

Autonomic Neuropathy in BB Rat

Assessment by Improved Method for Measuring Heart-Rate Variability

TOM A. J. McEWEN AND ANDERS A. F. SIMA

SUMMARY

Sinus arrhythmia is a normal respiration-related variation in heart rate that is diminished or absent in autonomic nerve dysfunction. The conventionally used measurements, R-R intervals and their standard deviations (RRSD), were made and compared with a computed statistic (\bar{R}) in spontaneously diabetic BB rats and in age-matched nondiabetes-prone control rats. In addition, the effects of pentobarbital sodium anesthesia on RRSD and \bar{R} values in normal control rats were compared with those of nonanesthetized animals.

BB rats demonstrated a decrease in \bar{R} values after 8 wk of diabetes. This decrease became more marked with increased duration of diabetes. No differences in mean R-R intervals or RRSD were obtained between diabetic and nondiabetic rats until 24 wk of diabetes. Normal control rats showed a decrease in sinus arrhythmia with age. Both RRSD and \bar{R} values were greatly decreased in anesthetized animals. *Diabetes* 36:251–55, 1987

Autonomic neuropathy is a common and severe complication of diabetes mellitus that leads to vagal denervation and sensorimotor and reflex dysfunction of the cardiovascular, urogenital, and gastrointestinal systems (1–6). Respiratory sinus arrhythmia is a well-documented normal phenomenon seen in both experimental animals and humans (7–10). Measurements of R-R interval variation provide a valuable index of autonomic dysfunction and may prove to be a sensitive noninvasive method of assessing early autonomic involvement (2,4,11–23).

The spontaneously diabetic BB rat is an authentic model

of human insulin-dependent diabetes mellitus (5,23–31). In previous ultrastructural and morphometric studies of the diabetic vagus nerve in the BB rat, we have demonstrated a progressive axonal atrophy of both unmyelinated visceral efferents and myelinated visceral afferents after 8 mo of diabetes. A loss of unmyelinated fibers was first demonstrated after 11 mo of diabetes. We have characterized R-R interval variation in this model with an improved method of assessment proposed by Weinberg and Pfeifer (32). This method takes into account the length of each respiration cycle and plots the time of occurrence of each cardiac event in this cycle. We demonstrate an early vagal functional deficit, suggesting a metabolic abnormality is responsible for vagal dysfunction, because it occurs before structural changes can be implicated.

MATERIALS AND METHODS

Animals. Twenty-eight prediabetic BB and 28 nondiabetes-prone age-matched male rats were obtained from the Department of Health and Welfare (Health Protection Branch, Ottawa, Canada) and from the University of Massachusetts Medical School (courtesy of Dr. A. A. Like, Department of Pathology, Worcester, MA). The animals were housed in individual metabolic cages with free access to food (Rat Chow, Wayne Lab Blox F-6; Wayne Laboratory Animal Diets, Winnipeg, Manitoba) and water. Urine glucose, ketones, and volume were monitored daily as previously described (23,24). Onset of diabetes occurred at 94 ± 7 (mean \pm SE) days. Diabetic animals were maintained at hyperglycemic levels (350–400 mg/dl) by administration of titrated daily doses of protamine zinc insulin (courtesy of Connaught Laboratories, Toronto, Ontario). Blood glucose levels and body weights were monitored regularly throughout the study as previously described (23,24).

R-R intervals. On the day before testing, animals were lightly anesthetized with diethyl ether (Fisher Scientific, Fair Lawn, NJ), and three small stainless steel electrodes (MikRon Autoclip, Becton Dickinson, Parsippany, NJ) were implanted subcutaneously. Two electrodes were placed transthoracically

From the Neuropathology Research Laboratory, Department of Pathology, University of Manitoba, Winnipeg, Canada.

Address correspondence and reprint requests to Dr. Anders A. F. Sima, Neuropathology Research Laboratory, Department of Pathology, University of Manitoba, 770 Bannatyne Avenue, Winnipeg, Manitoba, R3E 0W3.

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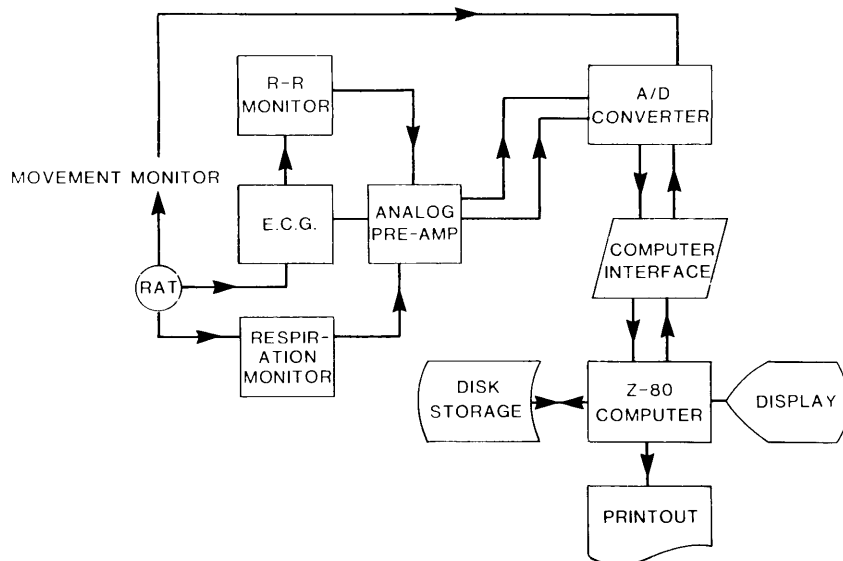


FIG. 1. Diagram of data collection system.

cally and were used to collect the electrocardiogram (ECG) and respiration data. The third electrode was placed at the lower right dorsum and acted as a ground lead. On the day of testing, the animals were jacketed (Harvard Rodent Jacket, Harvard Apparatus, Edenbridge, Kent, England) and moved to small transparent plastic cages. Animal movement was monitored by means of a miniature light-sensitive device (Tandy, Fort Worth, TX) attached to the side of the plastic cage. Simultaneous analog movement, ECG, and respiration signals were obtained from the movement monitor and the subcutaneous electrodes. The respiration data were amplified (33-1057A 4-channel amplifier, Tandy) and fed to a Wheatstone Bridge apparatus (Barclay Whyte, Winnipeg, Manitoba), which discriminated differences in transthoracic impedance due to respiration. The ECG signals were fed to an ECG recorder (Cardiofax ECG-6201, Nihon Kohden, To-

kyo, Japan, courtesy of N. Nakanishi, Eisai, Tokyo, Japan) for amplification. The output of the movement monitor was first amplified, then all three analog voltages were input to an 8-bit 16-channel analog-to-digital converter (A/D BWA 1002A, Barclay Whyte). Calibration of amplifier gain was assisted via an "electronic rat," which produced simulated ECG and respiration pulses, and a separate calibration program, which allowed the upper and lower output thresholds of the A/D converter to be preset before data were collected. Digital signals from the A/D converter were input to a microprocessor (TRS-80 Model I 64 Kilobytes RAM, 2-drive SDSS, Tandy). A diagrammatic representation of the data collection system is given in Fig. 1. The computer program monitored the animals' movement, respiration, and ECG until a 5-min, movement-free recording was obtained. This recording was then downloaded to a diskfile for later analysis.

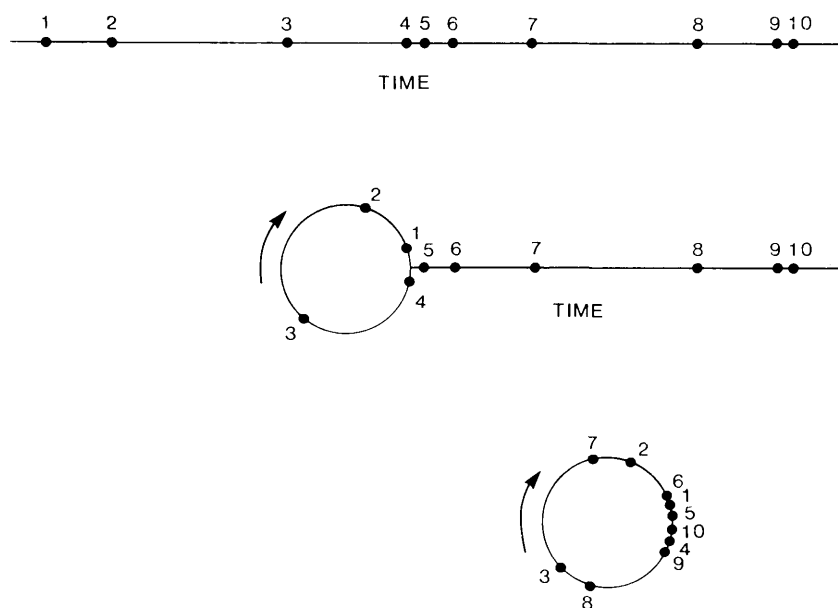


FIG. 2. Schema of circular time wrapping of R-R intervals around respiration.

TABLE 1
Mean blood glucose values (mg/dl) in control and diabetic animals during observation period

Duration of diabetes (wk)	Controls	N	Diabetics	N
3	86.7 ± 5.4	8	370.0 ± 25.7	11
4	98.1 ± 5.7	8	376.4 ± 11.6	11
8	90.6 ± 3.6	8	363.5 ± 19.2	8
12	81.9 ± 6.2	7	372.0 ± 19.5	10
16	94.3 ± 4.1	7	390.2 ± 14.6	7
20	85.8 ± 3.5	6	379.4 ± 10.6	5
24	97.5 ± 4.1	8	403.8 ± 16.3	4

Values are means ± SE. *N*, number of animals.
P < .001 between controls and diabetics at all durations.

Anesthesia experiment. Recordings were made on eight nondiabetes-prone control animals at 24 wk of age as described above. These same animals were then anesthetized by injection of pentobarbital sodium (50 mg/kg body wt, i.p.), and new recordings were taken 60 min after administration of the anesthetic.

Treatment of R-R interval data. The R-R interval is defined as the time between two successive R spikes in the QRS complex. The statistic \bar{R} is computed by mathematically wrapping these times onto a unit circle equal to the length of the respiration (Fig. 2). This wrapping takes the events to points around the unit circle. The vector mean of these points (\bar{R}/n), if they are evenly distributed, will be short; however, if the points tend to cluster toward one region of the circle, \bar{R}/n will be relatively long. The measurement that we have used as \bar{R} is the length of the vector mean.

If T_1, T_2, \dots, T_n are the times that R spikes are observed in the ECG and λ is the duration of the respiration, and we impose a scaling that forces the circle to have a radius of 1, then

$$X(T_i) = \cos(2\pi T_i/\lambda)$$

and

$$Y(T_i) = \sin(2\pi T_i/\lambda)$$

TABLE 2
Mean \bar{R} values ($\times 10^{-3}$) in control and diabetic animals during observation period

Duration of diabetes (wk)	Controls	N	Diabetics	N
3	32.6 ± 4.09	8	31.2 ± 1.88*	11
4	31.3 ± 2.62	8	28.4 ± 1.84*	11
8	29.4 ± 1.89	8	21.1 ± 1.70†	8
12	27.8 ± 2.28	7	14.4 ± 0.92‡	10
16	25.3 ± 2.77	7	11.9 ± 1.18‡	7
20	24.2 ± 1.96	6	11.4 ± 1.30‡	5
24	21.3 ± 1.98	8	10.8 ± 1.10‡	4

Analysis of covariance with body weight as covariate revealed no significant correlation ($F = 1.08$; *df* 1, 105; *P* < .5). Values are means ± SE. *N*, number of animals.

*Difference is not significant.

†*P* < .01 compared with controls.

‡*P* < .001 compared with controls.

TABLE 3
Mean R-R standard-deviation values (ms) in control and diabetic animals during observation period

Duration of diabetes (wk)	Controls	N	Diabetics	N
3	63.3 ± 4.75	8	68.8 ± 5.18*	11
4	67.3 ± 5.81	8	64.1 ± 4.21*	11
8	60.1 ± 4.10	8	52.9 ± 4.90*	8
12	57.3 ± 3.81	7	51.0 ± 3.64*	10
16	53.9 ± 4.18	7	52.0 ± 3.16*	7
20	48.6 ± 3.34	6	42.8 ± 3.27*	5
24	42.3 ± 2.14	8	31.6 ± 1.41†	4

Analysis of covariance with body weight as covariate revealed no significant correlation ($F = 0.89$; *df* 1, 105; *P* < .5). Values are means ± SE. *N*, number of animals.

*Difference is not significant.

†*P* < .002 compared with controls.

because

$$X(t_i)^2 + Y(t_i)^2 = 1$$

$$\bar{R} = \|\vec{R}/n\| = \left\{ \left[\sum X(T_i)/n \right]^2 + \left[\sum Y(T_i)/n \right]^2 \right\}^{1/2}$$

Statistical methods. All values are means ± SE. Statistical comparisons of differences were performed with Student's *t* test, one-way analysis of covariance, paired *t* test, and Spearman's rank-correlation coefficient.

RESULTS

Diabetic animals showed significant hyperglycemia throughout the observation period (Table 1). Evaluation of \bar{R} means demonstrated that after 8 wk of diabetes a significant deficit in sinus arrhythmia (*P* < .01) to 72% of normal values had already occurred (Table 2). The decrease in respiration-related arrhythmia (expressed as \bar{R}) progressed with duration of diabetes and reached 51% of normal values after 24 wk. Calculation of R-R interval standard-deviation (RRSD) values, on the other hand, first revealed significant differences (*P* < .002) between diabetic and nondiabetic control rats

TABLE 4
Mean weights (g) of control and diabetic animals during observation period

Duration of diabetes (wk)	Control	N	Diabetics	N
3	329.3 ± 6.4	8	295.4 ± 6.9*	11
4	331.9 ± 7.9	8	296.0 ± 9.7†	11
8	349.8 ± 6.2	8	283.0 ± 10.9†	8
12	367.8 ± 12.6	7	309.8 ± 14.2‡	10
16	382.7 ± 10.3	7	320.2 ± 12.9*	7
20	406.5 ± 11.5	6	336.4 ± 11.4§	5
24	427.6 ± 6.4	8	341.6 ± 8.7†	4

Values are means ± SE. *N*, number of animals.

**P* < .005 compared with controls.

†*P* < .001 compared with controls.

‡*P* < .01 compared with controls.

§*P* < .002 compared with controls.

TABLE 5

Mean \bar{R} ($\times 10^{-3}$) and RRSD (ms) values in control rats (24-wk-old) anesthetized (pentobarbital sodium) and awake

	Anesthetized	N	Awake	N
\bar{R}	14.65 \pm 1.53	8	28.43 \pm 3.34	8
RRSD	12.45 \pm 1.14	8	54.57 \pm 2.38	8

RRSD, R-R interval standard deviation.

Values are means \pm SE. Comparison was made by paired *t* test. *N*, number of animals. *P* < .001 between anesthetized and awake rats for both \bar{R} and RRSD.

after 24 wk of diabetes (Table 3). Both the \bar{R} and RRSD values declined with increasing age in nondiabetic control rats to 65 and 67%, respectively, of the initial values after 24 wk. (Tables 2 and 3). Spearman's rank-correlation applied to \bar{R} and RRSD data gave coefficients of $-.9998$ and $-.9643$, respectively (*P* < .001), when correlated to age.

Although the body weights of diabetic animals were significantly less than those of age-matched controls (Table 4), analysis of covariance between \bar{R} and RRSD with body weight as covariate revealed no significant relationships (*F* = 1.08; df 1, 105; *P* > .5; and *F* = 0.89; df 1, 105; *P* > .5)

Anesthesia with pentobarbital sodium had a profound effect on the heart-rate variation in normal control rats, with a reduction of 49% in the \bar{R} value and 81% in the RRSD value (Table 5).

Analysis of covariance of respiration data with age as covariate revealed no significant statistical difference between groups (*F* = 0.2340; df 1, 105; *P* > .5).

DISCUSSION

Monitoring of autonomic cardiac function has in the past relied heavily on comparisons between R-R intervals and RRSD, which in this study proved unreliable (8,12,14,16,18). Both of these parameters tend to be susceptible to external influences, such as premature ventricular contractions and episodes of tachycardia or bradycardia during assessment. Significant differences have generally been found only during deep breathing. The program used to analyze the R-R interval data was based on a pediatric \bar{R} program, developed and written in FORTRAN, in which the age of the subjects precluded controlled deep breathing. Our findings suggest that the calculation and subsequent analysis of \bar{R} values provide a more sensitive method for measuring heart-rate variability and thus of assessing cardiac autonomic function.

All of the R-R interval measurements were made while the animals were unanesthetized because pentobarbital sodium anesthesia significantly reduced variation. In the normal rat, we found a gradual decline in sinus arrhythmia with age, which parallels the human situation (3,15). It may be argued that the decrease in \bar{R} values in diabetic animals could be attributable to increased sympathetic tone, particularly if partially treated diabetic rats were allowed to become hypoglycemic. Because this was not the case in our study, this explanation is unlikely.

We have demonstrated significant differences in mean \bar{R} values in diabetic and nondiabetic BB rats after 8 wk of diabetes and a subsequent marked decline in sinus arrhythmia with duration of diabetes in the BB rat. Hence, functional changes in autonomic cardiac innervation are evident before

TABLE 6

Mean respiration rates (ms) of control and diabetic animals during observation period

Duration of diabetes (wk)	Controls		Diabetics	
	N		N	
3	8	607.3 \pm 42.1	8	491.0 \pm 31.4
4	8	751.6 \pm 44.8	8	686.7 \pm 29.7
8	8	921.1 \pm 63.4	8	804.3 \pm 35.6
12	7	505.4 \pm 39.2	7	486.8 \pm 52.4
16	7	823.7 \pm 44.6	7	784.2 \pm 37.2
20	6	843.6 \pm 49.2	6	691.5 \pm 40.8
24	8	742.3 \pm 61.4	8	582.3 \pm 32.8

Values are means \pm SE. One-way analysis of covariance revealed no significant statistical differences (*F* = 0.2340; df 1, 105). *N*, number of animals.

ultrastructural changes, which occur only after 8 mo of diabetes (31,33,34), can be demonstrated in the vagus nerve and in the sympathetic and parasympathetic reflex arcs. This parallels the situation in somatic diabetic neuropathy, where slowing of conduction velocities and reduced evoked muscle potentials can be related to metabolic abnormalities before structural defects are demonstrable (26,35).

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