Subcutaneous Nerve Activity and Mechanisms of Sudden Death in a rat Model of Chronic Kidney Disease

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Abstract

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

Objective—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 (6-OHDA).

Results—In normal rats, the baseline HR and SCNA were 351±29 bpm 5.12±2.97 mV-s, respectively; SCNA abruptly increased heart rate (HR) by 4.31% (95% confidence interval, CI, 4.15% to 4.47%). In comparison, the CKD rats had reduced baseline HR (336±21 bpm, p<0.01) and SCNA (4.27±3.19 mV-s, p<0.01). When SCNA was observed, the HR increased by only 2.48% (CI, 2.29% to 2.67%, p<0.01). All Cy/+ rats died suddenly, preceded by sinus bradycardia,

advanced (2nd and 3rd degree) atrioventricular (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<0.01) and sinus bradycardia (243±55 bpm, p<0.01). Histological studies showed myocardial calcification in CKD rats that involved the conduction system. Chemical sympathectomy resulted in progressive reduction of SCNA over 7 days.

Conclusions—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 the CKD rats.

Keywords

subcutaneous nerve activity; sudden death; AV block; Chronic Kidney Disease

In patients with chronic kidney disease (CKD) on dialysis, 25% of cardiovascular mortality were 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, atrioventricular (AV) block and ventricular tachyarrhythmia have been frequently observed.[2, 3] One of the possible pathophysiological changes associated with the 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 the 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. 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 that it would allow determination of the sympathetic nerve activity at the time of SCD. The purpose of the present study was to continuously record electrocardiogram (ECG) and subcutaneous nerve activity (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 but instead, binds to a 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 measured using hospital laboratory colorimetric techniques. Six normal Han:SPRD rats of similar (35-weeks) age underwent DSI monitoring 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 the implantation of a Data Science International (DSI; St Paul, Minnesota) model PhysioTel® ETA-F10 (5 CKD and 5 normal rats) or F50-W-F2 (1 CKD and 1 normal rat) radiotransmitters. The two bipolar recording wires were tunneled subcutaneously from the top of the dorsal incision subcutaneously to the forelimbs, with one 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 1,000 times/s 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-hydroxydopa (6-OHDA) (100 mg/kg) 1 and 4 days after baseline recording. The SCNA and ECG continued until 7 days after baseline recording.

Histological Examinations

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

Data Analyses

Using a custom-written software, we manually analyzed the recording from CKD rats at 5 days prior to death for baseline activity and also at 24 hours prior to 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-s 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 zero. We then determined the iSCNA and average heart rate (HR) 3-s prior to and 3-s after the time

zero to test the hypothesis that SCNA is associated with HR elevation. Figure 1 shows the methods used to determine whether or not SCNA increased HR.

Statistical Analysis

The descriptive data were expressed as mean ± SD while the data used for quantitative comparison were expressed as confidence interval.[17] Paired *t* tests were used to compare the means of two groups. Bonferroni correction was made for multiple comparisons. A two tailed *P* value of 0.05 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 22.0 (IBM SPSS, Chicago, IL) and R 3.0.1.

Results

Relationship between SCNA and heart rate

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% to 4.47%, P=0.027, Figures 1A and 1B). In comparison, SCNA increased from a HR of 356±16 bpm to 364±19 bpm in CKD rats over the 5 days prior to death. The average increase in the CKD rats was only 2.48% (CI, 2.29% to 2.67%, p<0.01 compared to normal rats). The effects of SCNA on HR were further reduced within 24 hours of death, when the SCNA showed an increased HR from 243±60 bpm to 245±64 bpm (increase by 0.54%, CI, 0.43% to 0.66%, p<0.01). These data show that SCNA can be used to measure sympathetic tone in both normal and CKD rats. The sinus node response to SCNA is reduced by CKD, especially prior to death. In two rats, the data were obtained using an F50-W-F2 transmitter that has a high sampling rate of 5000 Hz and band width of 50–1000 Hz, allowing us to high pass filter the signals at 150 Hz, 500 Hz and 700 Hz to further reduce the lower frequency signals such as ECG and muscle contractions. The results (Figure 2) show that significant high frequency signals were present even after 700 Hz high pass filtering. Figure 3 shows the number of SCNA episodes with various degrees of HR changes, using the baseline HR as 1. In normal rats (blue line), the SCNA shows invariable increases in HR. In CKD rats at baseline (5-d prior to death), the SCNA also showed nearly uniform increases in HR (red line). However, the magnitude of increase was smaller than normal. Within 24-hr before death (green line), the SCNA may be associated with either HR acceleration or reduction.

Bradycardia and sudden death in CKD rats

The average 24-hr HR and iSCNA for normal rats were 351±29 bpm and 5.12±2.97 mV-s, respectively. The HR and SCNA were, respectively, 336±21 bpm and 4.27±3.19 mV-s for CKD rats at baseline (5 days prior to SCD). During the 24-hr period before death in CKD rats, the HR was reduced to 243±55 bpm (p<0.001) while SCNA was reduced to 3.22±2.86 mV-s (p<0.001). Figure 4A and 4B, respectively, show averaged hourly HR and SCNA in normal rats and in CKD rats 5 days prior to death. There was significant (p<0.001) circadian variation of HR and SCNA in normal rats but not in the CKD rats. Figure 5 shows that average hourly HR (Figure 5A) and SCNA (Figure 5B) during the 24 hr period prior to death in 5 CKD rats. There was progressive bradycardia in these 5 rats. The 6th rat, which

died of spontaneous ventricular fibrillation (VF), did not show progressive HR or SCNA reduction within 24-hr of death.

Terminal rhythm in CKD rats

All 6 CKD rats died suddenly 23±14 days after surgery. The underlying rhythm of all rats was sinus. Second and third degree AV block occurred in all 6 rats, preceded by a period of progressive bradycardia that began within the previous 24 hours. Death, defined by a total absence of any synchronized cardiac electrical activity, occurred < 1 hour (range 9 min to 59 min) after the initial development of AV block. One rat developed 18 episodes of slow ventricular tachycardia (VT, Figure 6A) with a heart rate of 77±21 bpm and duration ranging from 1.1 s to 17.3 s before converting back to third degree AV block, bradycardia and death. Two rats died of ventricular fibrillation (VF, Figure 6B and F) 59 min and 9 min, respectively, after the onset of AV block. In the remaining 3 rats, the terminal rhythm was complete heart block, progressive bradycardia and asystole (Figures 6C, D and E).

SCNA during ventricular fibrillation

In CKD rat #6, accelerated HR occurred soon after onset of continuous SCNA (Figure 7A, arrow 1) and lasted for approximately 15 minutes. When SCNA spontaneously terminated (Figure 7B, arrow 3), the rat developed AV block and VF in approximately 3 s (Figure 7B, arrow 4). A large burst SCNA occurred roughly 7 s after the onset of VF (Figure 7B, arrow 5). That large bust persisted for approximately 12 s during continuous VF, most likely represents sympathetic responses to circulatory arrest.

Biochemistries

We performed tail vein collection at 35 weeks of age, near the time of DSI placement. The mean ± SD for the CKD animals was BUN 47 ± 8 mg/dl, calcium 9.1 ± 3.6 mg/dl (one animal was hypercalcemic), phosphorus 8.3 ± 1.7 mg/dl, parathyroid hormone 1,310 ± 1044 pg/ml, potassium 4.5 ± 1.2 mg/dl, and magnesium 2.9 ± 1.1 mg/dl. Thus, the CKD rats had hyperphosphatemia and secondary hyperparathyroidism as we have previously reported and is common with progressive CKD.[18, 19] Hyperkalemia and hypermagnesemia were not observed.

Histological changes

Trichrome staining showed obvious fibrosis in all CKD rats, especially surrounding AV node (Figure 8A) and in the subendocardium (Figure 8B). H&E staining showed calcification of all 6 CKD rats. The magnitudes of calcification were variable, including small isolated calcific deposits in 3 rats, and massive amount of calcific deposits in the remaining 3 rats. In the latter rats, the large areas of calcification were observed in the myocardium (Figure 8C) and endocardium, and disrupting the conduction system (Figure 8D). In comparison, no calcification was found in the conduction system or the myocardium of the normal rats (Figure 8E–F).

Effects of chemical sympathectomy on SCNA

The results of these studies were summarized in an online supplement. Supplemental Figure 1 shows examples of SCNA before (Panel A) and after (Panel B) 6-OHDA injection. To better estimate the effects of 6-OHDA on SCNA, we subtracted the baseline noise, including the unfiltered ECGs (red boxes) from the integrated SCNA. The SCNA was stable during the baseline recording, but progressively decreased after 6-OHDA (Supplement Figure 2A). The mean SCNA reduced from 2.08 mV-s [95% CI, 1.64 to 2.54] on baseline day 5 to 0.48 mV-s [95% CI, 0.02 to 0.96] (p<0.001) on day 7 after 6-OHDA injection in rat 1, and from 2.07 mV-s [95% CI, 1.58 to 2.56] on baseline day 5 to 0.44 mV-s [95% CI, 0.04 to 0.86] (p<0.001) after 7 days of injection in rat 2. The mean HR increased from 373 bpm [95% CI, 358 to 384] to 425 bpm [95% CI, 416 to 435] (p=0.016) in the first rat and from 368 bpm [95% CI, 352 to 382] to 392 bpm [95% CI, 374 to 411] (p=0.028) in the second rat (Supplemental Figure 2B). The increased HR is a typical response to 6-OHDA.[20] There were no circadian variations of SCNA (Supplemental Figure 2C) or HR (Supplemental Figure 2D) in Day7 after 6-OHDA injection.

Discussion

The major findings of the present study include the following: (1) SCNA can be used to estimate the sympathetic tone in rats. (2) Cardiac arrhythmias, including AV block, VT and VF underlie the mechanisms of SCD in CKD rats; (3) CKD rats had poor HR response to sympathetic activation, especially within 24-hr of SCD.

SCNA and sympathetic tone

Skin and muscle sympathetic nerve activity (SSNA and MSNA, respectively) can be recorded by microneurography techniques. Unlike MSNA, the SSNA is identified by broad-based bursts that are not pulse synchronous.[21] The SCNA recorded with widely spaced implanted electrodes[9–11] is also broad-based and does not track the respiratory modulation or beat to beat changes of blood pressure. Both techniques may be helpful in studying the sympathetic tone, but only SCNA can be used for long term recording in ambulatory animals. In the present study we demonstrated for the first time that SCNA can be recorded from a rat model of cardiac arrhythmia. Chemical sympathectomy resulted in a progressive reduction of the SCNA, strongly indicating that the SCNA contains a significant sympathetic component.

Mechanisms of Sudden Death in CKD rats

We defined SCD by the unexpected death from a cardiac cause within one hour of the onset of symptoms.[22] According to that definition, all CKD rats used in the present study died of SCD. Epidemiological studies have demonstrated the overall incidence of SCD in CKD population is significantly greater than the incidence of general population and that the incidence increases as CKD progresses.[1, 23] Once on dialysis, one in four patients dies suddenly and most do not fall into the high-risk categories that are associated with sudden death in the general population.[24] A recent study showed SCD in CKD was attributable to severe bradycardia and asystole during the long interdialytic period.[3] Another study demonstrated that 74.9% of the clinically significant arrhythmias in previously

asymptomatic hemodialysis patients were bradycardia or asystole events while only 1 sustained VT episodes were detected during the same monitoring period.[25] Continuous ECG recording in the present study showed that all SCD occurred < 1 hour after the development of AV block. While 1 rat developed slow VT and 2 rats died of VF, these tachyarrhythmia episodes occurred after the development of AV block. All but one rat developed progressive bradycardia prior to the onset of VT or VF, indicating that tachyarrhythmias may be part of the terminal events. These data suggest that defibrillation shocks that terminate VT and VF may not improve overall survival in patients with advanced CKD.

The causes of AV block remain unclear. Our previous study[8] found abnormal ion channel function and calcium handling in the CKD rats. These changes may play a role in producing sinus bradycardia and AV block. In addition, we found that there was increased fibrosis around the AV node and metastatic calcification in all 6 CKD rats, despite overt hypercalcemia in only one rat. Masanao et al[26] reported an autopsy case of renal transplant recipient who died suddenly after a good post-transplant course of 14 years. The pathophysiological findings of that case were very similar to our CKD rats, showing calcification in the central fibrous body surrounding the AV node and bundle of His, as well as in the origin of bifurcating bundle. Others have reported similar findings of calcification around AV node or myocardium in dialysis patients who died of AV block.[27,28] The etiology of this extra-skeletal calcification appears to be a result of disordered mineral metabolism (hyperphosphatemia and hyperparathyroidism) as was observed in our rats.[29] These case reports as well as our study suggest that calcification involving the cardiac conduction system in CKD may play an important role in the development of AV block and SCD. In addition to calcification, cardiac fibrosis is a common finding in pre dialysis and dialysis patients that die of cardiac death and undergo autopsy, although calcification was more prominent than fibrosis in the current rat study.[30, 31] However, because these structural changes are likely to have developed over a long time, additional factors are needed to explain the trigger of SCD.

SCD and sympathetic withdrawal

There is growing evidence that activation of the sympathetic nervous system contributes to the pathogenesis of CKD. Ishii et al[32] documented elevated concentrations of plasma catecholamines and enhanced sensitivity to norepinephrine in patient with CKD. Increased MSNA has also been reported in patients with end-stage renal disease (ESRD) undergoing dialysis.[33] Consequently, sympathetic activation was recognized as an early pathophysiological element of CKD which may have initiated various forms of renal damage. In our study, bipolar recording wires were inserted to upper thorax which is innervated by sympathetic nerves from the stellate ganglion.[34] In the normal rats, the SCNA was reliably followed by HR acceleration. However, there was significantly reduced SCNA and HR in CKD rats 5-d before death. In a majority of rats, SCD was preceded by further reduction of SCNA and HR 24-hr before death. These findings suggest that sympathetic withdrawal might have occurred in advanced CKD, and that sudden reduction of sympathetic nerve discharges and reduced sinus node response to norepinephrine may have facilitated the development of AV block and bradycardia as the terminal events.

Limitation of the study

We only measured the terminal stages of CKD. Therefore, our data do not imply an absence of increased sympathetic tone during early stages of CKD. The SCNA was followed by a HR rise of 4.13% in normal rats and 2.48% in CKD rats. The small difference may not have significant physiological consequence. However, poor HR response to sympathetic nerve activity may contribute to the reduced HR variability in patients with CKD.[35]

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Abbreviations

6-OHDA	6-hydroxy dopamine
AV	atrioventricular
BUN	blood urea nitrogen
CKD	chronic kidney disease
DSI	data science international
ECG	electrocardiogram
ESRD	end-stage renal disease
HR	heart rate
ICD	implantable cardioverter defibrillator
MSNA	muscle sympathetic nerve activity
SCD	sudden cardiac death
SCNA	subcutaneous nerve activity
SSNA	skin sympathetic nerve activity
VF	ventricular fibrillation
VT	ventricular tachycardia

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Clinical Perspectives

We reported the following new findings: (1) It was possible to record SCNA from rats, and that the SCNA might be used to estimate cardiac sympathetic tone. These findings are consistent with previous reports that documented the efficacy of SCNA in measuring sympathetic tone in dogs. Because sympathetic tone is important in the pathogenesis of cardiac arrhythmia, the SCNA might become a useful tool in basic science and clinical investigations of arrhythmia mechanisms. (2) SCD in Cy/+ rats is triggered invariably by AV block and bradycardia. Abrupted withdrawal of sympathetic tone preceded the onset of bradycardia and SCD. While VT and VF were also documented in half of the animals monitored, they were not triggered directly by sympathetic hyperactivity. Rather, those episodes occurred after the onset of AV block. Our findings are consistent with a recent report that showed bradycardia as the mechanism of SCD in CKD patients during the long interdialytic period. Clinical trials are needed to determine if prevention of bradycardia and/or avoiding excessive sympathetic blockade can reduce SCD in patients on dialyses.
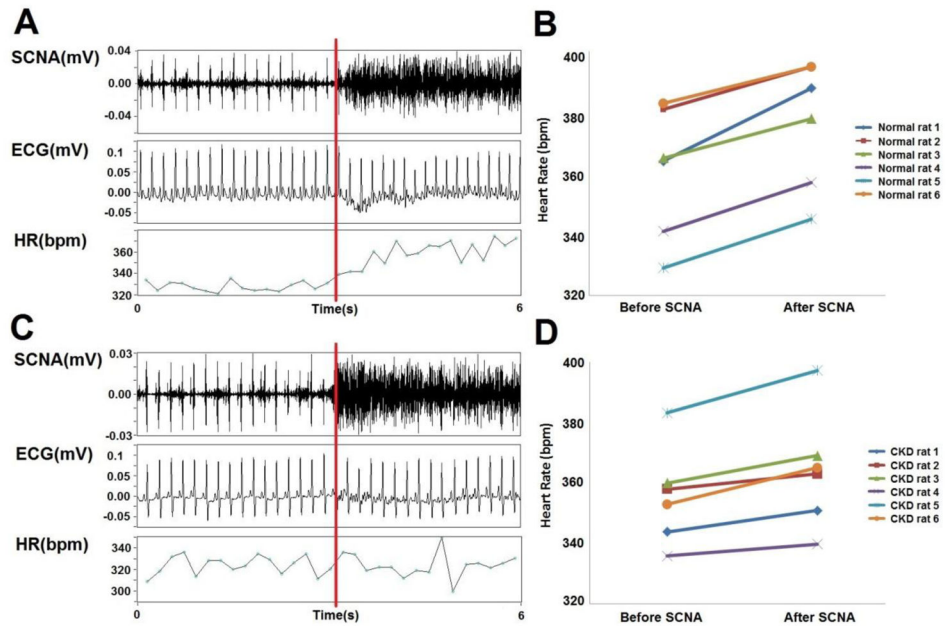
Figure 1.
Heart Rate (HR) and subcutaneous nerve activity (SCNA). **A** was recorded in a normal rat at baseline. We first identified the onset of SCNA (red bar), then determined the average HR 3-s prior to and 3-s after the onset of SCNA. **B** shows that in each rat, the HR 3-s after SCNA onset was faster than the HR before SCNA onset. **C,** The same analyses were done for HR and SCNA in CKD rats at baseline, showing SCNA induced very little HR change. **D** shows the results of all CKD rats at baseline. The HR changes were less apparent than that observed in normal rats.
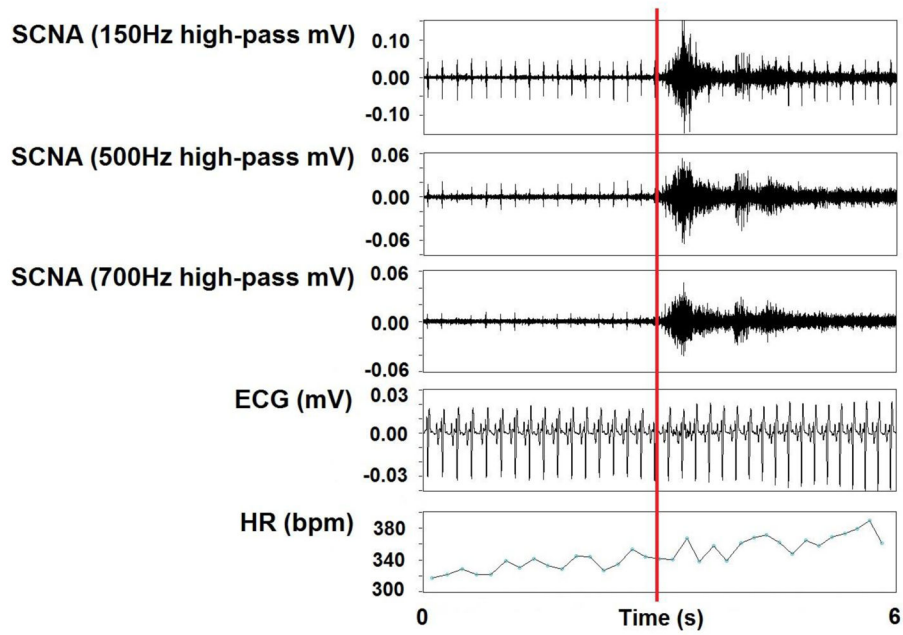
Figure 2.
Subcutaneous nerve activity (SCNA) high pass filtered at 150 Hz, 500 Hz and 700 Hz. Because the frequency of electromyogram does not exceed 400 Hz, most of the high frequency signals seen after 150 Hz filtering are likely nerve activities.
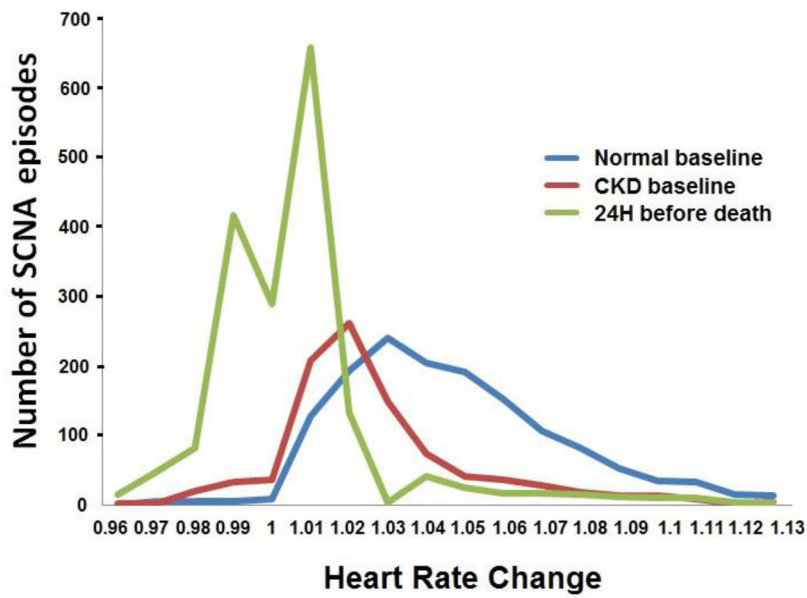
Figure 3.
Number of SCNA episodes associated with different degrees of heart rate change. Normal rats are shown in blue. CKD rats 5-d before death is shown in red and 24-hr before death is shown in green. The HR of "1" indicates that SCNA did not change the HR. In normal rats, practically all SCNA episodes were associated with significant HR increases. The magnitudes of change were smaller for CKD rats at baseline, and was even less for CKD rats 24-hr before death.
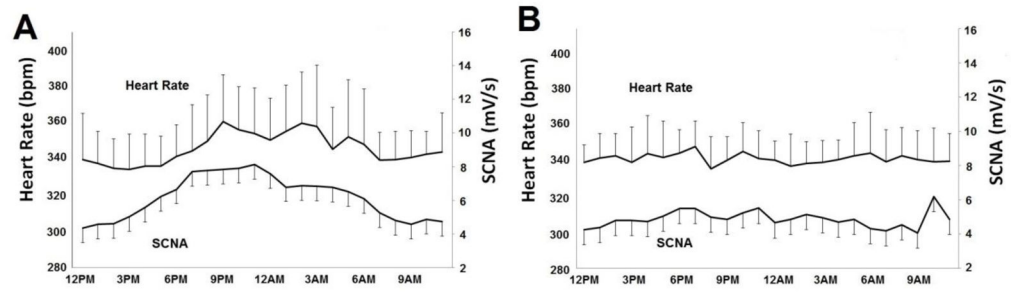
Figure 4.
HR and SCNA over a 24-hr period in all rats studied. **A,** There is significant circadian variation of HR and SCNA at baseline in normal rats. **B,** no circadian variations of HR or SCNA were observed in CKD rats 5-d before death.
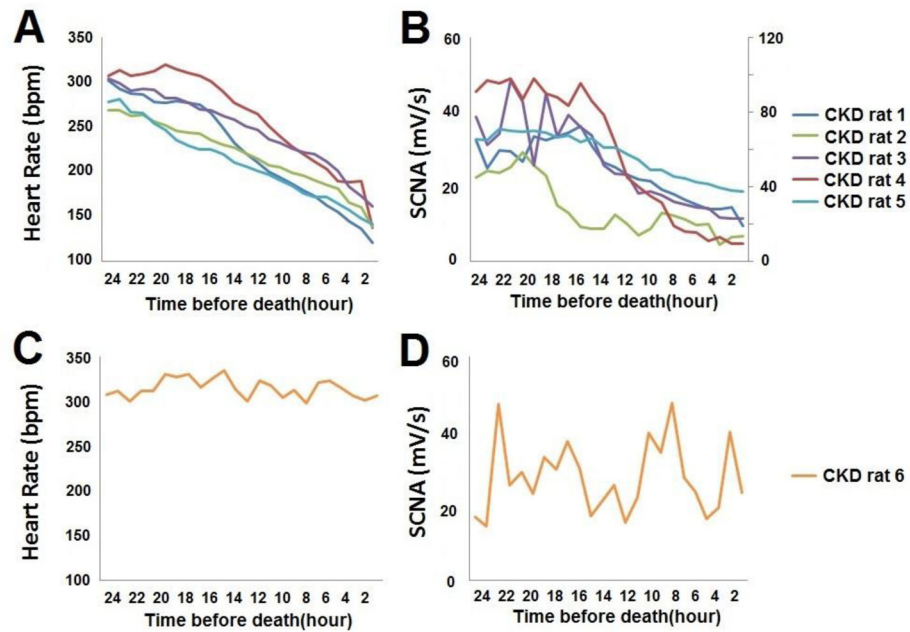
Figure 5.
HR and SCNA 24 hours before death. In 5 of 6 CKD rats, there were progressive HR (A) and SCNA (B) reduction over a 24-hr period before death. The bradycardias in these 5 rats were the results of complete AV block. In one CKD rat, no HR (C) or SCNA (D) changes were observed. The latter rat died of VF after a brief period of heart block (see Figure 6F).
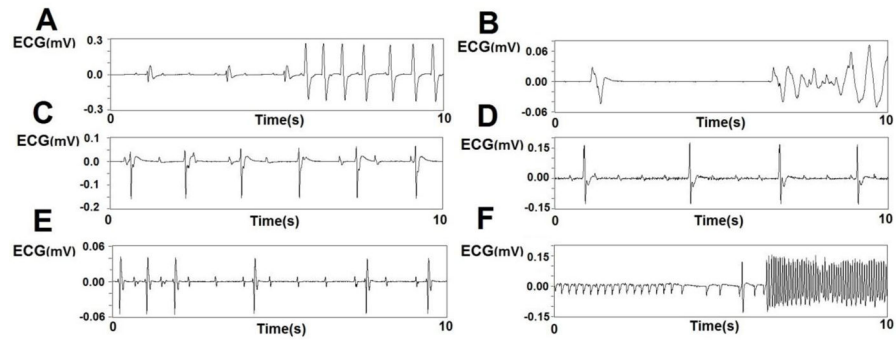
Figure 6.
Terminal Arrhythmias in CKD rats. **A,** AV block and slow ventricular tachycardia (VT). **B,** AV block and VF. **C,** Completed AV block. **D,** AV block. **E,** AV block. **F,** AV block and VF.
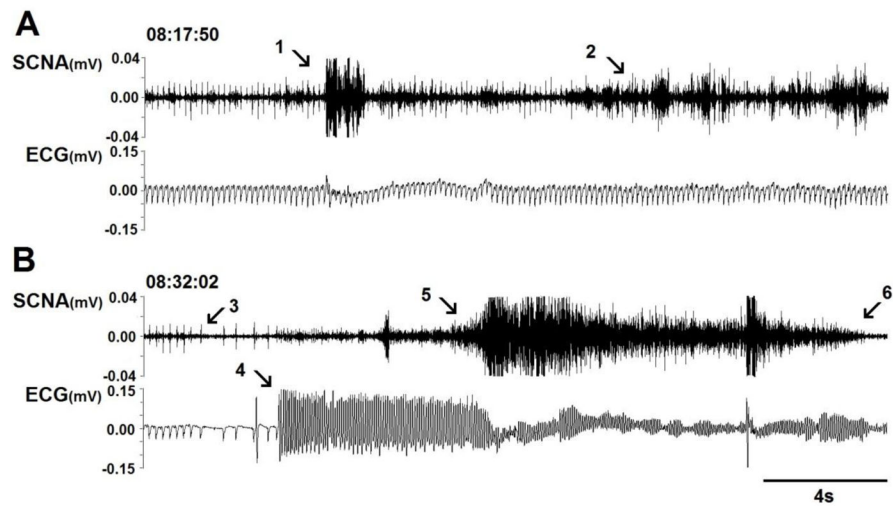
Figure 7.
SCNA and VF. **A,** Continuous SCNA started approximately 15-min prior to VF. Arrow 1 points to SCNA that accelerated the HR. The arrow 2 shows continuous SCNA. **B** shows the onset of VF, which was preceded immediately by SCNA withdrawal (arrow 3), AV block and onset of VF occurred (arrow 4). The total duration of AV block in this CKD rat was approximately 3-s. Arrow 5 shows a burst of massive SCNA which occurred 7 s after the onset of VF and persisted for 12 s (until arrow 6).
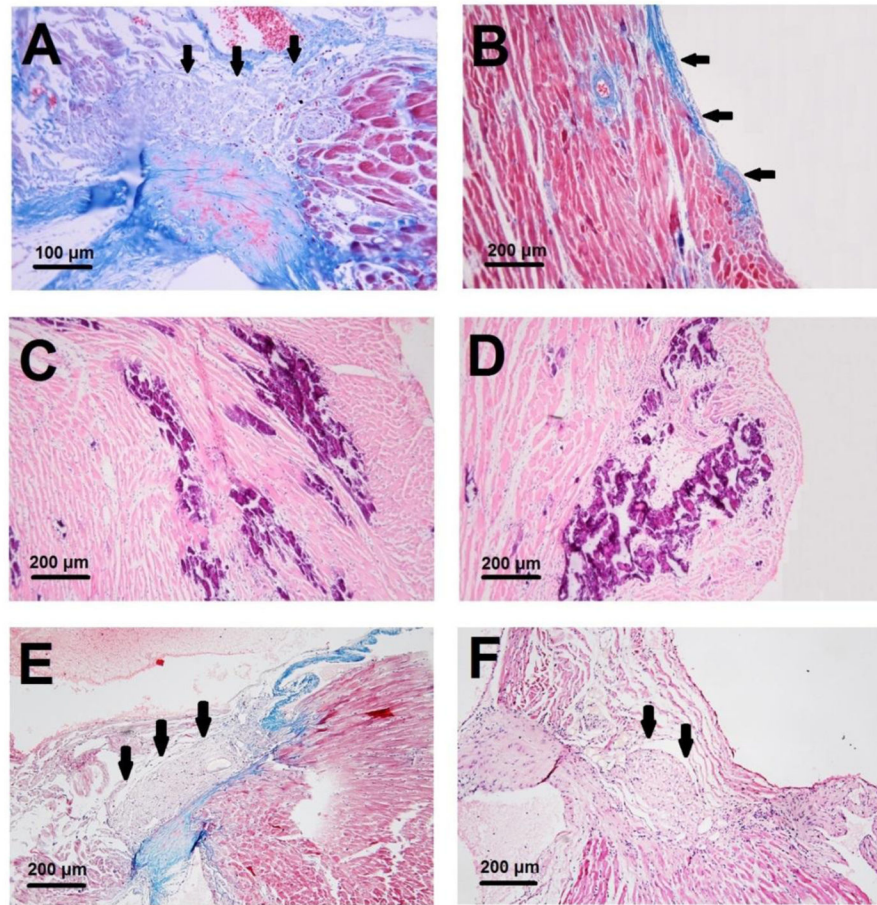
Figure 8.
Histological findings in CKD (A–D) and in normal (E–F) rats. **A,** Trichrome staining showed fibrosis surrounding AV node (arrows, X200). **B,** Trichrome staining showed fibrosis in endocardium (arrows, X100). **C and D,** H&E staining showed calcification (purple) within the myocardium (X100) and near the endocardium (X100), respectively. **E–F,** The AV node (arrows, X200) of normal rats stained with trichrome and H&E, respectively. There was no calcification or fibrosis.