Subcutaneous nerve activity and mechanisms of sudden death in a rat model of chronic kidney disease

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BACKGROUND The mechanisms of sudden death in chronic kidney disease (CKD) remain unclear.

OBJECTIVE The purpose of this study was to test the hypotheses that subcutaneous nerve activity (SCNA) can be used to estimate sympathetic tone in ambulatory rats and that abrupt reduction of SCNA precedes the spontaneous arrhythmic death of Cy/+ rats.

METHODS Radiotransmitters were implanted in ambulatory normal (N = 6) and Cy/+ (CKD; N = 6) rats to record electrocardiogram and SCNA. Two additional rats were studied before and after chemical sympathectomy with 6-hydroxydopamine.

RESULTS In normal rats, the baseline heart rate (HR) and SCNA were 351 ± 29 bpm and 5.12 ± 2.97 mV·s, respectively. SCNA abruptly increased HR by 4.31% (95% confidence interval 4.15%–4.47%). In comparison, the CKD rats had reduced baseline HR (336 ± 21 bpm, $P < .01$) and SCNA (4.27 ± 3.19 mV·s, $P < .01$). When SCNA was observed, HR increased by only 2.48% (confidence interval 2.29%–2.67%, $P < .01$). All Cy/+ rats died suddenly, preceded by sinus bradycardia, advanced (second- and third-degree) AV block (N = 6), and/or ventricular tachycardia or fibrillation (N = 3). Sudden death was preceded by a further reduction of SCNA (3.22 ± 2.86 mV·s, $P < .01$) and sinus bradycardia (243 ± 55 bpm, $P < .01$). Histologic studies in CKD rats showed myocardial calcification that involved the conduction system. Chemical sympathectomy resulted in progressive reduction of SCNA over 7 days.

CONCLUSION SCNA can be used to estimate sympathetic tone in ambulatory rats. CKD is associated with reduced HR response to SCNA and conduction system diseases. Abrupt reduction of sympathetic tone precedes AV block, ventricular arrhythmia, and sudden death of CKD rats.

KEYWORDS Subcutaneous nerve activity; Sudden death; Atrioventricular block; Chronic kidney disease

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In patients with chronic kidney disease (CKD) on dialysis, 25% of cardiovascular mortality was caused by sudden cardiac death (SCD), a 100-fold increase compared with the general population.[1] The mechanisms of SCD in patients with CKD were diverse, but sinus bradycardia, AV block, and ventricular tachyarrhythmia have been frequently observed.[2,3] One of the possible pathophysiologic changes associated with CKD is abnormal activity of the sympathetic nervous system.[4,5] Sympathetic hyperactivity is a cause of hypertension, which may contribute to the development of CKD. However, a direct relationship between sympathetic nerve activity and SCD in CKD has not been demonstrated. Cy/+ rat is a Han:SPRD rat with autosomal dominant polycystic kidney disease that is slowly progressive. These rats accurately reproduce many of the changes associated with human CKD, including hypertension, left ventricular hypertrophy, and mineral bone disorder.[6,7] Importantly, we observed a high incidence of sudden death of CKD rats after 35 weeks of age, prompting evaluation of possible cardiac etiologies. Cardiac ion channel and calcium handling are abnormal in these CKD rats.[8] These abnormalities may increase ventricular arrhythmogenesis and result in SCD.

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However, the terminal arrhythmia causing SCD remains unclear. We recently reported that subcutaneous nerve activity (SCNA) can be used to estimate sympathetic tone in ambulatory dogs.[9–11] We hypothesized that if this technique can be applied to rats, then it would allow determination of sympathetic nerve activity at the time of SCD. The purpose of this study was to continuously record ECG and SCNA in CKD rats to test the hypothesis that abnormal sympathetic nerve activities are immediate triggers of SCD.

Materials and methods

Animal model

Eight normal and six CKD male rats were used in this study. The CKD rat is a spontaneous cystic kidney disease model with a defect in the samcystin (Cy) gene. However, unlike other models of cystic kidney disease,[12] the samcystin protein does not affect the cilia; rather, it binds to the cytoplasmic RNA-binding protein bicc-1.[13,14] Male heterozygous rats develop characteristics of CKD (azotemia or elevated blood urea nitrogen [BUN]) around 10 weeks of ages, which thereafter slowly progresses to terminal uremia (symptomatic kidney disease) by about 40 weeks. This animal model spontaneously develops manifestations of CKD, including biochemical abnormalities, hypertension, left ventricular hypertrophy, renal osteodystrophy, and arterial calcification.[6,7,15,16] Animals had periodic blood tests for analyses of calcium, phosphorus, parathyroid hormone, and BUN using previously published methods.[6] Five animals also had potassium and magnesium measurements using hospital laboratory colorimetric techniques. Six normal Han: SPRD rats of similar age (35 weeks) underwent ECG and SCNA monitoring (see next section) to serve as control. All procedures were approved by the Indiana University School of Medicine Institutional Animal Care and Use Committee.

Continuous ECG and SCNA recordings

All rats underwent surgery at 35 weeks of age under isoflurane inhalation anesthesia. A 3.0-cm midline incision was made through the dorsal skin for implantation of a PhysioTel ETA-F10 (5 CKD and 5 normal rats) or F50-W-F2 (1 CKD and 1 normal rat) radiotransmitter (Data Science International, St. Paul, MN). The two bipolar recording wires were tunneled subcutaneously from the top of the dorsal incision subcutaneously to the forelimbs, with 1 wire for each limb. The transmitter was then secured in a subcutaneous pocket, and the rats were allowed to recover. ECG and SCNA signals were sampled continuously at 1000 times per second and transmitted to a receiver placed at the bottom of the rat cage. The data were then digitized for offline analyses. The recordings continued in CKD rats until the time of SCD. For comparison, 6 normal rats underwent the same surgical procedures performed by the same surgeon. After 2 weeks of recovery, continuous recordings were made for 3 weeks before euthanasia.

An additional 2 normal rats were studied to determine the effects of chemical sympathectomy on SCNA. These rats underwent 5 days of baseline recording, followed by intraperitoneal injection of 6-hydroxydopamine (6-OHDA; 100 mg/kg) 1 and 4 days after baseline recording. SCNA and ECG continued until 7 days after baseline recording.

Histologic examinations

The entire rat heart was fixed in 4% formalin for 45 minutss, followed by storage in 70% alcohol. The tissues were processed routinely, embedded in paraffin, and cut into 5-μm thick sections. Trichrome stains and hematoxylin and eosin stains were performed on all specimens. The slides were examined using a light microscope.

Data analyses

Using custom-written software, we manually analyzed the recording from CKD rats 5 days before death for baseline activity and again 24 hours before death. Data from normal rats were also analyzed manually for comparison. Noise and artifacts were eliminated during manual analyses. Low-frequency noise was eliminated with high-pass filters. The filtered signals were then rectified, integrated within 100-ms time windows, and summed to represent integrated subcutaneous nerve activity (iSCNA) of 10-second segments. Because integration was performed, the quantitative data considered both amplitude (voltage) and frequency of nerve activity. We also manually identified SCNA episodes by a 3-fold increase of SCNA amplitude over baseline noise. The onset of each SCNA episode was used as time 0. We then determined the iSCNA and average heart rate (HR) 3 seconds before and 3 seconds after time 0 to test the hypothesis that SCNA is associated with HR elevation. Figure 1 shows the methods used to determine whether SCNA increased HR.

Statistical analysis

Descriptive data are expressed as mean ± SD, and data used for quantitative comparison are expressed as confidence interval (CI).[17] Paired t tests were used to compare the means of 2 groups. Bonferroni correction was made for multiple comparisons. A 2-tailed P value $\leq .05$ was considered statistically significant. Generalized additive mixed-effects models were used to analyze circadian patterns of SCNA and HR. All analyses were performed using IBM SPSS software (version 22.0, IBM SPSS, Chicago, IL) and R 3.0.1.

Results

Relationship between SCNA and HR

SCNA increased an average HR of 360 ± 21 bpm to 376 ± 21 bpm in normal rats during the 5-day monitoring period. The average increase was 4.31% (CI 4.15%–4.47%, $P = .027$; Figures 1A and 1B). In comparison, SCNA increased from HR of 356 ± 16 bpm to 364 ± 19 bpm in CKD rats over the 5 days before death. The average increase in CKD