Changes in R-R and Q-T Intervals Following Cardiac Vagotomy in Neonatal Swine

MUHAMMAD S. KHAN,* NING ZHAO,† ANTHONY L. SICA,‡ NORMAN GOOTMAN,*,† AND PHYLLIS M. GOOTMAN*,†,[1]

Department of Pediatrics, SUNY Health Science Center Hospital, New York, New York 11203; †Department of Physiology and Pharmacology, SUNY Downstate Medical Center at Brooklyn, Brooklyn, New York 11203; ‡Department of Medicine, Long Island Jewish Medical Center, Clinical Campus of Albert Einstein College of Medicine, New Hyde Park, New York 11042

Asymmetric innervation of the myocardium, especially a predominance of sympathetic innervation, may establish conditions whereby electrical instability could result. Using a swine animal model, we studied the effect of right cardiac vagal denervation on the variability of R-R and Q-T intervals. Newborn pigs were assigned randomly to two groups: sham-operated controls (C), or denervation of the right cardiac vagus nerve (RCVX). EKGs were recorded weekly until the two groups exhibited significant heart rate differences. Analysis of the EKG included measurements of R-R and Q-T intervals and corrected Q-T intervals (QTc). Poincaré plots were used to display age-related differences in R-R and Q-T intervals. For RCVX animals, decreased QTc and R-R intervals were noted at 6 and 7 weeks after denervation, respectively. Unexpectedly, one RCVX animal exhibited marked sudden pauses in sinus rhythm. These data indicated that reduced vagal cardiac modulation during development might alter cardiac electrical stability in conscious swine. [E.B.M. 2001, Vol 226:32–36]

Functional maturation of the parasympathetic nervous system occurs earlier than the sympathetic nervous system in several species (1, 2). Anatomically, it has been found that both parasympathetic and sympathetic innervations are present at birth in piglets; however, functional development differs (3–5). Developmental abnormalities in the parasympathetic limb of the autonomic nervous system could result in unbalanced innervation of the heart, possibly leading to cardiac electrical instability and/or life-threatening arrhythmia (6–8). The impact of unbalanced cardiac control by vagally denervated heart with a relative predominance of sympathetic innervation can be monitored by long-term EKG recordings, and electrocardiac stability can be evaluated by analysis of Q-T intervals, a noninvasive indicator of ventricular vulnerability. Developing swine are considered to be a suitable animal model because the physiological level of maturity of swine at birth is similar to that observed in human infants (9, 10). In the present study, we used swine as our animal model (8, 11) and denervated the right cardiac vagus at 4–10 days of age. We examined the EKG at weekly intervals until significant R-R and Q-T differences were exhibited. Poincaré plots were used to display age-related differences in R-R and Q-T intervals.

Materials and Methods

This study was approved by our Institutional Animal Use Committee. The experimental procedures complied with the *Guiding Principles in the Use and Care of Animals* approved by the Council of American Physiological Society as well as with federal and state regulations. Swine used in these studies were raised in the Division of Laboratory Animal Resources of our Institution.

We randomly assigned piglets of either sex at age 4–10 days to one of two groups: sham-operated controls (C), or right cardiac vagotomy (RCVX). Lead II and III EKG were recorded at 25 mm/sec during 3-min epochs before and during surgery.

Surgical Procedures. The detailed surgical protocol for right cardiac vagotomy has been published elsewhere (11, 12). Briefly, animals were anesthetized initially with Saffan (2 ml/kg, im), and adequate depth of anesthesia was maintained by intermittent intravenous infusion at 6–8 mg/kg/30 min during surgery. Using sterile procedures, with electrocoagulation to control bleeding, the thoracic wall was opened along the mid-sternal line, and the entire sternum was divided. Right-side cardiac vagal branches were identified by their origins in the thoracic vagal trunk and by their distribution within the pericardium. To confirm

This research was supported by NIH grants HL-20864 and HD-28931.

[1] To whom requests for reprints should be addressed at the Department of Pharmacology and Physiology, Box 31, State University of New York Downstate Medical Center, 450 Clarkson Avenue, Brooklyn, NY 11203. E-mail: pgootman@netmail.hscbklyn.edu

Received February 2, 2000.
Accepted August 15, 2000.

0037-9727/01/2261-0000$15.00/0

physiologically that the proper nerves had been isolated, electrical stimulation (biphasic pulses in 5–20-sec trains; 0.5–2.5 msec pulse duration, 0.2–2 mA, 1–10 Hz) of the intact branches was carried out to determine whether typical bradycardia occurred. Nerve branches were then ligated and transected. For sham-operated control animals, the same surgical procedures were performed without the vagotomy.

Piglets were allowed to recover completely from anesthesia and were then returned to their sows. Postoperative antibiotics and analgesia were given for 4–7 consecutive days.

EKG Acquisition and Analysis. On each recording day, animals were removed from the sows to our laboratory and allowed to acclimatize. Adhesive leads were placed on the right forelimb and lower left hindlimb according to EKG lead II recording positions. Lead III was used as an alternative if lead II failed to provide clear observation of Q-T intervals. While animals rested quietly in a sling, or were judged to be behaviorally asleep, the EKG signal, monitored by an oscilloscope (Kenwood, 20 MHz, CS-4025), was amplified (Grass A.C. Pre-Amplifier), digitized (Digital Recorder, Instrutech Corp), and recorded for 896–928 sec on both VCR tape and computer. Continuous EKG recordings were made at weekly intervals until significant R-R and Q-T interval differences emerged. Between monitoring periods, the piglets remained with their sows. All raw data were carefully reviewed for the presence of ischemic injury and/or arrhythmias. Only continuous EKG epochs that were at least 256 sec in length or 512 consecutive cardiac cycles, stable, and free of movement artifacts were chosen for analysis.

All EKG data were sampled at 1024 Hz. The digitized EKGs were displayed, and pulses were placed appropriately to measure R-R and Q-T intervals. The duration of each Q-T interval was measured from the initial downward deflection of the QRS complex to the termination of the T wave at the point of its merge with the TP segment. The heart rate–corrected Q-T intervals (QTc) were calculated using Bazett's formula: $QTc = QT/(R\text{-}R)^{1/2}$ (13).

Poincaré plots were created by plotting each R-R interval against the preceding R-R interval as a dot (duration RiRi + 1, duration Ri + 1Ri + 2) using MS Excel 5.0. The Poincaré plots enabled visual judgments of heart rate variability (HRV) (14). Increased HRV gives a Poincaré plot with greater dispersion of data than that noted with decreased HRV (i.e., a Poincaré plot with a compact appearance) (Fig. 1).

Statistics. Statistical analysis was carried out with the SPSS computer program on calculated means ± SE. Between-group comparisons were made using a Student *t* test with Bonferroni correction for independent samples. The effects of postsurgery R-R and QTc intervals within the experimental group were examined by one-way ANOVA; if significance was obtained, a *posthoc* Neuman-Keuls test was used. The level of probability for statistical significance was established at $P < 0.05$.

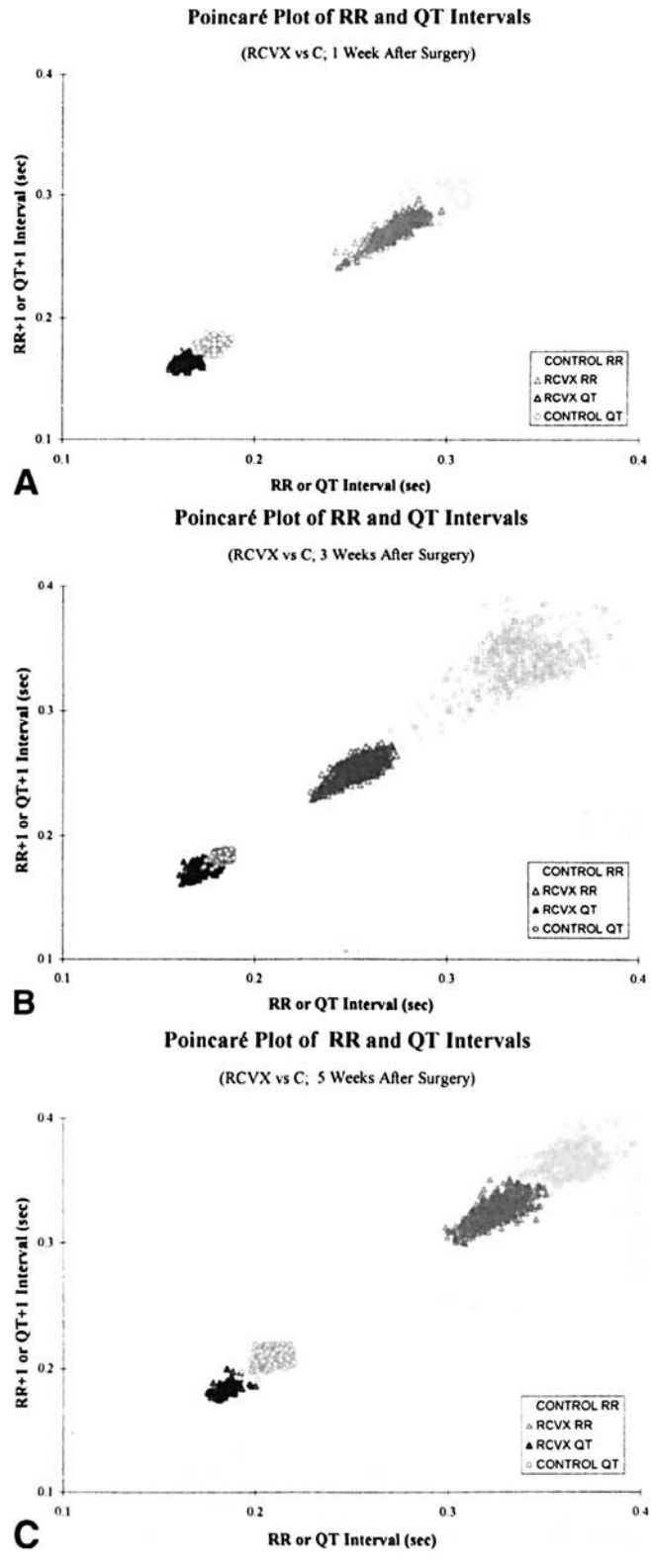

Figure 1. Poincaré plot of R-R and Q-T intervals from one right vagotomized animal (RCVX) versus one litter-mate sham-operated control animal at (A) 1 week, (B) 3 weeks, and (C) 5 weeks postsurgery. RCVX shows decreased R-R and Q-T intervals as well as compact plot compared with C at the same postsurgical week.

Table I. Comparison of R-R Intervals and QTc

	R-R Intervals		QTc	
	Control (n = 8)	RCVX (n = 5[a])	Control (n = 8)	RCVX (n = 5[a])
Presurgery	246 ± 19	237 ± 21	323 ± 5	313 ± 5
Postsurgery 1wk	264 ± 22	267 ± 21	335 ± 9	313 ± 9
2wk	294 ± 22	283 ± 33	347 ± 11	320 ± 5
3wk	318 ± 17[b]	281 ± 29	350 ± 6[b]	338 ± 8
4wk	325 ± 14[b]	296 ± 35	353 ± 12[b]	340 ± 9
5wk	344 ± 18[b]	314 ± 31	353 ± 17	339 ± 13[b]
6wk	363 ± 26[b]	333 ± 30[b]	361 ± 18[b]	340 ± 18[b,c]
7wk	398 ± 23[b]	351 ± 26[b,c]		

Note. ms, mean ± SE
[a] One animal with sinus pauses after surgery was excluded from statistics, postsurgery n = 4.
[b] Postsurgery significantly longer than presurgery, $P < 0.05$
[c] RCVX group significantly shorter than C, $P < 0.05$

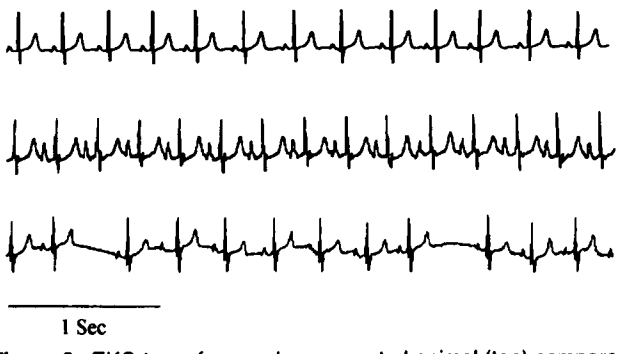

Figure 2. EKG trace from a sham-operated animal (top) compared with an RCVX animal (middle) and the RCVX animal with sinus pauses (bottom) at first postsurgical weeks.

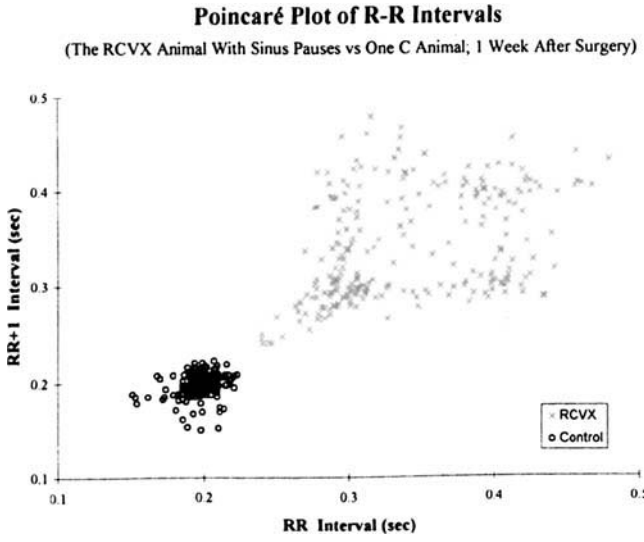

Figure 3. Poincaré plot of R-R intervals from the RCVX animal with sinus pauses versus a litter-mate control animal at first postsurgical weeks. Note the scatter of the plot from the RCVX animal.

Results

All 13 animals (C n = 8; RCVX n = 5) survived surgery.

EKG recording analysis verified the absence of any ischemic injury or arrhythmia before surgery. Averaged R-R intervals and QTc of control and RCVX groups for presurgery and weeks postsurgery are summarized in Table I.

Sham Control Group. Significant increases in R-R intervals occurred with age in all eight animals. Within-group statistical analyses revealed that significant differences in R-R intervals first appeared at postsurgical Week 3. In addition, QTc increased with age in all animals; within-group comparisons revealed that significant differences occurred by postsurgical Week 3.

RCVX Group. Significant increases in R-R intervals also occurred with age in all five animals. The age of appearance of significant differences was postsurgical Week 6. QTc also was prolonged with age. Within-group comparisons revealed that the first significant differences occurred in the fifth postsurgical week.

When between-group comparisons were made, a significant shortening in R-R intervals in the RCVX group was found by 7 weeks after surgery. A significantly decreased QTc from the RCVX group was observed at postsurgical Week 6 compared with the C group. Poincaré plots of R-R intervals from RCVX animals showed a more compact pattern compared with that of C animals (Fig. 1).

In addition to the changes in R-R and Q-T intervals, one RCVX animal also exhibited sudden sinus pauses starting the first week after surgery. These pauses were not preceded by changes in heart rate (Fig. 2). When Poincaré plots were applied to the R-R intervals from this piglet, widely scattered plots were observed. The overall length and width of the plots were much greater when compared with those obtained in control animals (Fig. 3).

Discussion

The parasympathetic limb of the autonomic nervous system innervating the heart has long been the focus of basic and clinical research, especially during early development. For example, reduced parasympathetic modulation of the heart can result in arrhythmias (7, 15) and has been postulated as a cause of the Sudden Infant Death Syndrome (SIDS) (16). The present study continues our investigation

of the cardiac effects of selected cardiac autonomic denervation in our swine model (8, 11). This model allows acquisition of EKG recordings from conscious animals, eliminating possible effects from drugs or anesthesia.

A significant increase in R-R interval duration in the sham-operated control group was first observed during postsurgical Week 3, indicating that considerable functional maturation of cardiac autonomic innervation occurred during postnatal Weeks 3–4, consistent with our earlier findings (3, 5). In the RCVX group (i.e., after elimination of right cardiac vagal innervation), sympathetic innervation dominated SA nodal control causing R-R intervals to decrease. Therefore, a statistically significant difference in R-R intervals was delayed by 3 weeks compared with the C group. Previous studies have established a strong correlation between decreased levels of cardiac vagal tone and increased susceptibility to ventricular fibrillation (17, 18). Relative right sympathetic dominance because of insufficiency in cardiac vagal tone not only decreases R-R intervals, but also decreases the ventricular fibrillation threshold, thus increasing susceptibility to ventricular fibrillation and sudden cardiac death (19).

During the postsurgery period, QTc values lengthened with age in both groups, as had been observed in infants (20) and puppies (16). However, the time of onset of the prolongation of QTc in the RCVX was observed by the fifth postsurgical week and was significantly delayed when compared with the controls. This result agreed with our earlier QTc observation on swine (11) and reports that vagotomy protected against prolongation of Q-T in dog and puppies (17).

Sudden sinus pauses were detected in one of the RCVX animals in the present study. When the SA node fails to initiate an impulse, and escape pacemaker sites (AV junction, ventricles) also fail to pace the heart, PQRST complexes are absent. Sinus pauses have been reported both in congenital Long QT Syndrome (LQTS) patients as well as in experimental animals (21) and are considered very arrhythmogenic (22). The incidences of sinus pauses are commonly considered to be related closely to vagal dysfunction. One explanation is that they reveal a self-adjusting mechanism of the autonomic nervous system to restore a more or less regular heart rate when cardiac autonomic control is out of balance (23). Since sinus pauses were observed in just one RCVX animal and the R-R interval plots from this animal were much expanded compared with those from the rest of the RCVX animals, determining the mechanism involved is still difficult. Insufficient vagal innervation of the SA node without altering the characteristic left vagal control of the AV node may account for these pauses. It may be possible to determine the mechanisms by increasing the number of subjects and by comparing the resulting data with our findings from studies of afferent stimulation and cardiac denervation (24, 25).

In conclusion, the decreased R-R interval and shortened QTc duration observed during postnatal maturation after right cardiac vagal denervation reflects decreased parasympathetic modulation of the heart. These data support our hypothesis that the lack of appropriate vagal cardiac modulation during development may impair cardiac electrical stability in conscious swine.

1. Gootman PM, Cohen HL, Gootman N. Autonomic nervous system regulation of heart rate in the perinatal period. In: Liebman J, Plomsey R, Rudy Y, Eds. Pediatric and Fundamental Electrocardiography. Hague: Martinus Nijhoff Publishers, pp137–159, 1987.
2. Castellini MA, Rea LD, Sanders JL, Castellini JM, Zento-Savin T. Developmental changes in cardiorespiratory patterns of sleep-associated apnea in northern elephant seals. Am J Physiol **267:**R1294–R1301, 1994.
3. Gootman PM, Gandhi MR, Coren CV, Kaplan NM, Pisana FM, Buckley BJ, Armour JA, Gootman N. Cardiac responses elicited by stimulation of loci within stellate ganglia of developing swine. J Auton Nerv Syst **38:**191–200, 1992.
4. Gootman PM, Hopkins DA, Buckley NM, Gootman N. Vagal cardiac nerves of the developing swine. In: Ferrans VJ, Rosenquist G, Weinstein C, Eds. Cardiac Morphogenesis. New York: Elsevier Scientific Publishers, pp267–274, 1985.
5. Buckley NM, Gootman PM, Yellin EL. Age-related cardiovascular effects of catecholamines in anesthetized piglets. Circ Res **45:**282–292, 1979.
6. Harper RM, Leake B, Hoppenbrouwers T, Sterman M, McGinty DJ, Hodgmen J. Polygraphic studies of normal infants and infants at risk for the sudden infant death syndrome: Heart rate and variability as a function of state. Pediatr Res **12:**778–785, 1978.
7. Schwartz PJ, Stramba-Badiale M. Parasympathetic nervous system and malignant arrhythmias. In: Kulbertus HE, Frank MN, Eds. Neurocardiology. New York: Futura Publishing Company, pp79–200, 1987.
8. Tong S, Ingenito S, Frasier ID, Gootman N, Gootman PM. Differential effects of left and right cardiac sympathetic denervation on ventricular fibrillation threshold in developing swine. In: Advances in Swine in Biomedical Research. New York: Plenum Press, pp131–140, 1996.
9. Dobbing J. The later development of the brain and its vulnerability. In: Davis JA, Dobbing J, Eds. Scientific Foundations of Pediatrics. London: Heineman Medical, pp744–759, 1991.
10. Pond WG, Boleman SL, Fiorotto ML, Ho H, Knabe DA, Mersmann HJ, Savell JW, Su DR. Perinatal ontogeny of brain growth in the domestic pig. Proc Soc Exp Biol Med **223:**102–108, 2000.
11. Tong S, Ingenito S, Anderson JE, Gootman N, Sica AL, Gootman PM. Development of a swine animal model for the study of sudden infant death syndrome (SIDS). Lab Anim Sci **45:** 398–403, 1995.
12. Lipsitz LA, Pincus SM, Morin RJ, Tong S, Eberle LP, Gootman PM. Preliminary evidence for the evolution in complexity of heart rate dynamics during autonomic maturation in neonatal swine. J Auton Nerv Syst **65:**1–9, 1997.
13. Bazett HC. An analysis of time relations of electrocardiograms. Heart **7:**353–370, 1920.
14. Malik M. Geometrical methods for heart rate variability assessment. In: Malik M, Ed. Heart Rate Variability. New York: Futura Publishing Co, pp47–61, 1995.
15. Yamaha KA, Witkowski FX. Mechanisms controlling cardiac autonomic functions and their relation to arrhythmogenesis. In: The Heart and the Cardiovascular System. New York: Raven Press, pp1343–1404, 1986.
16. Stramba-Badiale M, Lazzarotti M, Schwartz PJ. Development of cardiac innervation, ventricular fibrillation and sudden infant death syndrome. Am J Physiol **263:**H1514–H1522, 1992.

17. Billman GE, Hoskins RS. Time-series analysis of heart rate variability during submaximal exercise: Evidence for reduced cardiac vagal tone in animals susceptible to ventricular fibrillation. Circulation **80:**146–157, 1989.
18. Tong S, Gootman PM, Ingenito S. Effects of cardiac autonomic denervation on ventricular fibrillation threshold (VFT) in neonates. Clin Res **42:**455A, 1994.
19. Moise NS, Riccio ML, Kornreich B, Flahive WJ Jr., Gilmour RF Jr. Age dependence of the development of ventricular arrhythmia in a canine model of sudden cardiac death. Cardiovasc Res **34:**483–492, 1997.
20. Schwartz PJ. Quest for the mechanism of SIDS: Doubts and progress. Circulation **75:**677–683, 1987.
21. Schwartz PJ. The long QT syndrome. In: Kulbertus HE, Wellens HJJ, Eds. Sudden Death. The Hague: Martinus-Nijhoff Publishers, pp358–378, 1980.
22. Moise NS, Dugger DA, Brittain D. Relationship of ventricular tachycardia to sleep/wakefulness in a model of sudden cardiac death. Pediatr Res **40:**344–350, 1996.
23. Schwartz PJ, Locati E, Napolitana C, Prior SG. The long QT syndrome. In: Zipes DP, Jalife J, Eds. Cardiac Electrophysiology: From Cell to Bedside. Philadelphia: W. B. Saunders, pp788–811, 1995.
24. Khan MS, Zhao N, Ingenito S, Sica AL, Gootman N, Gootman MP. Chronic cardiac vagotomy or stellectomy on the ECG responses to baroreceptor stimulation in neonatal swine. FASEB J **13:**453, 1999.
25. Zhao N, Khan MS, Sica AL, Ingenito S, Gootman N, Gootman MP. Effects of baroreflex stimulation on the ECG in chronic cardiac sympathetic-denervated neonatal swine. Clin Auton Res **8:**287, 1998.