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Early cardiac electrographic and molecular remodeling in a model of status epilepticus and acquired epilepsy

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Summary

Objectives—A myriad of acute and chronic cardiac alterations are associated with status epilepticus (SE) including increased sympathetic tone, rhythm and ventricular repolarization disturbances. Despite these observations, the molecular processes underlying SE-associated myocardial remodeling remain to be identified. Here we determined early SE-associated myocardial electrical and molecular alterations using a model of SE and acquired epilepsy.

Methods—We performed electrocardiography (ECG) assessments in rats beginning at 2 weeks following kainate-induced SE, and calculated short-term variability (STV) of the corrected QT intervals (QTc) as a marker of ventricular stability. Using western blotting, we quantified myocardial β 1-adrenergic receptors (β 1-AR) and ventricular gap junction protein connexin 43 (Cx43) levels as makers of increased sympathetic tone. We determined the activation status of three kinases associated with sympathetic stimulation and their downstream ion channel targets: extracellular signal-regulated kinase (ERK), protein kinase A (PKA), Ca^{2+} /calmodulin-dependent protein kinase II (CamKII), hyperpolarization-activated cyclic nucleotide-gated channel subunit 2 (HCN2), and voltage-gated potassium channels 4.2 ($\text{Kv}_{4.2}$). We investigated whether SE was associated with altered Ca^{2+} homeostasis by determining select Ca^{2+} -handling protein levels using western blotting.

Results—Compared with the sham group, SE animals exhibited higher heart rate, longer QTc interval, and higher STV beginning at 2 weeks following SE. Concurrently, the myocardium of SE rats showed lower β 1-AR and higher Cx43 protein levels, higher levels of phosphorylated ERK, PKA, and CamKII along with decreased HCN2 and $\text{Kv}_{4.2}$ channel levels. In addition, the SE rats

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Disclosure

The authors declare no conflicts of interest. We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

had altered proteins levels of Ca^{2+} -handling proteins, with decreased $\text{Na}^+/\text{Ca}^{2+}$ exchanger-1 and increased calreticulin.

Significance—SE triggers early molecular alterations in the myocardium consistent with increased sympathetic tone and altered Ca^{2+} homeostasis. These changes, coupled with early and persistent ECG abnormalities, suggest that the observed molecular alterations may contribute to SE-associated cardiac remodeling. Additional mechanistic studies are needed to determine potential causal roles.

Keywords

Cardiac remodeling; Epilepsy; Intracellular signaling; Ion channelopathy; Status epilepticus

Status epilepticus (SE), defined as seizure activities lasting longer than 30 min,¹ is a prevalent neurologic emergency and constitutes a predisposing condition to the development of epilepsy.² In experimental rodent models, initial SE is often followed by neuropathologic changes leading to chronic, spontaneous seizures later in life (epilepsy).³ In addition to increased brain excitability, SE triggers a substantial activation of the autonomic nervous system that is implicated in increased cardiac excitability and seizure-induced arrhythmias,^{4–6} thus demonstrating that SE can acutely destabilize cardiac electrophysiology. Furthermore, evidence of altered cardiac structures and electrophysiology have been observed in chronically epileptic rats in models of SE and acquired epilepsy.^{7–9} It is notable that the cardiovascular alterations observed in the experimental SE models recapitulate many salient ictal and interictal cardiac findings in individuals with epilepsy.¹⁰ Taken together, these observations suggest that SE may promote acute and long-term myocardial pathologic changes and thereby cardiac morbidity in epilepsy.

A myriad of acute and chronic cardiac alterations has been associated with SE that include increased sympathetic tone,^{4–8,11} rhythm and ventricular repolarization disturbances,^{5,7,9,12} myocardial injury, and cardiac hypertrophy.^{8,13} Few studies have demonstrated decreased myocardial expression of hyperpolarization-activated cyclic nucleotide-gated channel subunit 2 (HCN2) and voltage-gated potassium channels 4.2 ($\text{Kv}_{4.2}$) early in the development of epilepsy^{7,9} with corresponding electrocardiography (ECG) abnormalities in the SE animals. $\text{Kv}_{4.2}$ channels have predominant activity during early repolarization represented by the transient outward current (I_{TO}),¹⁴ whereas HCN channels play a predominant role in the automaticity of the cardiac pacemaker cells.¹⁵ Therefore, these findings suggest that arrhythmogenic molecular remodeling may occur early in the development of epilepsy. However, despite these observations, the initiating events and intracellular signaling cascades underlying SE-associated myocardial remodeling are unknown.

Increased sympathetic tone, reflected in direct sympathetic nerve measurements, as well as heart rate and blood pressure changes, represents a common finding during SE.^{4–7} Furthermore, elevated cardiac sympathetic tone also has been observed weeks to months beyond SE cessation, the period that is characterized by spontaneous recurrent seizures.^{8,11} In primary cardiac pathology, increased sympathetic stimulation leads to activation of intracellular signaling cascades such as protein kinase A (PKA), extracellular signal-

regulated kinase (ERK), and Ca^{2+} /calmodulin-dependent protein kinase II (CamKII).^{16,17} These signaling molecules can serve as initiators of arrhythmogenic molecular remodeling in the myocardium by modulating ion channel function and intracellular Ca^{2+} homeostasis.¹⁸ Taken together, these findings suggest the possibility that increased cardiac sympathetic tone in the SE animals may similarly lead to aberrant activation of PKA, ERK, and CamKII in the myocardium that may contribute to epilepsy-associated cardiac pathology.

Therefore, the objectives of this study were to determine the temporal profile of cardiac electrical changes following an episode of SE, and to determine early myocardial molecular alterations following SE. These included SE-associated alterations in the protein levels of β -adrenergic receptors, candidate intracellular signaling cascades, and ion channels in the myocardium.

Materials and Methods

Ethics statement

Animal procedures used throughout this study were approved by the Baylor College of Medicine Institutional Animal Care and Use Committee and conformed to National Institutes of Health (NIH) guidelines.

Animals

Male Sprague-Dawley animals (150–200 g) were kept at an ambient temperature of 22°C, with a 14-h light and 10-h dark diurnal cycle with unrestricted access to food and water.

Kainate-induced SE

Intraperitoneal (i.p.) injections of kainate (KA, 18 mg/kg) (Abcam, Cambridge, MA, U.S.A.) were administered to adult animals. Age-matched, saline-treated sham animals served as controls. Behavioral seizures and SE were scored using the Racine scale. SE onset was indicated by the appearance of class 5 limbic motor seizures (rearing and falling). Sodium pentobarbital (20 mg/kg, i.p.) was administered following 1 h of SE to terminate seizures. Following KA-induced SE, animals were monitored for adequate food and water intake. All animals were monitored for behavioral seizures either by real-time observation, or by retrospective review of video recordings, between 1 and 2 months ($n = 14$), and then between 7 and 10 month following SE ($n = 13$) for a total of 427 h.

Electrocardiography (ECG) acquisition and analysis

Two weeks following SE, animals were anesthetized using 2% isoflurane and stabilized for 5 min before ECG recordings were acquired. This procedure was repeated at 1, 2, 3, and 5 months after SE to acquire ECG recordings at these time points. Both saline-treated sham ($n = 7$) and experimental SE ($n = 9$) groups were subjected to the same procedure for ECG acquisition. In subsequent experiments, ECG recordings were obtained only at 2 weeks following SE. Single-channel ECG in lead II configuration was recorded for 5 min using VSM7 multiparameter monitor (VetSpecs, Canton, GA, U.S.A.) and analyzed at 10 min following the induction of anesthesia. Automated heart rate (HR) measurements were

obtained from the VSM7 monitor. An investigator blinded to the group assignment manually measured PR, QRS, and QT intervals based on the best-available ECG waveform. Bazett's formula was used to calculate corrected QTc (QTc) interval. Short-term variability (STV) of the QTc interval was calculated based on 10 consecutive QTc values using the formula:

$$STV = \left(\sum |QT_{c_{n+1}} - QT_{c_n}| \right) / \left(N \times \sqrt{2} \right), \text{ where } |QT_{c_{n+1}} - QT_{c_n}| \text{ is the absolute difference between the two successive beats and } N \text{ is number of heartbeats.}^{19}$$

Immunoblotting

Two weeks following SE, whole heart samples were processed for western blotting using previously described protocols with minor modifications.⁷ Briefly, hearts were homogenized in ice-cold phosphate-buffered saline (PBS) solution (1 ml/1 g of heart tissue) containing protease inhibitor cocktail (Roche, Alameda, CA, U.S.A.) using a handheld VWR 200 homogenizer, followed by glass homogenizer. Samples were centrifuged at 2,000 *g* and 4°C for 10 min. Pellets were suspended in ice-cold PBS and re-homogenized. Total protein concentration of whole heart homogenates was determined using the Bradford protein assay (Bio-Rad, Hercules, CA, U.S.A.). Equal amounts of total protein (20–40 µg) for each sample were separated by size and charge using sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to polyvinyl difluoride membranes (GE Healthcare, Piscataway, NJ, U.S.A.). Membranes were incubated in blocking solution (5% nonfat milk in 1× Tris-buffered saline [TBS] + 0.1% Tween 20) and blotted at 4°C overnight with the following primary antibodies (antibodies were diluted to 1:1,000 unless otherwise indicated.): β1-adrenergic receptors (β1-AR, 1:500), connexin 43 (Cx43), P-PKA (Thr197), PKA, P-ERK (T202/Y204), ERK, P-CamKII (Thr286), CamKII, K_v4.2, HCN2, Na⁺/Ca²⁺ exchanger-1 (NCX-1), calreticulin (CaR), calmodulin (CaM), P-S6 (S240/244), S6, and glyceraldehyde 3-phosphate dehydrogenase (GAPDH; 1:20,000). Horseradish peroxidase (HRP)-conjugated secondary antibodies (1:5,000) and Pierce enhanced chemiluminescence (Thermo Scientific, Rockford, IL, U.S.A.) were used to capture the immunoreactive bands on autoradiography film (Double Emulsion Blue Autoradiography Film; MISC, St. Louis, MO, U.S.A.). A Protec EcoMax X-Ray Processor System (ClassicXray, Rolla, MO, U.S.A.) was used to develop the films. The mean gray density of immunoreactive bands was obtained and analyzed using the ImageJ (NIH, Bethesda, MD, U.S.A.) and GraphPad Prism (GraphPad Software, La Jolla, CA, U.S.A.) software as previously described.⁷ The immunoreactive bands from the proteins of interest were normalized to the GAPDH levels for the corresponding protein. Those of the phosphoproteins were normalized to the respective total protein.

Statistical analyses

Comparisons of HR, PR, QRS, and QTc intervals between sham animals and epileptic animals over time were performed using two-way analysis of variance (ANOVA). Comparisons of protein levels between sham and epileptic animals were performed using Student's *t*-test; *p* < 0.05 represents statistical significance. Data are shown as mean and standard deviation.

Chemicals and primary antibodies

All chemicals were purchased from Fisher Scientific unless otherwise specified. β 1-AR was from Santa Cruz Biotechnologies (Dallas, TX, U.S.A.). Cx43, PKA, P-PKA (T197), ERK1/2, P-ERK1/2 (T202/Y204), P-CamKII (T286), CamKII, calreticulin, calmodulin, P-S6 (S240/244), S6, and HRP-conjugated mouse and rabbit secondary antibodies were from Cell Signaling Technology (Boston, MA, U.S.A.). HCN2 and Kv_{4.2} antibodies were from Neuro-Mab (Davis, CA, U.S.A.). NCX-1 was from Alomone Labs (Jerusalem, Israel).

Results

Electrocardiographic alterations and ventricular instability occur early after SE

Previous studies demonstrated resting tachycardia and prolonged QTc interval at 1–2 weeks following chemocon-vulsant-induced SE or multiple brief kindling-induced seizures in rats.^{7,12,13} However, whether these SE-associated ECG alterations persist for weeks and months was not known. Therefore, we prospectively monitored a cohort of age-matched sham and SE rats from 2 weeks to 5 months following SE, a period that encompasses the early and chronic phases of epilepsy.³ Serial ECG recordings demonstrated a significant decrease in HR and constant QTc interval from 2 weeks to 5 months in sham animals ($n = 7$, $p < 0.01$, Fig. 1A,B). The SE animals also exhibited a significant decrease in HR and constant QTc intervals from 2 weeks to 5 months following SE ($n = 9$, $p < 0.05$, Fig. 1A,B). However, compared with the sham animals, the SE animals exhibited higher HR ($p < 0.05$, Fig. 1A) and longer QTc intervals ($p < 0.01$, Fig. 1B) that were evident as early as 2 weeks following SE and persisted throughout the monitoring period, during which spontaneous recurrent seizures and epilepsy develop (Fig. 1C). We monitored these animals for the development of behavioral seizures and found that between 6 and 8 weeks after SE (1–2 months) SE rats (13/14) displayed unprovoked seizures. These animals continued to show recurrent seizures for up to 10 months after SE and therefore were epileptic.

In this model of SE and acquired epilepsy, recurrent electrographic and motor seizures, as well as hippocampal molecular alterations implicated in epileptogenesis appear at 2 weeks following SE.^{3,20–22} Therefore, we focused our investigation at this time point corresponding to the early stage of epilepsy in this model. Additional ECG studies at 2 weeks following SE further demonstrated higher HR (303 ± 38 vs. 335 ± 47 bpm, sham vs. SE, $n = 28$ – 29 /group, mean $\pm$ standard deviation of the mean [SEM], $p < 0.01$) (Fig. 1D) and longer QTc (239 ± 34 vs. 295 ± 41 msec, sham vs. SE, $n = 28$ – 29 /group, $p < 0.001$) (Fig. 1E) in the SE group as compared with the sham animals. Furthermore, SE animals exhibited significantly higher STV values as compared with sham animals (11.58 ± 5.74 vs. 16.92 ± 9.11 msec, sham vs. SE, $n = 28$ – 29 /group, $p < 0.05$) (Fig. 1F), indicating more beat-to-beat variability in QTc intervals. Increased STV of the QTc intervals has been observed in congenital long-QT syndrome and nonischemic heart failure, and is thought to reflect increased risk for ventricular arrhythmias.^{19,23} Concurrently in these animals, we confirmed hippocampal alterations in signaling cascades and ion channel levels that have been implicated previously in epileptogenesis (Fig. S1; Appendix S1). Together, these findings suggest that commonly observed resting tachycardia and prolonged QTc interval, as well as

ventricular repolarization instability that could contribute to increased excitability and arrhythmias may occur at the outset and parallel the development of epilepsy.

Increased β -adrenergic stimulation in the myocardium of the SE animals

Persistently elevated HR suggests ongoing cardiac sympathetic predominance in the myocardium of the SE animals. A molecular signature of chronic myocardial sympathetic stimulation is the desensitization of β 1-AR, represented by decreased β 1-AR protein levels.²⁴ Therefore, we used immunoblotting to determine β 1-AR protein levels in the myocardium of SE and sham rats. Densitometry analysis of the immunoreactive bands showed that the protein levels of β 1-AR were significantly reduced at 2 weeks after SE relative to the sham group ($100.3 \pm 8.78\%$ vs. $80.75 \pm 4.78\%$, sham vs. SE, $n = 5-7/\text{group}$, $p < 0.01$, Fig. 2A). Furthermore, sympathetic stimulation mediated through β 1-AR can increase Cx43 expression, the predominant ventricular gap junction protein, to enhance myocardial electrical coupling.²⁵ Therefore, we also determined the protein levels of Cx43 and found a significant increase in the myocardium of SE animals compared with the sham group ($100.0 \pm 9.38\%$ vs. $134.5 \pm 16.7\%$, sham vs. SE, $n = 5-7/\text{group}$, $p < 0.001$, Fig. 2B). Taken together, these molecular alterations along with the cardiac electrophysiologic observations (Fig. 1) suggest a persistently elevated cardiac sympathetic input after SE.

Myocardial intracellular signaling alterations following SE

In myocardium, sympathetic activation represents a common initiator of ERK, PKA, and CamKII signaling cascades.^{16,17} Therefore, we examined the activation status of these signaling pathways in the myocardial whole cell homogenates by measuring the phosphorylation of ERK, PKA, and CamKII proteins in the sham and SE groups at 2 weeks following SE. Immunoblotting revealed that the myocardium of SE animals exhibited a significant increase in the phosphorylation (P) levels of all three proteins, with increased levels of P-ERK ($100.0 \pm 21.71\%$ vs. $202.1 \pm 25.58\%$, sham vs. SE, $n = 4-5/\text{group}$, $p < 0.001$), P-PKA ($100.0 \pm 86.18\%$ vs. 435.5 ± 194.3 , sham vs. SE, $n = 4/\text{group}$, $p < 0.05$), and P-CamKII ($100.0 \pm 139.7\%$ vs. $735.4 \pm 612.7\%$, sham vs. SE, $n = 9/\text{group}$, $p < 0.01$) as compared with the sham animals, suggesting that these kinases are activated (Fig. 3). Activation of these kinases can modulate the expression and function of HCN2 and $Kv_{4.2}$ channels in myocardium,²⁶⁻²⁸ the levels of which are decreased in epilepsy models.^{7,9} Therefore, we investigated whether decreased myocardial levels of these ion channels occur concurrently with PKA, ERK, and CamKII activation in the SE animals. In addition to the increased P-PKA, PERK, and P-CamKII levels, we found a significant reduction in the protein levels of HCN2 ($100.0 \pm 7.68\%$ vs. $48.89 \pm 11.82\%$, sham vs. SE, $n = 5-6/\text{group}$, $p < 0.01$) and $Kv_{4.2}$ ($100.0 \pm 9.99\%$ vs. $70.89 \pm 7.04\%$, sham vs. SE, $n = 6/\text{group}$, $p < 0.05$) channels in the myocardium of the SE animals as compared with the sham animals (Fig. 4A,B). Therefore, our findings provide several signaling cascades that may potentially contribute to acquired HCN2 and $Kv_{4.2}$ channelopathies in epilepsy.

Altered levels of Ca^{2+} -handling proteins in the myocardium of the SE animals

The robust increase in CamKII activation suggests that intracellular Ca^{2+} homeostasis may be altered in the SE animals. Comparative analysis of the myocardial whole cell homogenates using unbiased protein profiling between the SE and sham animals revealed

multiple candidate proteins that are involved in the intracellular Ca^{2+} homeostasis: $\text{Na}^+/\text{Ca}^{2+}$ exchanger-1 (NCX-1), calreticulin (CaR), and calmodulin (CaM) (data not shown). Therefore, we determined the protein levels of these candidate proteins by western blotting and found that the protein levels of NCX-1 were significantly decreased in the myocardium of SE animals ($100.0 \pm 16.58\%$ SE, vs. $64.57 \pm 24.96\%$, sham vs. SE, $n = 9/\text{group}$, $p < 0.01$), whereas those of CaR were significantly increased relative to sham animals ($100.0 \pm 41.46\%$ vs. $199.1 \pm 78.97\%$, sham vs. SE, $n = 8/\text{group}$, $p < 0.01$), and no differences were observed in the protein levels of CaM between the groups ($100.0 \pm 33.27\%$ vs. $105.7 \pm 23.69\%$, sham vs. SE, $n = 8/\text{group}$) (Fig. 5). NCX-1 is the ion channel primarily responsible for Ca^{2+} extrusion following myocardial excitation-contraction.²⁹ CaR serves as a major Ca^{2+} -binding protein in the endoplasmic reticulum and regulator of Ca^{2+} homeostasis.³⁰ These data support that SE promotes acute changes in myocardial Ca^{2+} homeostasis, which could subsequently lead to aberrant cardiac physiology.

Discussion

In this model of SE and acquired epilepsy we found early electrical and molecular alterations in the myocardium of the SE animals that may contribute to arrhythmogenesis in epilepsy. Previous studies have demonstrated tachycardia and QTc interval prolongation at 1–2 weeks following SE,^{7,12,13} whereas others reported similar ECG alterations in chronically epileptic animals.^{8,9} However, no information was available regarding the temporal evolution of these ECG abnormalities in relation to the progression of epilepsy. In this study, we described higher HR and longer QTc interval in the SE animals occurring as early as 2 weeks, and persisting for at least 5 months following SE. This period encompasses epileptogenesis with the onset of electrographic and motor seizures, and epilepsy with the recurrence of unprovoked seizures.³ Longer QTc interval reflects altered ventricular depolarization-repolarization sequence,³¹ which may increase the risk for developing ventricular arrhythmias. Similarly, increases in beat-to-beat variability of the QTc intervals, represented by higher STV, suggests ventricular repolarization instability and may serve as an additional risk biomarker for ventricular arrhythmias.^{19,23} Therefore, our findings suggest that epilepsy-associated electrocardiographic alterations, as well as increases in arrhythmogenic potential, may occur early after SE and parallel the development of spontaneous recurrent seizures and epilepsy.

Cardiac sympathetic activation represents a common finding during SE.^{4–7} Persistently elevated sympathetic tone also has been observed weeks to months beyond SE cessation.^{8,11} Furthermore, pharmacologic blockade of the β -adrenergic receptors prior to seizure induction have prevented QTc prolongation and T-wave abnormalities, decreased arrhythmogenic potential and myocardial damage, and preserved left ventricular function in SE animals.^{32,33} Together, these findings suggest that cardiac sympathetic activation may play a significant role in SE-associated pathologic changes of the myocardium. Indeed, in our study, the SE animals exhibited decreased β_1 -AR protein levels, a finding that is consistent with adrenergic receptor desensitization due to persistent sympathetic stimulation and a common pathologic alteration in heart failure.²⁴ Furthermore, β_1 -AR stimulation and subsequent PKA activation can increase Cx43 expression, a major gap junction protein in the ventricle, resulting in enhanced electrical coupling between cardiomyocytes.²⁵

Therefore, we speculate that increased Cx43 levels may reflect a response to increased sympathetic stimulation and an adaptation necessary to accommodate the increased HR that are observed in the SE animals.

Classically, stimulation of $\beta 1$ -AR leads to increased secondary messenger cAMP levels and activation of cAMP-dependent kinase (PKA). Others have demonstrated that $\beta 1$ -AR stimulation increases ERK phosphorylation in adult mouse cardiomyocytes.¹⁶ In addition, sustained $\beta 1$ -AR stimulation leads to increased intracellular Ca^{2+} transients and persistent CamKII activity.¹⁷ In cardiomyocytes, ERK, PKA, and CamKII signaling pathways are essential for normal development and function such as cellular homeostasis, growth, and survival; and their aberrant activation in the myocardium contributes to many pathologic changes seen in cardiac diseases.³⁴ In cardiomyocytes, these signaling molecules can phosphorylate $\text{Kv}_{4.2}$, leading to altered current density and biophysical properties.^{26–28} Furthermore, HCN2 contains multiple putative PKA phosphorylation sites, raising the possibility that HCN2 expression and function may be modulated by PKA activation. Here we described for the first time that SE triggered ERK, PKA, and CamKII activation in cardiomyocytes. This finding, coupled with decreased myocardial HCN2 and $\text{Kv}_{4.2}$ protein levels in the same animals, suggests that ERK, PKA, or CamKII activation may be involved in modulating expression and function of HCN2 and $\text{Kv}_{4.2}$ channels in the heart. Additional studies are needed to identify the potential role that each of these pathways may play in SE-induced cardiac channelopathies and arrhythmogenesis.

Phosphorylation of CamKII at T286, which we investigated, occurs under conditions of high intracellular Ca^{2+} and is highly dependent on the formation of the Ca^{2+} /CaM complex and binding to CamKII.³⁵ Therefore, we speculate that increased P-CamKII at T286 may reflect underlying alterations in intracellular Ca^{2+} content and homeostasis. We found decreased NCX-1 along with increased CaR levels in the myocardium of SE animals, consistent with this hypothesis. Because NCX-1 represents the major ion channel for Ca^{2+} extrusion following myocardial excitation-contraction,²⁹ decreased NCX-1 protein levels could result in increased intracellular Ca^{2+} content that requires increased intracellular Ca^{2+} buffering capacity. Accordingly, elevated protein levels of CaR, a major endoplasmic reticulum Ca^{2+} binding protein, in the myocardium of SE rats may reflect a homeostatic response to the increased intracellular Ca^{2+} content. In primary cardiac pathology such as heart failure, dysregulated intracellular Ca^{2+} homeostasis and CamKII activation mediated through persistent sympathetic stimulation have been associated with diverse pathologic alterations including decreased systolic function, ventricular hypertrophy, and arrhythmias.³⁶ These cardiac pathologies also have been observed in experimental models of epilepsy,^{7–9} thus raising the possibility that aberrant intracellular Ca^{2+} homeostasis and CamKII activation may play an important role in cardiac abnormalities seen after SE and in epilepsy.

In summary, herein we demonstrated that a single episode of SE can trigger an array of electrophysiologic and molecular changes in the heart that are evident during the period of epileptogenesis. Therefore, these findings suggest that cardiac alterations may occur at the outset and may closely parallel the development of epilepsy. Furthermore, our findings provide multiple candidate mechanisms underlying SE-associated pathologic cardiac alterations that include persistent sympathetic stimulation, aberrant activation of intracellular

signaling cascades, altered intracellular Ca^{2+} homeostasis, and acquired HCN2 and $\text{Kv}_{4.2}$ channelopathies. Future mechanistic studies are needed to further delineate the contributions of these SE-induced changes to the observed cardiac pathology associated with SE and epilepsy.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Biography

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Key Points

- SE can trigger electrophysiologic changes in the heart that become evident during epileptogenesis and persist thereafter
- Early myocardial molecular alterations following SE suggest increased sympathetic tone and altered Ca^{2+} homeostasis
- These molecular alterations, coupled with electro-physiologic changes, provide candidate mechanisms for cardiac remodeling in epilepsy

Figure 1.

Kainic acid (KA)-induced status epilepticus promotes acute and long-lasting electrocardiography (ECG) alterations and ventricular instability. (A, B) Lead II ECG recordings were obtained serially in a cohort of sham ($n = 7$) and KA ($n = 9$) animals between 2 weeks and 5 months following KA-induced status epilepticus (SE). (A) The heart rate, shown as beats per minute (bpm), is significantly increased in animals from the KA group as early as 2 weeks and for up to 5 months following SE compared with the age-matched sham group. (B) The QTc interval is significantly longer in animals from the KA group compared to the sham group. (C) Quantification of behavioral seizures between 1 and 2 months and between 7 and 10 months after KA-induced SE. (D) ECG recordings were obtained in a larger cohort of sham ($n = 28$) and KA ($n = 29$) animals at 2 weeks post-SE. The heart rate of animals from the KA group is significantly elevated compared to the sham group. (E) The QTc interval is significantly prolonged in animals from the KA group compared to the sham group. (F) The beat-to-beat variability of the QTc intervals quantified as short-term variability is shown (described in Materials and Methods). The KA group exhibits significantly larger short-term variability as compared with the sham group. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Data are shown as mean standard deviation.

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Figure 2.

KA-induced status epilepticus promotes alterations in β 1-adrenergic receptors and connexin 43 (Cx43) in the myocardium. (A, B) Quantitative analyses of the mean gray density of immunoreactive bands normalized to percent of controls (% Control) are shown for β 1-adrenergic receptors (β 1 AR) (A) and Cx43 (B) in sham and 2 weeks post KA-induced SE (KA) groups. Representative immunoblots from cardiac tissue homogenates probed with antibodies against β 1-AR, Cx43, and GAPDH (loading control) are shown. A significant decrease in the protein levels of β 1-AR (A), and significant increase Cx43 (B) are evident in the KA group compared to the sham group. The protein levels of GAPDH are not different between the groups. ** $p < 0.01$. Data are shown as mean $\pm$ standard deviation, $n = 5-7$ /group.

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Figure 3.

KA-induced status epilepticus promotes alterations in ERK, PKA, and CamKII pathway activation in the myocardium. (A–C) Quantitative analyses of the mean gray density of immunoreactive bands normalized to percent of controls (% Control) are shown for P-ERK (A), P-PKA (B), and P-CamKII (C) in sham and 2 weeks post KA-induced SE (KA) groups. Representative immunoblots from cardiac tissue homogenates probed with antibodies against phospho- (P) and total- (T) ERK, PKA, and CamKII are shown. Levels of phosphoproteins were normalized to those of the corresponding total protein. A significant increase in the phosphorylation levels of P-ERK, P-PKA, and P-CamKII is evident in the KA group compared to the respective sham group. Protein levels of T-ERK, T-PKA, and T-CamKII were not different between the groups. *p < 0.05. Data are shown as mean ± standard deviation, n = 4–7/group.

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Figure 4.

KA-induced status epilepticus promotes alterations in the protein levels HCN2 and Kv_{4.2} channels in the myocardium. (A, B) Quantitative analyses of the mean gray density of immunoreactive bands normalized to percent of controls (% Control) are shown for HCN2 (A) and Kv_{4.2} (B) in sham and 2 weeks post KA-induced SE (KA) groups. Representative immunoblots from cardiac tissue homogenates probed with antibodies against HCN2, Kv_{4.2}, and GAPDH (loading control) are shown below the corresponding graphs. A significant decrease in the protein levels of HCN2 (A) and Kv_{4.2} (B) channels is evident in the KA group relative to the sham group. The protein levels of GAPDH are not different between the groups. *p < 0.05. Data are shown as mean standard deviation, n = 5–6/group.

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Figure 5.

KA-induced status epilepticus promotes alterations in calcium handling proteins in the myocardium. (A–C) Quantitative analyses of the mean gray density of immunoreactive bands normalized to percent of controls (% Control) are shown for the $\text{Na}^+/\text{Ca}^{2+}$ exchanger (NCX-1) (A), calreticulin (CaR) (B), and calmodulin (CaM) (C) in sham and 2 weeks post KA-induced SE (KA) groups. (A) A significant decrease in the protein levels of NCX-1 is evident in the KA group relative to the sham group. (B) A significant increase in the protein levels of CaR is evident in the KA group relative to the sham group. (C) Protein levels of CaM are not different between the groups. The protein levels of GAPDH are not different between the groups. * $p < 0.05$, ** $p < 0.01$. Data are shown as mean $\pm$ standard deviation, $n = 6-9/\text{group}$.

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