Original Article

Prolongation of Action Potential Duration and QT Interval During Epilepsy Linked to Increased Contribution of Neuronal Sodium Channels to Cardiac Late Na^+ Current

Potential Mechanism for Sudden Death in Epilepsy

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Background—Arrhythmias associated with QT prolongation on the ECG often lead to sudden unexpected death in epilepsy. The mechanism causing a prolongation of the QT interval during epilepsy remains unknown. Based on observations showing an upregulation of neuronal sodium channels in the brain during epilepsy, we tested the hypothesis that a similar phenomenon occurs in the heart and contributes to QT prolongation by altering cardiac sodium current properties (I_{Na}).

Methods and Results—We used the patch clamp technique to assess the effects of epilepsy on the cardiac action potential and I_{Na} in rat ventricular myocytes. Consistent with QT prolongation, epileptic rats had longer ventricular action potential durations attributable to a sustained component of I_{Na} (I_{NaL}). The increase in I_{NaL} was because of a larger contribution of neuronal Na channels characterized by their high sensitivity to tetrodotoxin. As in the brain, epilepsy was associated with an enhanced expression of the neuronal isoform $Na_V1.1$ in cardiomyocyte. Epilepsy was also associated with a lower I_{Na} activation threshold resulting in increased cell excitability.

Conclusions—This is the first study correlating increased expression of neuronal sodium channels within the heart to epilepsy-related cardiac arrhythmias. This represents a new paradigm in our understanding of cardiac complications related to epilepsy. **(*Circ Arrhythm Electrophysiol*. 2015;8:912-920. DOI: 10.1161/CIRCEP.114.002693.)**

Key Words: action potentials ■ arrhythmias, cardiac ■ death, sudden, cardiac ■ electrophysiology ■ epilepsy ■ sodium channels

Human mortality associated with epilepsy in North America is 21% to 22%.[1] ECG abnormalities occur in 35% of generalized seizures[2–4] and are characterized by cardiac rhythm and repolarization changes.[3,5] Sudden death caused by epilepsy (SUDEP) accounts for 17% of all epilepsy-related casualties. Clinical evidence has linked SUDEP to arrhythmias[1] associated with different conditions, including conduction block[3,5–7] and prolongation of the QT interval on the ECG,[6,7] the latter being an indication of a prolonged action potential. These causes of arrhythmias are commonly associated with alterations of the cardiac sodium current I_{Na}. The rat model of epilepsy used in this study faithfully reproduces these cardiac arrhythmias.[8,9]

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I_{Na} displays the following 3 phases: (1) a fast activating component responsible for the peak inward (depolarizing) current associated with the peak of the action potential, (2) rapid inactivation of most of the peak current, and (3) a sustained late component (I_{NaL}). In most cases, conduction disturbances are associated with alterations of peak I_{Na}. The high density of fast Na channels (Na_Vs) within the ventricles and His-Purkinje system insures a rapid initial depolarization rate during an action potential (AP) and, thereby, a rapid conduction velocity. The voltage dependence of I_{Na} activation determines excitability. Therefore epilepsy-induced changes in the activation voltage of I_{Na} could explain changes in cardiac conduction and excitability. The duration of the QT interval, however, is mostly determined by a balance between the inward late sodium current (I_{NaL}) that tends to lengthen the AP duration (APD) and the outward potassium currents that tend to shorten APD. An increase in I_{NaL} will therefore have a tendency to prolong the QT interval. The important role of I_{NaL} in regulating repolarization time is highlighted by studies of long-QT syndrome,[10–12] the use of ranolazine (a I_{NaL} blocker) to treat arrhythmias,[13–15] and by early experiments showing that application of low concentrations of the sodium channel blocker tetrodotoxin (TTX) shortens APD.[16] This latter result

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WHAT IS KNOWN

- Human mortality associated with epilepsy in United States and Canada is 21% to 22%. Sudden unexpected death during epilepsy accounts for 17% of all epilepsy-related casualties and is associated with cardiac arrhythmias, such as heart rhythm disturbances and prolongation of the QT interval on the ECG (an index of the ventricular action potential duration).
- The mechanisms leading to prolongation of the QT interval are unknown but the duration of the cardiac action potential and QT is governed by a fine balance of outward potassium currents and, among others, the late sodium current.

WHAT THE STUDY ADDS

- Epilepsy induces a molecular remodeling of the cardiac ventricle characterized by expression of neuronal type sodium channels ($nNa_V s$).
- Overexpression of the $nNa_V s$ lead to a significant augmentation of the late sodium current thus favoring prolongation of the action potential duration and arrhythmias.
- This represents a new paradigm in our understanding of sudden unexpected death during epilepsy whereby arrhythmias are not centrally mediated (brain) as currently thought but result from adaptive cardiac defects.

is explained by the much higher affinity of the neuronal isoforms of the Na channel for TTX compared with the primary isoform in cardiomyocytes ($Na_V 1.5$).

Recent findings indicate that expression of neuronal sodium channel isoforms ($nNa_V s$) in the rat hippocampus increases during epilepsy.[17,18] This enhanced expression of TTX-sensitive $Na_V s$ is thought to potentiate seizures by increasing I_{NaL} and by also increasing excitability in brain cells. Missense mutations in $Na_V 1.1$ and $Na_V 1.2$ that increase I_{NaL} amplitude[19–21] in hereditary epilepsy seem to confirm this hypothesis. However, epilepsy patients display prolonged QT interval and enhanced expression of $Na_V 1.1$ even in absence of mutations in neuronal $Na_V s$.[22,23] These observations raise the possibility that, as in the brain, epilepsy enhances expression of TTX-sensitive $Na_V s$ in cardiac cells thereby modifying their excitability and increasing I_{NaL}. In the heart, we showed that an increase in I_{NaL} is associated with long-QT syndrome[10,11,24] and sudden death. In this study, we wanted to test the hypothesis that upregulation of $nNa_V s$ channels may, in part, explain the cardiac arrhythmias associated with the increased risk of SUDEP in epileptic patients.

Our results indicate that ventricular APs are longer in epileptic rats because of a TTX-sensitive increase in I_{NaL} amplitude. Epilepsy also resulted in an increased contribution of TTX-sensitive channels to peak I_{Na}, a reduced activation threshold, and a slower recovery from inactivation. All of these effects on I_{Na} can be explained by an increase in neuronal Na-channel expression levels. Consistent with this possibility, we report here that epilepsy was associated with enhanced expression of the neuronal sodium channel isoform $Na_V 1.1$ in cardiac cells. Overall, our study confirms the important role of neuronal sodium channels in establishing the duration of the cardiac action potential, and our results indicate that epilepsy-related arrhythmias and SUDEP are likely because of enhanced expression of these channels.

Methods

Animal Model

We used the kainic acid (KA)-induced epilepsy model which is the most widely used model to study chronic epilepsy. Briefly, adult male Sprague–Dawley rats weighing between 200 and 250 g were housed 1 per cage on a 14-hour/10-hour light/dark cycle, with free access to food and tap water. Seizures were induced by intraperitoneal injection of KA (12 mg/kg). This proconvulsant agent was injected (intraperitoneally) and the status epilepticus was stopped 2 hours after KA injection with diazepam (25 mg/kg; IP). Status epilepticus is characterized by limbic seizures which start 10 to 15 minutes after KA injection. Only animals showing seizure behavior consisting of rearing or rearing and falling, which correspond to stages 4 to 5 on the Racine scale[25],were used for this study. Control animals (sham) received diazepam and saline in lieu of KA. All animals were used between 36 and 40 days after treatment.

Cell Dissociation

Ventricular myocytes from adult rats were isolated by enzymatic dissociation as previously described.[26,27]

Electrophysiology

APs were measured using the perforated patch technique with amphotericin (Sigma; 6 mmol/L) as the ionophore.[28] For AP measurements, cardiomyocytes were superfused at room temperature with a solution containing (in mmol/L): 126 NaCl, 5.4 KCl, 2.0 $CaCl_2$, 1.0 $MgCl_2$, 20 4-(2-hydroxyethyl)piperazine-1-ethanesulfonic acid (HEPES), and 11 glucose (pH 7.4 with NaOH). Pipette solution contained in mmol/L: 90 K-aspartate, 30 KCl, 10 NaCl, 5.5 glucose, 1.0 $MgCl_2$, 10 ethylene glycol tetraacetic acid (EGTA), 4 Na_2-ATP, 10 HEPES (pH 7.2 with KOH). The I_{to} specific blocker 4-aminopyridine (4 mmol/L) was used where indicated. Tip potential cancellation procedure, methodological considerations, and quality selection criteria are described in the Data Supplement.

I_{Na} was measured at room temperature using the patch clamp technique in voltage-clamp mode as previously described.[27] The extracellular solution contained (in mmol/L): 125 choline-Cl, 2.5 NaCl, 2.5 NaOH, 2.8 Na acetate, 4 KOH, 0.5 $CaCl_2$, 1.5 $MgCl_2$, 20 HEPES, and 10 glucose (pH 7.4 with NaOH). The concentration of Na^+ was reduced to 7.8 mmol/L to prevent loss of voltage control during I_{Na} measurements. Tetraethyl ammonium (5 mmol/L), $CoCl_2$ (1 mmol/L), and $BaCl_2$ (5 mmol/L) were used to block I_{to}, I_{CaL}, and I_{K1} currents, respectively. For I_{NaL} recordings, perfusion solution contained (in mmol/L): 125 NaCl, 5 NaOH, 2.8 Na acetate, 4 KOH, 0.5 $CaCl_2$, 1.5 $MgCl_2$, 20 HEPES, and 10 glucose (pH 7.4 with NaOH). For both I_{Na} and I_{NaL} recordings, the patch pipette (1–3 MΩ) solution contained (in mmol/L): 15 NaCl, 5 KCl, 120 CsF, 1.0 $MgCl_2$, 4 Na_2-ATP, 10 EGTA, and 10 HEPES (pH 7.2 with CsOH). All solutions were adjusted at 300 mOsm with sucrose. Data acquisition and analysis were performed using the pCLAMP program suite V9.2 (Axon instruments).

Dose–Response Curve Analysis

The dose–response curve was obtained using a sum of 2 Langmuir isotherms $I_{Na,TTX}/I_{Na,Ctrl}=f_{TTXs}\{1/(1+[TTX]/IC_{50fTTXs})\}+(1-f_{TTXs})$

{1/(1+[TTX]/$IC_{50/fTTXr}$)} fitted to data. The 50% blocking concentration of TTX-sensitive channels ($IC_{50/fTTXs}$) was determined by fitting data points between 0.1 and 100 nmol/L TTX with the absorption isotherm: $I_{Na,TTX}/I_{Na,Ctrl}=f_{TTXs}$ {1/(1+[TTX]/$IC_{50/f\ TTXs}$)} with f_{TTXs} defined as the fraction of TTX-sensitive channels given by difference between 100% and the value of the plateau observed at 30 nmol/L which blocks essentially all of the TTX-sensitive and almost none of the cardiac isoform of the Na channel (which is blocked at much higher concentrations of TTX). The f_{TTXs} and $IC_{50/fTTXs}$ values were then used as seed value to fit the entire curve keeping the assumption of a 1:1 binding of TTX to all receptors.

Quantitative Real-Time Reverse Transcription Polymerase Chain Reaction

Sodium channel cDNA was measured as previously published.[29,30] Total RNA was isolated (Total RNA Isolation Kit, Ambion) and reverse transcribed (RT) using Superscript II (Invitrogen). One µg of RT-cDNA was used as template for the amplification. Primers specific to each of the rat Na_V isoforms tested were used to amplify fragments. Each amplicon was subcloned into pUC119 for sequencing and for amplification efficiency controls. Real-time PCR was performed using the Rotor-Gene 3000 Cycler from Corbett Research (United States) with the Platinum SYBR Green Kit (Invitrogen). The specificity of the PCR reactions was verified by sequencing of the amplicon. The amount of cDNA in each reaction was calculated by comparing the results with calibration curves obtained by simultaneous amplification of known concentrations of a construct containing the amplicon of each gene in pUC119 using the Comparative Quantification feature of the Rotor-Gene software. β-actin served as an endogenous control for loading of the cDNA sample. Each experiment was repeated 4× for SCN5A and 3× for SCN1A. PCR was performed in triplicate for each sample.

Western Blot Analysis

Myocardial extracts were prepared through homogenization of dissected ventricles (left and right) as described previously.[27,30] Proteins from the plasma membrane, cytosolic, and endosomal fractions were separated by centrifugation at 5000 to 10 000*g*, 20 000*g*, and 100 000*g*, respectively, as previously described by us[27,31] and others,[32] and 100 µg of proteins from the plasma membrane fraction was used in the Western blot assay. Detection was performed using the following primary antibodies at a dilution of 1:200: SP19 antipan sodium channel (ACS-003, Alomone Laboratories), $Na_V1.5$ (ASC-013, Alomone), $Na_V1.1$ (ab24820, Abcam), and Calnexin (Abcam). Horseradish peroxidase–conjugated antirabbit (1:5000; Cell Signaling) was used as secondary antibody.

Statistics

Data are expressed as mean±SEM (SE of the mean). Statistics were performed using a Student *t* test on paired data. Data that could not be paired were pooled. We therefore had 2 variables (factors) such as type of animal and concentration of TTX to take into account and a 2-way ANOVA was performed in those cases. When applicable (Figures 1–3), statistical significance was verified by a second test (Kruskal–Wallis, standard χ^2). In all cases, the second tests confirmed the validity of the 2-way ANOVA.

Study Approval

All animal protocols, care, and maintenance were approved by the ethics review board of the Faculty of Medicine of the Université de Sherbrooke and follow the ARRIVE guidelines (Animal Research: Reporting of In vivo Experiments).

Results

Epileptic patients have longer QT intervals (indicating longer ventricular action potential) which have been associated with SUDEP. As seen in Figure 1, our epileptic rat model reproduced the increased APD observed in human epileptic patients (Figure 1). Application of 4-aminopyridine (4-AP) to eliminate the contribution of the transient outward potassium current (I_{to}) further prolonged the APD in epileptic animals thus confirming an increased contribution of depolarizing currents to APD. We next assessed whether the prolongation of APD during epilepsy was because of an increase in I_{Na} using TTX, a specific blocker of sodium channels that has a much higher affinity for binding and blocking neuronal sodium channels compared with the main cardiac Na channel (Figure 2). TTX was more potent at reducing the APD in epileptic animals. In control conditions and following perfusion with 4-AP, a TTX concentration of 1 nmol/L reduced the APD_{30} (measured at 30% repolarization) in epileptic rats but not in sham cardiomyocytes (Figure 2C). This effect on cardiomyocytes from epileptic rats was more pronounced in the presence of 4-AP, whereas those from sham animals showed no significant reduction in APD_{30} with 1 or 50 nmol/L TTX (Figure 2D). These results indicate an increased contribution of TTX-sensitive sodium channels in cardiomyocytes from epileptic rats.

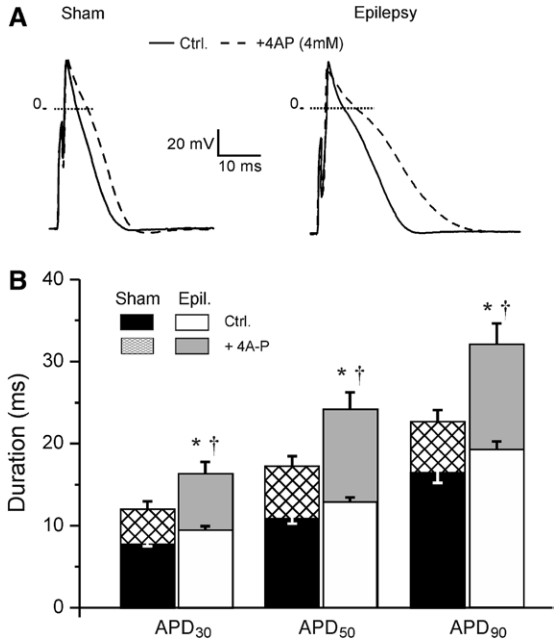

Figure 1. Duration of the cardiac action potential increased during epilepsy. **A**, Typical recordings of cardiac action potentials in cardiomyocytes from sham and epileptic (Epil.) rat in control and after perfusion with 4-aminopyridine (4-AP) to block the contribution of the potassium transient outward current I_{to}. **B**, Augmentation of the action potential duration at 30% (APD_{30}), 50% (APD_{50}), and 90% (APD_{90}) repolarization in sham and epileptic animals in control and after perfusion of 4-AP. Statistical significance (2-way ANOVA): **P*<0.001 (APD Epil. vs sham), †*P*<0.001 (prolongation by 4-AP Epil. vs sham). Sham: n=21, +4-AP: n=15; Epil.: n=22, +4-AP: n=17.

We next compared properties of I_{Na} in sham and epileptic animals (Figure 3). The maximum amplitude of I_{Na} was increased by 18±6%, from −75±5 pA/pF in sham ventricular myocytes to −92±6 pA/pF (Figure 3C) in those from epileptic rats. In addition, myocytes from epileptic rats were more excitable as indicated by a ≈5 mV negative shift in I_{Na} activation threshold (Figure 3B). The mid-activation potentials

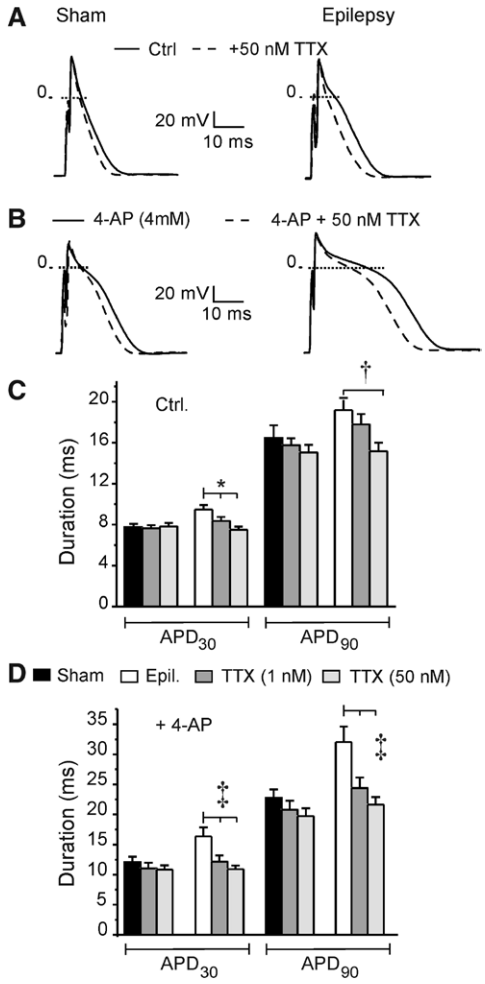

Figure 2. The contribution of tetrodotoxin (TTX)-sensitive currents to the action potential duration (APD) increased during epilepsy. **A**, Typical AP recordings in cardiomyocytes from sham (**left**) and epileptic (Epil., **right**) rat exposed to the specific sodium channel blocker TTX in control conditions. **B**, AP recordings as presented in **A** in presence of 4-aminopyridine (4-AP). **C**, APD at 30% and 90% repolarization after perfusion with TTX. **D**, APD in presence of 4-AP as presented in **C**. Statistical significance (2-way ANOVA): **P*<0.05, †*P*<0.01, ‡*P*<0.001 (Student *t* test on paired values). Number of sham cells: (1 nmol/L TTX) n=15; (50 nmol/L TTX) n=13; (4-AP+1 nmol/L TTX) n=14; and (4-AP+50 nmol/L TTX) n=13. Number of cells from Epil.: (1 nmol/L TTX) n=18; (50 nmol/L TTX) n=19; (4-AP+1 nmol/L TTX) n=18; and (4-AP+50 nmol/L TTX) n=17.

($V_{1/2}$) give a more quantitative value associated with this shift (Figure 3D); this value shifted from −42.8±0.3 mV in myocytes from sham animals to −48.6±0.3 mV in those from epileptic rats. There was no significant difference in the maximum conductance ($G_{Na,Max}$; Figure 3E). These changes indicate that I_{Na} activates at voltages closer to the resting membrane potential during epilepsy indicating greater excitability in ventricular cardiomyocytes from epileptic rats.

We next tested if an increase in the availability of the sodium channels could explain the gain in I_{Na} amplitude associated with epilepsy. However, this does not seem to be the case because the availability of I_{Na} shifted by only 2 mV during epilepsy, from a mid inactivation voltage of −81.8±0.1 mV in sham animals to −83.8±0.1 mV in epileptic animals (Figure 4). Although significant (*P*<0.05, *t* test), this change is in the wrong direction because hyperpolarizing steady-state inactivation will reduce the number of sodium channels available at resting membrane potential. This last result combined with the observations of a shift in activation of the channel without significant changes in the maximum conductance suggests that epilepsy altered the gating of channels responsible for I_{Na} rather than increasing their number. These results combined with data presented in Figure 2 indicate that a shift of the sodium channel population toward TTX-sensitive channels might be responsible for the changes observed in APD and I_{Na}. To test this hypothesis, we assessed the sensitivity of I_{Na} to low doses of TTX.

Figure 5A shows that cardiomyocytes from epileptic rats are more sensitive to TTX. Cells from both sham and epileptic animal show a biphasic TTX dose response with a plateau for concentrations between 30 and 300 nmol/L (Figure 5B).

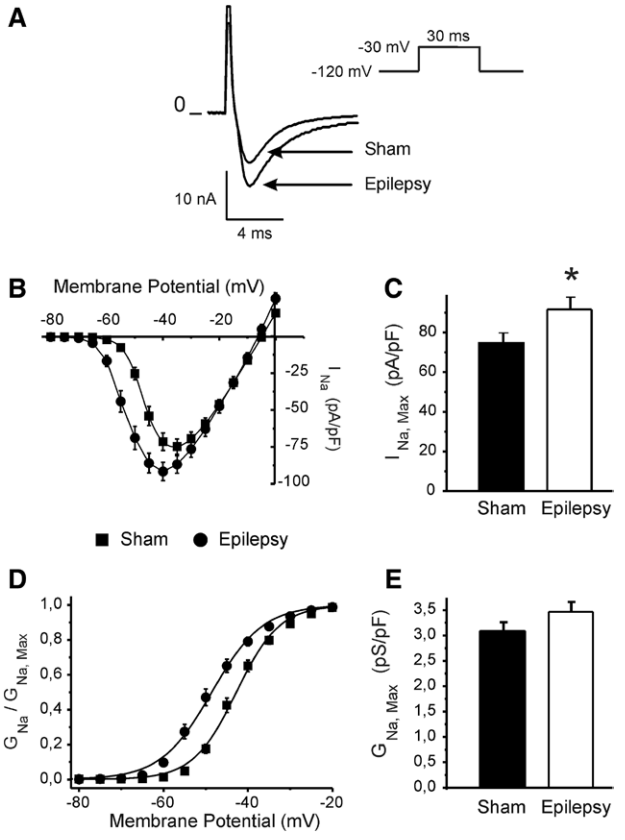

Figure 3. The amplitude of I_{Na} increased during epilepsy. **A**, Typical recordings of I_{Na} in sham and epileptic animals during a depolarization to −30 mV from a holding potential of −120 mV (inset). **B**, Current–voltage relationship following stimulations from −90 to +40 mV in 5-mV increments from a holding potential of −120 mV. Peak currents were normalized to cell capacitance (pA/pF). **C**, Average maximum peak current density. **D**, Voltage dependence of activation of I_{Na}. Sodium conductance G_{Na} was calculated as the ratio of $I_{Na}/(V_m-E_{Na})$, where V_m represents the membrane test potential and E_{Na} represents the reversal potential for I_{Na}. G_{Na} was then normalized to the maximum conductance ($G_{Na,max}$) for each individual cell and data were averaged to yield the activation curve. Data were fitted to a standard Boltzmann distribution. Half-activation potential was hyperpolarized during epilepsy (*P*<0.05, *F* test from ANOVA). **E**, Maximum conductance obtained from the slope of the linear portion of the I/V curve presented in **A**. Sham: n=21. Epileptic: n=35. **P*<0.05 vs sham.

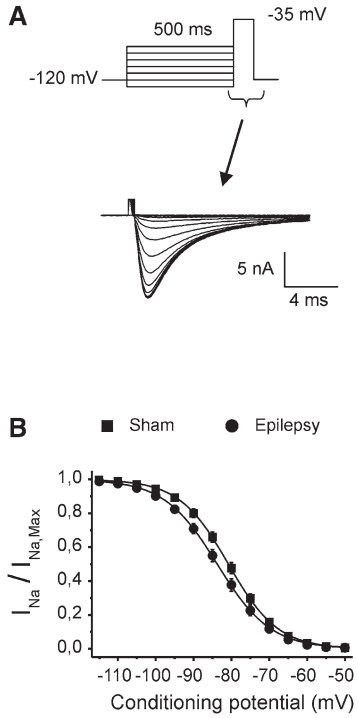

Figure 4. Availability of sodium channels shifted toward more negative voltages during epilepsy. **A**, Standard inactivation protocol consisting of a series of 500-ms inactivating pulses from −130 to 50 mV in increment of 5 mV from a holding potential of −120 mV followed by a 25-ms test pulse to −35 mV to open all available channels. Representative I_{Na} recordings during the test pulse. **B**, Inactivation curve obtained from the ratio ($I_{Na}/I_{Na,Max}$) for each conditioning potential. Data were fitted to a standard Boltzmann distribution. Epilepsy was associated to a shift in availability of channels ($P<0.05$, F test, ANOVA). Number of cells (from 6 epileptic and 3 sham animals)—sham: n=21 and epileptic: n=17.

However, epilepsy was associated with a lower plateau level thus indicating that a larger fraction of Na^+ channels was blocked at low TTX concentrations. To emphasize this aspect of the TTX block, we analyzed our measurements using a sum of 2 Langmuir isotherms (see Methods) assuming a 1:1 binding ratio of TTX to TTX-sensitive (f_{TTXs}) and TTX resistant (f_{TTXr}) receptors. The TTX concentrations of half-maximal block ($IC_{50/TTX}$) obtained from the fit to data were not different between cells from sham and epileptic animals with respective values of 1100 and 1250 nmol/L for the TTX-resistant and 5.3 and 2 nmol/L for the TTX-sensitive channels, respectively. The plateau observed at the lower concentration of TTX in the dose–response curve (Figure 5B) dropped from 76±3% to 65±4% indicating an 11% increase in f_{TTXs} during epilepsy. The increase in the fraction of I_{Na} blocked by low concentration of TTX in epileptic animals is consistent with an augmentation of TTX-sensitive channels (IC_{50} ≈ 2–5 nmol/L)[33] without significant effect of the cardiac sodium channel isoform $Na_V1.5$ (IC_{50} ≈2–5 µmol/L). This result therefore indicates an increased contribution of TTX-sensitive channels to I_{Na}.

The late component of I_{Na} (I_{NaL}) is known to modulate APD. As seen in Figure 6, I_{NaL} was measured by subtracting the current measured at the end of a 300-ms test pulse in the presence of 25 µmol/L TTX (inhibiting all of I_{Na}) from that measured in the absence of TTX. I_{NaL} density increased from 1.5±0.2 pA/pF in sham animals to 2.3±0.3 pA/pF during epilepsy. Application of 1 nmol/L TTX to block TTX-sensitive channels reduced the amplitude of I_{NaL} more importantly in myocytes from epileptic animals (1.12 pA/pF) versus sham (0.52 pA/pF). Following application of 1 µmol/L, the amplitude of I_{NaL} in cells from sham and epileptic animals were reduced to the same level. However, because I_{NaL} amplitude was larger in epileptic rats, TTX reduced I_{NaL} by 75±15% versus 59±16% in sham cells (Figure 6B), thus indicating a larger contribution of TTX-sensitive channels during epilepsy.

TTX-sensitive (brain type) and TTX-resistant (cardiac type) sodium channels differ by their activation voltage. TTX-resistant channels ($Na_V1.5$) are largely activated between −60 and −30 mV, whereas TTX-sensitive channels are not fully activated in that range of potentials.[29,34–36] However, at −10 mV, all channels are activated. Therefore, a contribution of TTX-sensitive channels to I_{NaL} should translate in a smaller amount of block by TTX at −30 mV compared with the one measured at −10 mV. In agreement, Figure 7 shows that 1 nmol/L and 1 µmol/L TTX blocked a larger portion of I_{NaL} at voltages above −30 mV, thus creating an inflection point at −35 mV in the sham current–voltage relationship. Compared with sham myocytes, TTX blocked a larger portion of I_{NaL} at −30 mV in epileptic rats but blockade remained more pronounced at −10 mV, consistent with an increased contribution of TTX-sensitive channels.

Previous studies have reported that epilepsy increases the amplitude of I_{NaL} in rat brains[37–39] by enhancing expression of TTX-sensitive channels, including $Na_V1.1$ in rat hippocampus.[17,18,40] To verify if epilepsy was also associated with an

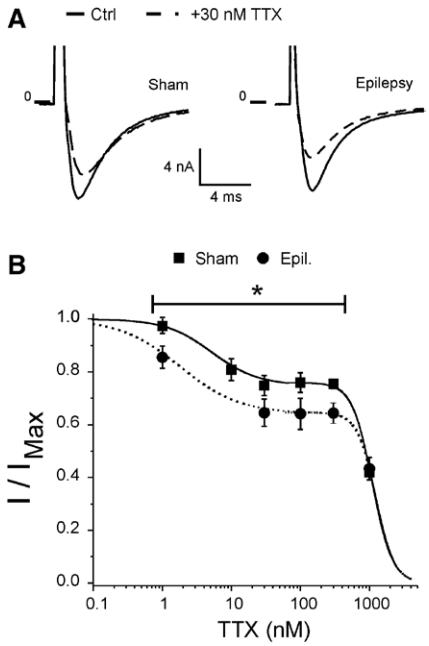

Figure 5. Peak sodium current is more sensitive to tetrodotoxin (TTX) in epileptic heart. **A**, Representative current recordings of the sodium current in rat cardiomyocytes following application of 30 nmol/L TTX. **B**, Dose–response curve for the effect of TTX on I_{Na}. A sum of 2 Hill equations fitted to data yielded respective Kd of 1100 and 5.3 nmol/L in sham cells and 1250 and 2 nmol/L in epileptic (Epil.) rat myocytes. Statistical significance (t test): *$P<0.05$ (Epil. vs sham). Sham: n=8; Epil.: n=8.

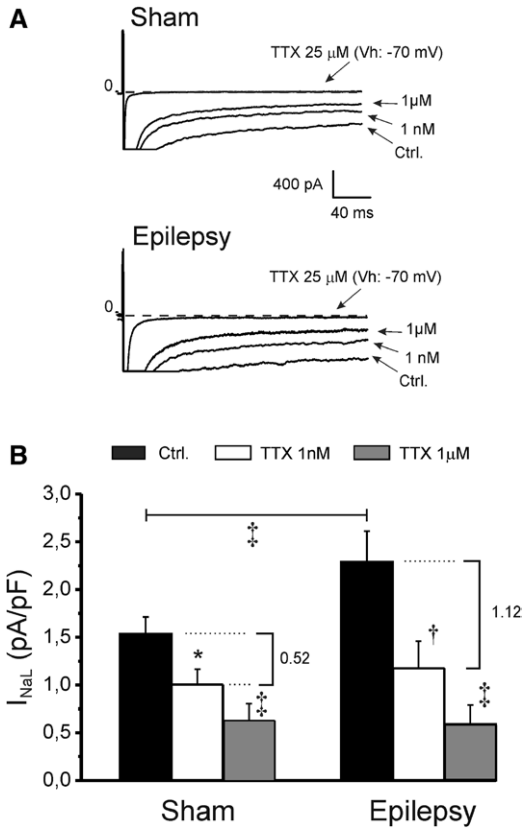

Figure 6. Contribution of tetrodotoxin (TTX)-sensitive sodium channels to I_{NaL} was increased during epilepsy. Late sodium current (I_{NaL}) was measured as the amplitude of the current blocked by TTX 300 ms after the start of test pulses between −100 mV and +10 mV elicited from a holding potential of −120 mV. **A**, Representative I_{NaL} recordings during a test pulse to −10 mV in control (Ctrl.) and after sequential addition of 1 nmol/L and 1 μmol/L TTX to the perfusate. Baseline was obtained by depolarizing the holding membrane potential to −70mV and adding 25 μmol/L TTX. **B**, Average maximum I_{NaL} density (per capacitance of the cell) for conditions illustrated in **A**. I_{NaL} amplitude was obtained by subtraction of baseline from each recording. Statistical significance (*t* test): **P*<0.05, †*P*<0.01, ‡*P*<0.001. Number of cells (from 4 animals) sham: n=13, epileptic: n=12.

increased expression of $Na_V1.1$ in the heart, we measured the amount of SCN1A and SCN5A cDNA, respectively, coding for the α-subunit of $Na_V1.1$ and $Na_V1.5$. Quantitative real-time RT-PCR experiments (Figure 8A) using specific primers to each gene show a significant increase (49%) in expression of the $Na_V1.1$ cDNA in hearts from epileptic animals. Surprisingly, cDNA for $Na_V1.5$ was however reduced by 54% during epilepsy. However, no difference in expression of either gene was observed between sham-operated and control animals (no injections). To correlate these results with changes in expression of sodium channels, we performed Western blot experiments on proteins extracted from the plasma membrane of ventricular myocytes. Figure 8B shows that overall expression of sodium channel proteins (SP19), including $Na_V1.5$ and $Na_V1.1$, was increased during epilepsy. Changes in the expression of sodium channels were determined by densitometric measurements of each band and comparison with the loading control calnexin (Figure 8C). Detection with the antipan SP19 antibody showed a trend to increase overall expression of sodium channels but the difference between sham and epileptic animals did not reach a significance level (*P*=0.06) within the number of animals used (n=6). However, expression of $Na_V1.1$ and $Na_V1.5$ was significantly increased in epileptic animals.

Conduction block is a frequent occurrence in epilepsy and can be caused by an increase in the heart refractory period because of delayed recovery of I_{Na} from inactivation. In agreement, we found that epilepsy was associated with a longer I_{Na} recovery (*P*<0.05, Figure in the Data Supplement). A sum of 2 exponentials fitted to data yielded time constants of 14.0±0.2 ms (85%) and 63.2±8 ms (15%) in sham animals and 18.4±0.2 ms (84%) and 119±6 ms (14%) in epileptic rats. This result indicates that an increased refractory period may contribute to conduction anomalies during epilepsy.

Discussion

Our measurements indicate that epileptic conditions increase I_{Na} peak amplitude by 18±6% and hyperpolarized its threshold voltage by 6 mV in cardiac ventricles. These changes are strikingly similar to the findings of Guo et al[18] who reported a 16% increase and a negative shift of 7 mV in steady state activation of I_{Na} in the hippocampus of epileptic rats. In brain, these gating alterations were mostly attributed to enhanced expression of nNa_Vs isoforms, $Na_V1.1$ and $Na_V1.2$.[17,40] Results in this article combined with our earlier study showing the presence of neuronal TTX-sensitive sodium channels within the heart[41,42] led to our hypothesis that a common mechanism is responsible for increasing the expression of nNa_Vs in both brain and heart during epilepsy.

Our RT-PCR and Western blot analysis confirmed that both cDNA and protein expression of $Na_V1.1$ are enhanced in the heart. This direct correlation between the change in mRNA and protein level suggests that epilepsy is acting at the genomic level to promote expression of neuronal channels. One puzzling observation (Figure 8) was a decreased amount of $Na_V1.5$ mRNA but a doubling of the expressed protein in cells from epileptic animals. One potential explanation is that reduction in $Na_V1.5$ mRNA is compensated by a slower internalization (recycling) of the mature proteins expressed at the surface of cardiomyocytes. Thus, epilepsy may act by directly altering gene expression and protein trafficking, perhaps by prolonging $Na_V1.5$ half-life at the plasma membrane. $Na_V1.2$ is another neuronal channel involved in epilepsy, however, it does not seem to be expressed in the cardiac ventricle of rodents.[42–44] Consistent with these observations, we could not detect $Na_V1.2$ mRNA in rat ventricle (data not shown).

Our measurements with the SP19 antibody indicated a trend to increase overall expression of sodium channels (*P*<0.06) in the plasma membrane (Figure 8B). This translated into a rather modest augmentation of I_{Na} peak current (Figure 3A–3C). Although the semiquantitative nature of Western blot measurements preclude a direct correlation with the number of functional proteins participating to I_{Na}, these results nonetheless suggest that cellular mechanisms altering the gating of other Na_Vs are contributing to limit the increase in I_{Na} during epilepsy. Moreover, epilepsy did not change I_{Na} maximal conductance $G_{Na,Max}$, thus indicating that the overall number of channels contributing to I_{Na} remained

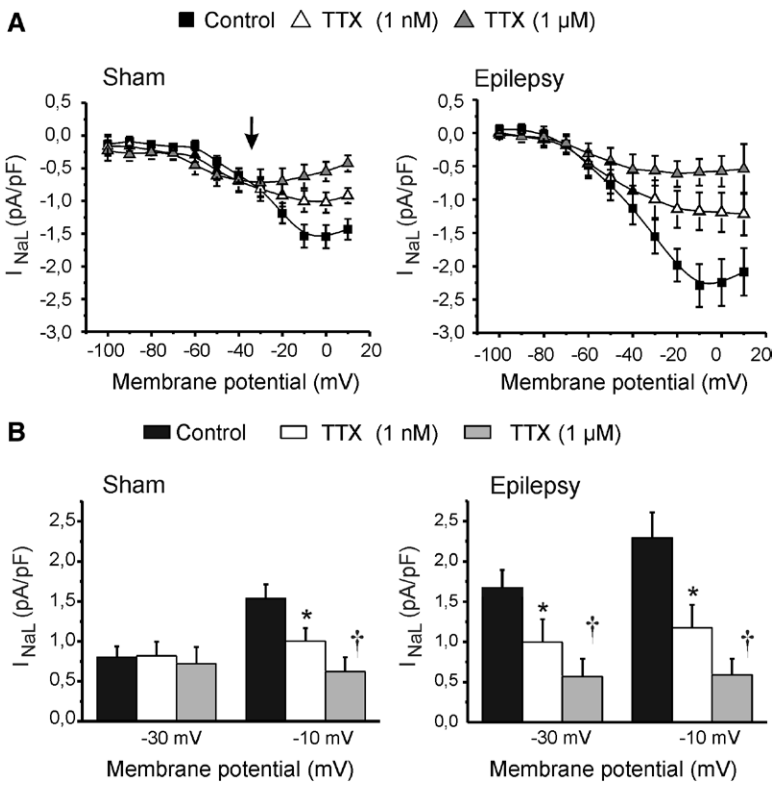

Figure 7. Tetrodotoxin (TTX)-sensitive channels increase the amplitude of I_{NaL} at negative membrane potentials. I_{NaL} was measured as described in Figure 6. **A**, Current–voltage (I/V) relationship for I_{NaL} in sham (**left**) and epileptic (**right**) cardiomyocytes after sequential perfusion of 1 nmol/L and 1 µmol/L TTX. An inflection point at –30 mV (arrow) in sham I/V relationship indicates 2 populations of channels with different sensitivities to TTX. In epileptic rats, the larger contribution of TTX-sensitive channels masks the inflection point at –30 mV observed in sham cardiomyocytes. **B**, Quantitative evaluation of the TTX block at –30 mV and –10 mV. Statistical significance (*t* test): **P*<0.01, †*P*<0.001, vs control. Sham: n=13. Epileptic: n=12.

the same. Changes in activation voltage (Figure 3) because of enhanced expression of sodium channels having slightly different biophysical properties may, in part, explain the increase in I_{Na} amplitude, a hypothesis supported by our results showing that epilepsy is associated with increased expression of TTX-sensitive channels. Recent findings also suggest that phosphorylation of sodium channels by calmodulin kinase II (CaMKII) also contribute to modulation of I_{Na} amplitude in epileptic brain.[40] Whether a similar mechanism also exists in epileptic heart remains to be determined.

Despite the absence of alteration in $G_{Na,Max}$ arguing against a net increase in the total number of sodium channels participating to I_{Na}, the TTX dose–response curve we obtained clearly indicate an augmentation of the contribution of TTX-sensitive channels during epilepsy. At first glance, the contribution of TTXs-Na_Vs to I_{Na} during epilepsy seems relatively modest (≈25%, Figure 5B) and makes it difficult to conceive that this alone could trigger cardiac arrhythmias ultimately leading to SUDEP. However, TTX-sensitive channels contribute much more significantly to the late sodium current.[41] This larger contribution to I_{NaL} can be explained from the fact that the fraction of persistent current to peak current is 10 times larger for TTXs-Na_Vs compared with $Na_V1.5$; ranging from 0.05 to 0.13 for TTXs-Na_Vs and 0.002 to 0.005 for $Na_V1.5$.[10,41,45–48]

Our study revealed that I_{NaL} increased by 30±2% during epilepsy. Consistent with the contribution of TTXs-Na_Vs to I_{Na} peak, application of 1 nmol/L TTX (a concentration ≈1000-fold less than the Kd for $Na_V1.5$ channels) blocked 49±3% of I_{NaL} in epileptic rat myocytes versus 35±2% in sham cells (Figure 6). Because of the larger I_{NaL} in epileptic animals, this translated into a doubling of the density of the TTX-sensitive current from 0.52 pA/pF in sham to 1.12 pA/pF in epileptic rat myocytes. With 1 µmol/L TTX, the amplitude of I_{NaL} in sham and epileptic rat myocytes were reduced by 59±16% and 75±15%, respectively, but the final amplitudes were not different, indicating no change in the number of TTX-resistant Na channels because only TTX-resistant channels contribute to I_{NaL} after application of 1 µmol/L TTX. Further support for an important contribution of TTXs-Na_Vs to I_{NaL} during epilepsy comes from the high sensitivity of I_{NaL} to TTX in a range of membrane potentials where TTX-s Na_Vs are maximally activated (V_m > –30 mV, Figure 7). In summary, the I_{NaL} measurements are consistent with an enhanced expression of TTX-sensitive Na_Vs and no change in TTX-resistant Na_Vs.

The important role of I_{NaL} in regulating repolarization time and QT interval is exemplified by studies of long-QT syndrome[10–12] or the use of ranolazine (a I_{NaL} blocker) to treat arrhythmias in myocardial ischemia and atrial fibrillation[13–15,49] and early experiment showing that specific sodium channel blocker TTX shortens normal APD.[16] Severe prolongation of the ventricular repolarization time is associated with a longer QT interval on the ECG and lethal torsade de pointe arrhythmias as well as conduction anomalies all of which can result in sudden death. Our results show that epilepsy correlated with an increase in I_{NaL} amplitude which likely explained the prolongation of the APD_{30} by ≈20% (Figure 1). In human, a similar effect would increase the APD from a standard value of 440 to 528 ms, the latter well within the arrhythmogenic range, which would explain the epilepsy-induced prolongation of the QT interval observed in humans.[6,7,50] We were able to demonstrate that epilepsy was associated with an increase in I_{NaL} contributing to the plateau of the action potential. This was confirmed by application of TTX at low concentration

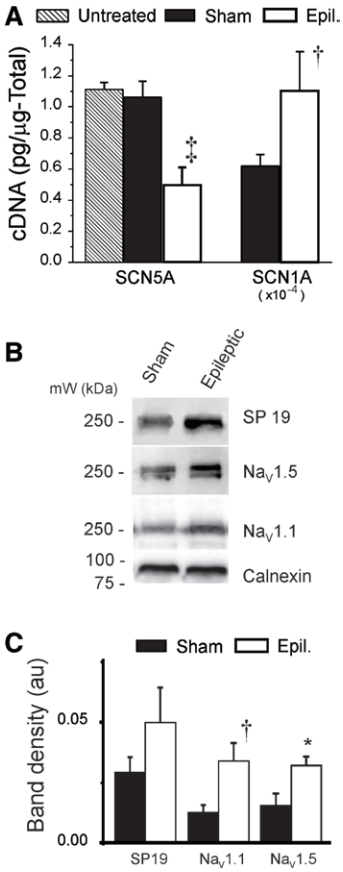

Figure 8. Expression of the neuronal sodium channel isoform $Na_V1.1$ (SCN1A) is increased during epilepsy. **A**, Quantitative real-time reverse transcription polymerase chain reaction measurements of reversed transcribed cDNA for the cardiac sodium channel isoform $Na_V1.5$ and the TTX-sensitive channel $Na_V1.1$ in untreated, sham, and epileptic animals. Statistical significance (ANOVA): ***P*<0.01, ****P*<0.001; epileptic vs sham. Number of samples (animals): sham, 4; control, 3; and epileptic, 4. Each experiment was repeated in triplicate. **B**, Representative Western blot assay on ventricular plasma membrane proteins showing the total amount of sodium channels (SP19) and expression of the $Na_V1.5$ and $Na_V1.1$ channels in sham and epileptic rats. Calnexin was used as loading controls. **C**, Averaged density ratio obtained by normalizing the intensity of each sodium channel band to calnexin. Statistical significance (ANOVA): **P*<0.05, †*P*<0.01, ‡*P*<0.001 (data±SEM, epileptic vs sham).

which produced a greater reduction in the APD in epileptic rat cells compared with control cell (Figure 6).

Conduction anomalies are also observed in epilepsy.[3,5–7] Conduction velocity and the ventricular contraction rate depend on the time needed for I_{Na} to recover from inactivation between APs, the so-called refractory period of the heart. Prolongation of I_{Na} recovery time (Figure in the Data Supplement) because of changes in the biophysical properties of the sodium channels will prolong the refractory period and may lead to conduction anomalies such as skipped heartbeat or bradycardia similar to the ones observed during epilepsy. Longer APDs allowing a greater fraction of sodium channels to become inactivated will further potentiate the influence of I_{Na} recovery on conduction. Our results show that I_{Na} recovery from inactivation is slower in epileptic animals. Therefore, the combined effects of epilepsy on APD and the slower recovery of I_{Na} are likely to increase the risk of conduction disturbances during epilepsy.

We show for the first time that epilepsy alters cardiac I_{Na} and I_{NaL} in a manner consistent with QT prolongation and conduction anomalies observed clinically. Previous studies have shown that epileptic conditions enhance expression of $Na_V1.1$ and its contribution to I_{NaL} in the brain hippocampus. A new finding also reported here is that overexpression of $Na_V1.1$ in epileptic cardiomyocytes may also play a role in these effects on I_{NaL}. This suggests that similar but yet unknown adaptation mechanisms to epilepsy exist in the brain and the heart. Our results also raise the possibility that systemic changes in neurotransmitter levels or other signaling process during status epilepticus are acting as a trigger for overexpression of TTXs-Na_Vs in both tissues and this contributes to cardiac arrhythmias observed during epilepsy in humans.[17,18] Based on our findings, we propose a new paradigm whereby an increased contribution of neuronal sodium channels alters the conduction properties of cardiomyocytes and the cardiac ventricular action potential duration both of which can contribute to the known risk of epileptic patients to SUDEP.

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Disclosures

None.

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Prolongation of Action Potential Duration and QT Interval During Epilepsy Linked to Increased Contribution of Neuronal Sodium Channels to Cardiac Late Na^+ Current: Potential Mechanism for Sudden Death in Epilepsy

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SUPPLEMENTAL MATERIAL

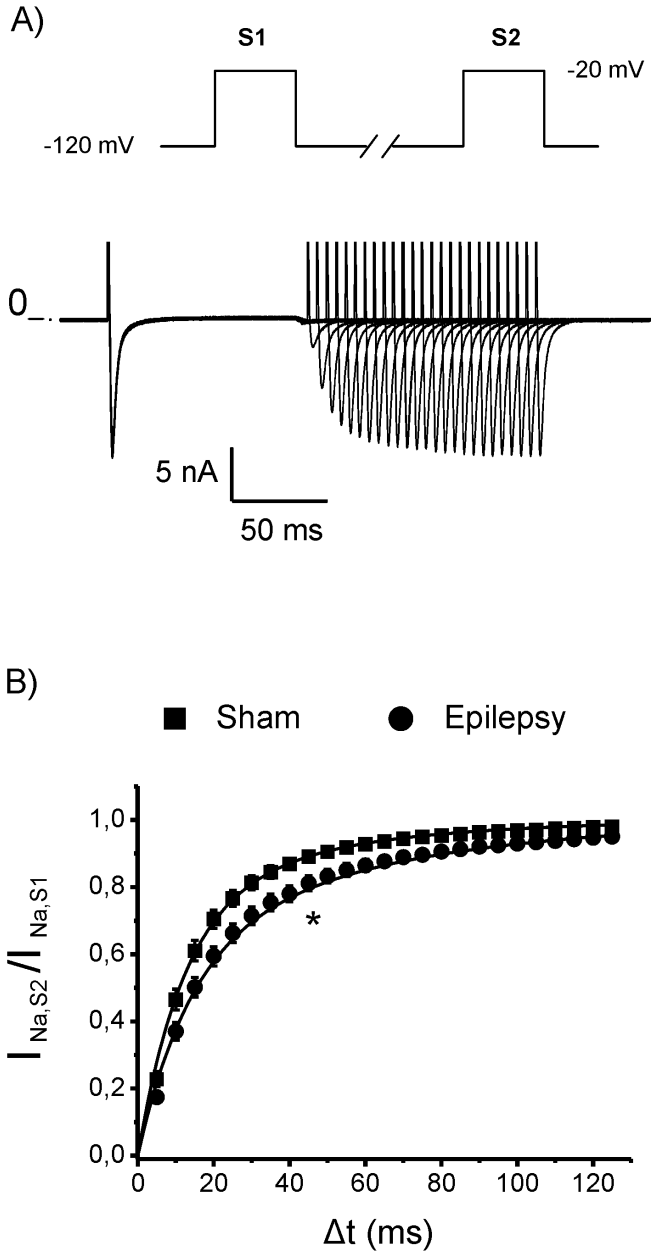
Supplemental methods

Animal model: Almost all rats experiencing SE for at least 60 min after injection of kainic acid developed chronic epilepsy. Average latency to the first spontaneous seizure was approximately 37 days. Animals showing spontaneous seizures were sacrificed 50 to 60 days after the initial SE and compared to sham animals to evaluate the progression of SE and its effect on the parameters studied.

Cell dissociation: Briefly, whole hearts were perfused at 35°C through the aorta for 10 minutes with Ca-free Tyrode solution supplemented with EGTA 2 mmol/l and 0.1% of BSA. Perfusion was switched to Tyrode solution containing 0.1 mmol/L Ca and 230 U/ml collagenase (CLS 2, Worthington, Freehold, NJ) and recirculated for 10-20 minutes until the tissue became discolored and mushy. The heart was then removed and both ventricles (without the atria) were minced and gently stirred in beakers containing the enzymatic solution. The supernatant containing dissociated cells was kept in 10 ml tubes and stored in Krebs solution containing (in mmol/l): 100 Potassium glutamate, 10 Potassium Aspartate, 25 KCl, 10 KH_2PO_4, 2 $MgSO_4$, 20 Taurine, 5 Creatine, 0.5 EGTA, 20 Glucose, 10 HEPES, 2% BSA, supplemented with 0.2 mmol/L $CaCl_2$.

Electrophysiology: For whole cell patch clamp measurements, tip potential, typically between 9 and 15 mV was nulled before seal formation. Recordings were acquired at 10 kHz and filtered online at 5 kHz (Bessel filter). Electrical stimuli were given at a rate of 1 per 15s to avoid use-dependent block by TTX. All recordings were obtained at room temperature (22°C) using an Axopatch 200B amplifier (Axon instruments, Union City CA) equipped with a CV-201A head stage (Axon Instruments, Foster City, CA). Whole cell capacitance and series resistance compensation (85%) were optimized to minimize the capacitive artifact and reduce voltage errors. For AP recordings, only cells displaying a resting membrane potential (Vr) between -90 mV and -70 mV in current clamp (I=0) were selected for the experiments (average Vr: -92 ± 10mV; n=17). Membrane potential was maintained at -100 mV in current clamp during recordings and series of 2 ms pulses of varying intensity in increments of 0.01 nA were applied at a frequency of 0.1 Hz to determine their voltage threshold. Parameters were measured on fully activated AP.

Supplemental Figure 1



Supplemental Figure 1. I_{Na} recovery from inactivation is slower during epilepsy. **A)** Standard double pulse protocol (S1-S2) to -20 mV with incremental recovery time interval (Δt=5 ms). **B)** Recovery from inactivation curves obtained from the ratio of I_{Na} amplitudes (S2/S1) plotted against Δt. Number of cells: Sham n=22, Epileptic n=33. Data were fitted to a sum of two exponential. * $p < 0.05$ (F-test, comparison of time constants for fit to data. see text).