the study. Pellets, which contained protein 14.5%, and salt 0.5%, and hay were added to their diet from the age of 3 wk. The growth of the lambs was normal during the study period.

Instrumentation was performed under local anesthesia with 1% lidocaine solution (8). Polyvinyl catheters filled with heparin solution and sealed with a metal plug were inserted into an external carotid artery and jugular vein. Bilateral silver ECG electrodes were implanted s.c. into the thoracic wall at the midaxillary line and tunneled to the neck of the animal. They were deposited with the catheters in a plastic pocket attached to the skin for the 10-wk study period. The animals were given parenteral gentamicin and penicillin for 3–4 d postoperatively. No recordings were done during this recovery period.

Signal acquisition and study protocol. During the 1–2 h recording periods the lambs were placed upright in a sling with freedom to sleep or stand comfortably in a quiet study room with an environmental temperature of 24 to 27°C. Sequential recordings of ECG, heart rate, arterial blood pressure, and transthoracic electrical impedance respirograms were made using a Corometrics 512 Neonatal Monitor (Corometrics Medical System Inc., Wallingford, CT) and Statham pressure transducers. The signals were recorded continuously on paper and simultaneously stored on magnetic tape with an Analog 7 FM tape recorder (Philips) equipped with a storage monitor (Advance 2200, Advance, Essex, UK) for quality control. All recordings were made by one of the authors (A.S.I.S.), who documented the sleep state and motor activity of the lambs during the study sessions (8).

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The heart rate of the lambs decreased markedly with age, more during the 1st than the 2nd mo (Fig. 1). The break point (minimum residual variance) with two linear regression lines was at 30 d. Therefore, the animals were divided into two subgroups, lambs <30 and >30 d of age. Vagal blockade was induced with atropine sulfate injected as a bolus into the jugular vein at 0.1 or 0.2 mg/kg (15 and 21 experiments, respectively). The mean dose in the younger lambs was 0.15 mg/kg and in the older lambs 0.18 mg/kg (nonsignificant difference). β -Adrenergic sympathetic blockade was induced with a propranolol bolus at a dose of 1 or 2 mg/kg (16 and 10 experiments, respectively). The mean dose for the younger group was 1.2 mg/kg and for the older lambs 1.6 mg/kg; there was no significant difference between the groups. All autonomic blockades were considered total, because no further change of heart rate could be produced by additional administration of the drugs.

The study protocol comprised a 30-min control period and a 10-min postdrug study period, during which a maximal (90 s) and a mean effect (10 min) of vagal or sympathetic blockade were documented. Arterial blood pH and gas tensions were measured before, 10 min, and 1 h after the blockades were administered. One study, either with atropine or propranolol, was carried out each day (at most five blockade studies during the 10-wk period) on one lamb. Inasmuch as the intervals between the experiments were long it was not considered necessary to randomize the order of the blockades.

Signal analysis. The R-R intervals were measured from the ECG and processed by a system consisting of two minicomputers (NOVA 3 and Eclipse C/150, Data General Corporation, Southboro, MA). During analogue preprocessing, correct R wave detection was verified visually with an oscilloscope. The timebase

stability of the magnetic tape recorder was 0.6% including the triggering error. RMSM and RMSSD were computed for successive 90-s periods of heart rate signal (8, 9):

$$\text{RMSM} = \sqrt{1/N \sum_{i=1}^N (\text{RR}_i - \text{RR})^2}, \text{ and } \text{RMSSD} = \sqrt{1/(N-1) \sum_{i=1}^{N-1} (\text{RR}_i - \text{RR}_{i+1})^2}.$$

where RR_i is the i th interval, RR the mean R-R interval, and N the number of intervals in the sample. The corresponding coefficients of variation were used to estimate HRV in relation to the mean R-R interval: $\text{CV} = 100 \times \text{RMSM}/\text{RR}$, and $\text{CVS} = 100 \times \text{RMSSD}/\text{RR}$. In addition, the R-R interval sequence was detrended by computing a 4th order polynomial least-squares approximation and subtracting the polynomial trend from the signal to avoid the effect of slow trends on the HRV indices and any possible discontinuity between the break point of two successive 90-s period signals. The overall residual HRV index computed for the detrended signal was called RMSR and the corresponding coefficient of variation CVR.

For power spectral analysis the R-R interval sequence was low-pass filtered and equispaced (sampling frequency 2.2 Hz), using a $\sin(X)/X$ digital convolution filter. The signal was detrended using an up to 4th order polynomial approximation as described above. This detrending did not affect any data above 0.02 Hz in the power spectra of heart rate. The HRV power was computed for the frequency range of 0.0 to 1.0 Hz, using a Fast Fourier Transformation algorithm. The individual HRV spectra, subdivided into 10 frequency bands, were band-integrated for intergroup comparisons (8, 10). Both the high-frequency and the low-frequency area were divided into five bands, of which the lowest band, <0.02 Hz, had no power after the trend correction (Figs. 3 and 5). The distribution of the power of HRV for each band was also computed in relative units, *i.e.* as percentages of the total area under the spectral curve. This relative band-integrated HRV displayed the distribution of power within the spectral range and allowed a better comparison of records with high and low HRV.

Each animal served as its own control. Therefore, we compared heart rate and HRV values during control periods and blockades, using the paired t test or the Wilcoxon test for paired observations. The latter test was used for comparison of power spectra. Differences between age groups were compared using the respective nonpaired tests. Linear and multiple regression analysis were used to correlate heart rate, HRV, and the change in heart rate and HRV during autonomic blockades with age and with the mean R-R interval or mean heart rate.

RESULTS

Arterial blood pressure and acid-base balance values of the lambs remained within normal limits during the experiments. The heart rate and HRV responses to the blockades were computed separately for each sleep state. Despite small differences in the distribution of HRV in the different sleep states, the mean responses were similar in each state. Therefore, they were pooled to assess the average response of heart rate and HRV variables to each blockade. However, the maximal HRV response was examined by comparing a 90-s epoch at the maximal heart rate response with a control epoch in the same sleep state.

The heart rate versus age is depicted in Figure 1. Multiple regression analysis showed that the autonomic modulation of heart rate and HRV varied in relation to age and the mean heart rate or the mean R-R interval (Table 1). Either the mean R-R interval or the mean heart rate was used for analysis, whichever gave the better fit. RMSM, RMSR, and RMSSD showed positive associations with the mean R-R interval ($p < 0.001$). CV, CVR, and CVS were independent of the mean heart rate and the mean

Fig. 1. Mean heart rate as a function of age of the animals, depicted using two linear regression analyses. The break point that yielded the best statistical difference (the least residual variance) between the two age groups was 30 d.

Table 1. Basal condition; regression analysis of HRV related to age and to mean R-R interval

RMSM	=	- 0.214 ^{0.03} × age + 0.064 ^{0.001} × mean R-R interval - 0.33
RMSR	=	- 0.202 ^{0.04} × age + 0.061 ^{0.001} × mean R-R interval - 1.70
CV	=	- 0.048 ^{0.03} × age + 0.002 ^{NS} × mean R-R interval + 5.55
CVR	=	- 0.045 ^{0.03} × age + 0.003 ^{NS} × mean R-R interval + 4.55
RMSSD	=	- 0.210 ^{NS} × age + 0.084 ^{0.001} × mean R-R interval - 12.94
CVS	=	- 0.041 ^{NS} × age + 0.100 ^{NS} × mean R-R interval + 0.88

R-R interval. A negative association was observed between the age of the animals and the overall HRV, RMSM, RMSR, CV, and CVR ($p < 0.05$, Table 1). There was no such association between age and the beat-to-beat HRV.

Vagal Blockade. Heart rate and HRV indices. Examples of instantaneous heart rate tracings and transthoracic electrical impedance respirograms in two lambs before and after administration of atropine are shown in Figure 2. In response to vagal blockade there was an increase in heart rate and almost complete disappearance of visible "short-term" HRV. Especially in the older lamb, within a few minutes of administration of atropine, the heart rate underwent slow sinusoidal accelerations at the rate of 1–5 oscillations/min (Fig. 2B).

The heart rate response to atropine had independent negative associations with both age and the mean heart rate of the lambs in multiple linear regression analysis ($p < 0.001$; Table 2).

Vagal blockade led to a 41–65% reduction in the overall HRV indices ($p < 0.001$) in the <30-d-old lambs and to a 22–38% reduction in the older lambs ($p < 0.05$; Table 3). The reduction was greatest in the residual HRV (RMSR and CVR *versus* RMSM and CV, respectively, $p < 0.001$). There was also an extensive reduction of 69–84% in the beat-to-beat HRV (RMSSD and CVS), which was independent of the age of the animals ($p < 0.001$; Table 4). The proportional changes in RMSM, RMSR, and CVR, which were unrelated to the mean R-R interval or the mean heart rate, were age related ($p < 0.05$; Table 2). However, there was no statistically significant difference in HRV between the two age groups (Table 4). The changes in CV and CVS were smaller than those in RMSM and RMSSD, respectively ($p < 0.001$).

The change in the maximal heart rate during vagal blockade was similar to the change in the average heart rate. Simultaneously, the overall HRV was 60–70% and the beat-to-beat HRV was 90% smaller than the respective indices for controls in the younger group ($p < 0.001$, data not shown). The respective values, were 50–60 and 85% for the >30-day-old lambs ($p < 0.01$).

Spectral components of HRV. In the younger lambs, the spectral density of HRV, depicted as the area under the spectral curve, decreased at the frequency ≥ 0.05 Hz during the first 10 min after atropine administration ($p < 0.01$, Fig. 2A). A similar reduction of HRV was found at frequencies of >0.2 Hz (>12 cycles/min) in the older lambs ($p < 0.05$ – 0.01 , Fig. 3C), whereas the low-frequency HRV (<0.1 Hz) remained stable even though there was a systematic sinusoidal pattern of heart rate (Fig. 2). In both age groups, the percentage of low frequencies (less than six oscillations per min), measured as relative power density, increased. The relative spectral HRV power decreased at frequencies of >0.1 Hz in the younger animals and at frequencies of 0.5–0.7 Hz in the older ones (Fig. 3B and D). Results of spectral analysis of HRV during the maximal response of heart rate were similar to the mean responses during the 10-min analysis.

β -Adrenergic Blockade. Heart rate and HRV indices. Propranolol administration induced a 16% decrease in the average heart rate of the younger lambs ($p < 0.001$), which was smaller in the older lambs ($p < 0.02$, Table 3 and 5; Fig. 4). The heart rate change had a negative and independent association with both age and the mean heart rate (Table 3). Both the overall and the beat-to-beat HRV decreased by 47–59% in the <30-d-old lambs ($p < 0.001$, Table 5). In the older lambs the beat-to-beat HRV

did not change, and the CV and CVR decreased less than in the younger lambs (about 20%, $p < 0.05$, Table 5). A difference was found between the age groups in the response of all HRV ($p < 0.001$). Multiple linear regression analysis showed that the proportional changes in RMSM, RMSR, CV, CVR, RMSSD ($p < 0.05$), and CVS ($p = 0.06$) were positively associated with the age of the animals but not with the mean heart rate (Table 3). The changes in CV and CVS were greater than those in RMSM and RMSSD, respectively ($p < 0.001$, Table 5).

In the younger lambs the maximal change in heart rate and HRV caused by propranolol administration did not differ from the mean response (data not shown). In contrast, in the older lambs, the maximal response of all HRV indices showed a more than 20% rise compared with the mean response: RMSM decreased maximally by 37% and CV by 44% ($p < 0.01$). The reduction in beat-to-beat HRV was insignificant.

Spectral components of HRV. All spectral components of HRV decreased during the 10-min period of β -blockade in the <30-d-old group ($p < 0.01$, Fig. 5A). In the older lambs, the reduction was less uniform, although significant, except at the frequency band of 0.5 Hz (Fig. 5C). The respective relative spectral densities in the two subgroups were different. In the younger lambs, the distribution was similar in β -blockade and in control situations (Fig. 5B). In the older lambs, propranolol induced a decrease of relative HRV power at frequencies of ≤ 0.2 Hz (<12 oscillations/min), and shifted it to 0.3–0.5 Hz (Fig. 5D).

Associated with the maximal heart rate reduction during β -blockade the spectral densities of HRV were similar to the mean responses. In both age groups the relative spectral distribution of the HRV remained unchanged.

DISCUSSION

Spontaneous oscillations in heart rate are caused by complex nervous control mechanisms adjusting cardiac output to meet the requirements of the peripheral circulation. Three features of the circulatory control mechanism are prerequisites for generating oscillatory HRV: 1) negative feedback via baroreceptors, 2) time delay, and 3) dual efferent nervous control consisting of fast vagal and slower sympathetic pathways (11, 12). The vagal system reduces the heart rate and cardiac output with a reaction time of less than 1 s, whereas the sympathetic system increases them, acting in 2–3 s in adults (13, 14). In neonates, the time delay in baroreflex is longer (12, 15) and the sensitivity of baroreflex is weaker than in adults (16). There has been controversial information about whether HRV is, as with the sino-atrial node, under dual autonomic control (17–24). Our results indicate that in young neonatal lambs both divisions of the autonomic nervous system regulate HRV. Concerning the control systems, some questions may arise in relation to the neonatal period, such as the advantage of this dual autonomic control, the roles of the two divisions in development during the postnatal period, and whether disturbed or abnormal autonomic control causes specific patterns in perinatal cardiopulmonary adaptation that could be used for diagnostic or monitoring purposes.

Some earlier studies show that the vagal, but not the β -adrenergic system, has a regulatory role on HRV in adult humans or animals (17, 18, 21). Surprisingly few attempts have been made to document the role of the autonomic nervous system in the regulation of HRV in the peri- and postnatal periods, and the results on the role of β -adrenergic sympathetic division are

Fig. 2. Original tracings of instantaneous heart rate and respirogram before and after administration of atropine (arrow) at a dose of 0.2 mg/kg in a 10-d-old lamb (A) and a 36-d-old lamb (B). Note that in the younger lamb the heart rate rose to 260 bpm after vagal blockade. In the display of the commercial monitor the heart rate readings exceeding 240 bpm (solid line below the heart rate tracing) were automatically scaled to half size.

Table 2. Vagal blockade; multiple regression analysis of responses of heart rate and HRV to age and the mean heart rate or mean R-R interval during vagal blockade

Δ heart rate	$= -1.128^{0.001} \times \text{age} - 0.258^{0.01} \times \text{mean heart rate} + 109.5$
Δ % heart rate	$= -0.828 \times \text{age} - 0.333^{0.0001} \times \text{mean heart rate} + 100.6$
Δ % RMSM	$= 1.357^{0.05} \times \text{age} - 0.032^{\text{NS}} \times \text{mean R-R interval} - 65.66$
Δ % RMSR	$= 1.373^{0.03} \times \text{age} - 0.045^{\text{NS}} \times \text{mean R-R interval} - 71.73$
Δ % CV	$= 0.973^{\text{NS}} \times \text{age} + 0.010^{\text{NS}} \times \text{mean R-R interval} - 62.41$
Δ % CVR	$= 1.369^{0.05} \times \text{age} - 0.009^{\text{NS}} \times \text{mean R-R interval} - 75.98$
Δ % RMSSD	$= 0.540^{0.08} \times \text{age} - 0.023^{\text{NS}} \times \text{mean R-R interval} - 84.00$
Δ % CVS	$= 0.390^{\text{NS}} \times \text{age} + 0.013^{\text{NS}} \times \text{mean R-R interval} - 90.21$

Table 3. β -Blockade; multiple regression analysis of change in heart rate and HRV during β -blockade related to age, and to mean heart rate

Δ heart rate	=	$-0.342^{0.04} \times \text{age} - 0.477^{0.0001} \times \text{mean heart rate} + 64.58$
Δ % heart rate	=	$-0.204^{0.04} \times \text{age} - 0.201^{0.001} \times \text{mean heart rate} + 25.57$
Δ % RSM	=	$0.988^{0.02} \times \text{age} + 0.109^{\text{NS}} \times \text{mean heart rate} - 81.24$
Δ % RMSR	=	$0.175^{0.03} \times \text{age} + 0.023^{\text{NS}} \times \text{mean heart rate} - 15.69$
Δ % CV	=	$0.745^{0.04} \times \text{age} - 0.040^{\text{NS}} \times \text{mean heart rate} - 58.46$
Δ % CVR	=	$0.756^{0.04} \times \text{age} - 0.069^{\text{NS}} \times \text{mean heart rate} - 55.86$
Δ % RMSSD	=	$0.226^{0.05} \times \text{age} + 0.031^{\text{NS}} \times \text{mean heart rate} - 14.54$
Δ % CVS	=	$1.134^{0.06} \times \text{age} - 0.139^{\text{NS}} \times \text{mean heart rate} - 41.24$

Table 4. Absolute and percentage changes in mean heart rate (HR), mean R-R interval (RR), and indices of HRV induced by vagal blockade in lambs less than and more than 30 d old; means $\pm$ SEM

	Overall HRV						Beat-to-beat HRV	
	HR (1/min)	RR (ms)	RMSM (ms)	RMSR (ms)	CV (%)	CVR (%)	RMSSD (ms)	CVS (%)
Age <30 d (<i>n</i> = 20)								
Atropine	234.2 ± 9.3	266.9 ± 13.0	8.2 ± 0.9	5.4 ± 0.8	3.00 ± 0.25	1.95 ± 0.20	2.2 ± 0.7	0.80 ± 0.23
Control	187.9 ± 10.7	345.8 ± 22.8	18.6 ± 1.6	16.2 ± 1.3	5.44 ± 0.32	4.74 ± 0.27	13.0 ± 2.0	3.63 ± 0.41
<i>p</i>	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Age >30 d (<i>n</i> = 16)								
Atropine	156.8 ± 5.4	390.0 ± 12.8	12.0 ± 1.8	8.7 ± 1.4	3.02 ± 0.41	2.18 ± 0.32	3.1 ± 0.5	0.76 ± 0.10
Control	130.1 ± 4.7	472.2 ± 17.8	20.5 ± 2.7	18.2 ± 2.6	4.24 ± 0.42	3.73 ± 0.41	18.4 ± 3.3	3.70 ± 0.54
<i>p</i>	<0.001	<0.001	<0.016	0.005	NS	0.014	<0.001	<0.001
<i>p</i> *	<0.001	<0.001	NS	NS	0.026	0.040	NS	NS
% change								
Age <30 d	28.4 ± 4.1	−20.9 ± 2.2	−54.2 ± 5.5	−65.3 ± 5.2	−41.0 ± 7.2	−55.8 ± 6.2	−83.7 ± 2.5	−78.4 ± 4.0
Age >30 d	20.7 ± 2.8	−16.9 ± 1.8	−33.6 ± 10.9	−38.4 ± 12.2	−22.3 ± 12.3	−27.5 ± 13.8	−73.4 ± 6.0	−69.4 ± 6.5

p^* Compared with the control value <30 d.

Fig. 3. Band-integrated power spectra of HRV for lambs less than 30 d old ($n = 20$) computed in arbitrary units (A; left upper) and percentages (B; right upper) and, respectively, for lambs more than 30 d old ($n = 16$) (C; left lower and D; right lower) before (solid line) and after vagal blockade (dotted line). Mean $\pm$ SEM. $*p < 0.05$, $**p < 0.01$.

Fig. 4. Tracings of heart rate and respirogram before and after administration of propranolol (arrow) at a dose of 1 mg/kg in an 8-d-old lamb (A) and at a dose of 2 mg/kg in a 67-d-old lamb (B). As in Figure 1, the heart rate values exceeding 240 bpm (indicated with a solid line) were automatically scaled to half size by the monitor.

controversial (20, 25). In fetal lambs, propranolol administration induced no response, but in neonatal lambs an increasing response of the HRV indices with age was reported. In our study, even though the proportional change in the overall HRV indices after β -blockade was positively associated with age, all HRV responses were significantly smaller in the older lambs. The HRV indices used by Zugaib *et al.* (25) may not quantify increasing short-term heart rate irregularities, *e.g.* oscillations occurring with increasing frequency (26) and, unlike spectral analysis, they do not measure the frequency distribution of HRV. Further, statistical indices are sensitive to transients and trends in the sample of heart beats; they are only rough estimates of the overall and beat-to-beat variability. For these reasons, in addition to HRV indices we used spectral analysis to quantify HRV. We found

the method of spectral band integration (8–10) convenient for studying frequency-selective responses to autonomic blockades. We analyzed five bands above and five bands below 0.15 Hz, because several important physiologic mechanisms have been reported to act at low frequencies, including baroreceptor control of blood pressure at 0.1 Hz, vasomotor thermoregulation, and the renin-angiotensin system at 0.04–0.05 Hz (10, 24, 27). The high-frequency HRV component (>0.20 Hz) is influenced only by the respiration, and in neonates even less than in adults (11, 12, 27). We used a polynomial approximation up to the 4th order for trend removal in successive 90-s time series. The relatively small change in HRV indices after correction indicates good stationarity of the source time series. We lost data less than 0.02 Hz (9), but this frequency is not of interest to us.

Table 5. Respective data as in Table 1 before and after β -blockade; means $\pm$ SEM

	HR (1/min)	RR (ms)	Overall HRV				Beat-to-beat HRV	
			RMSM (ms)	RMSR (ms)	CV (%)	CVR (%)	RMSSD (ms)	CVS (%)
Age <30 d (<i>n</i> = 14)								
Propranolol	157.2 ± 6.3	391.0 ± 17.0	9.3 ± 1.0	7.5 ± 0.8	2.44 ± 0.32	1.97 ± 0.25	4.9 ± 0.6	1.27 ± 0.15
Control	188.1 ± 9.0	330.2 ± 18.1	17.4 ± 0.89	15.1 ± 0.8	5.47 ± 0.42	4.73 ± 0.36	9.9 ± 0.5	3.10 ± 0.24
<i>p</i>	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Age >30 d (<i>n</i> = 12)								
Propranolol	111.5 ± 4.0	547.0 ± 19.0	18.0 ± 3.7	15.9 ± 3.8	3.17 ± 0.58	2.79 ± 0.60	18.9 ± 5.7	3.25 ± 0.93
Control	123.6 ± 4.2	493.0 ± 16.5	20.6 ± 4.3	17.9 ± 4.4	4.15 ± 0.86	3.60 ± 0.89	18.0 ± 7.1	3.54 ± 1.41
<i>p</i>	<0.001	<0.001	NS	NS	0.031	0.065	NS	NS
<i>p</i> *	<0.001	<0.001	NS	NS	<0.01	<0.01	NS	NS
% change								
Age <30 d	−15.8 ± 1.9	19.8 ± 2.8	−47.3 ± 4.8	−50.7 ± 4.6	−56.6 ± 3.7	−58.9 ± 3.6	−51.5 ± 5.8	−54.5 ± 5.8
Age >30 d	−9.6 ± 1.4	10.9 ± 1.7	−12.0 ± 6.6	−5.9 ± 7.1	−20.7 ± 6.1	−23.1 ± 7.1	17.3 ± 10.6	−8.5 ± 10.0
<i>p</i>	0.019	0.014	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

 p^* As in Table 1.

Fig. 5. Band-integrated power spectra of HRV for lambs less than 30 d old ($n = 14$) in arbitrary units (A; left upper) and percentages (B; right upper), and, respectively, for lambs more than 30 d old ($n = 12$) (C; left lower and D; right lower) before (solid line) and after β -blockade (dotted line). Mean $\pm$ SEM. * and ** as in Figure 3.

It appears that in neonatal lambs, the vagal division has a stronger influence on HRV than the sympathetic division, in particular affecting the rapid changes in heart rate. The β -adrenergic system, however, seemed to account for half of the total variability measured by the HRV indices after birth and without any frequency selection. These results differ from earlier findings in adult man, dog, and cat; in these the high-frequency HRV was found to be only under vagal control, whereas the β -adrenergic division showed its effect only on the low-frequency HRV, and even then in situations with high adrenergic tone (17–19, 21–23). In our study, the different response times of the two autonomic systems were more evident in the older lambs than in younger animals. Even so, the β -adrenergic system showed

some effect on the slow changes of heart rate, whereas the vagal system affected not only the rapid changes, but also, although less strongly, the slow ones. Vagal blockade also shifted the relative HRV power from higher to lower frequencies. This indicates that the control system became unstable and may have started to oscillate, which was in fact demonstrated in the heart rate tracings as slow accelerations at a rate of one to five cycles per min in all lambs (Figs. 2 and 3). This phenomenon may be related to a reciprocal increase in sympathetic nervous system activity or in plasma vasopressin concentration (28, 29), because a sinusoidal heart rate curve has been experimentally produced by vasopressin infusion into fetal lambs during vagal blockade (30). Reacting rapidly, the vagal system induces high-frequency

components of HRV, but it also seems to damp slow sinusoidal accelerations of the heart rate. The tendency of the indices RMSM, RMSR, and RMSSD to increase with declining heart rate, and vice versa, also reported earlier (31, 32), may mask the effect of β -blockade on HRV during bradycardia, or the effect of atropine may be visually overestimated. This problem should be avoided by using HRV indices properly corrected for the length of the R-R intervals, in this study CV, CVR, and CVS, although not in visual HRV estimations of the clinical heart rate tracings. Therefore, for comparison, we also wished to show uncorrected indices such as RMSM, RMSR, and RMSSD.

The impact of the sympathetic system on heart rate decreased with age, but its influence on the HRV did so even more strongly. The development of vagal predominance with age, but a high β -adrenergic state in lambs during the first few weeks of life, as evidenced by the response of the heart rate to propranolol (25, 33, 34), also found here, may explain why propranolol had a greater effect on HRV in the younger lambs than in the older lambs or fetal lambs (20). Our study does not exclude either myocardial depression induced by administration of propranolol to immature animals, as described in puppies, kittens, and lambs (35, 36), or a possible central inhibitory effect of propranolol. If the peripheral resistance of the vasculature operates as a low-pass filter (12) and the filtering effect of the peripheral sympathetic tone declines with age, the high-frequency irregularity in the heart rate may increase (8). The postnatal maturation of the control of the central circulation, cardiac output, and peripheral vasculature, as well as developmental changes in the sinus node itself (11, 16, 36), may also contribute to changes in heart rate and HRV. Thus, during the neonatal period the autonomic nervous control of the heart is modified by the development of both regulatory and effector organs.

In man, the normal development of heart rate control may be seriously affected by several perinatally occurring diseases. Although perinatal asphyxia and depression of the CNS in respiratory distress syndrome and brain death may result in a flat heart rate curve, our present and earlier findings in lambs indicate that blocking autonomic control may reduce neonatal HRV by 80–90% without causing any changes in the arterial pH or gas tensions (9). In human neonates however, a negative correlation has been found between the PCO_2 and HRV (37). Abnormal development of autonomic nervous control of autonomic imbalance in the neonate during an acute illness and convalescence may produce clinically useful diagnostic patterns of heart rate. Our results suggest that in vagal failure a sinusoidal heart rate pattern may appear, a flat curve after suppression of the entire autonomic nervous system, or increased HRV may result from its stimulation in neonatal lambs. Further information is needed on human neonates, especially, because both atropine and propranolol are clinically used drugs.

In lambs, the most important mechanism regulating HRV after birth seems to be the dual autonomic nervous control with vagal dominance, and this mechanism is needed to ensure adequate stability of the heart rate during early cardiovascular adaptation. With age, the decrease in direct autonomic control of HRV is followed by accentuation of specific frequency selections of its two divisions.

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