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_{V}1.1 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 seizures2-4 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 arrhythmias1 associated with different conditions, including conduction block1,3,7 and prolongation of the QT interval on the ECG,6,8 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_{V}s) 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 arrhythmias13,15 and by early experiments showing that application of low concentrations of the sodium channel blocker tetrodotoxin (TTX) shortens APD.16 This latter result
WHAT IS KNOWN

- Human mortality associated with epilepsy in the United States and Canada is 21% to 22%. Sudden unexpected death during epilepsy accounts for 17% of all epilepsy-related fatalities 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 (nNaVs).
- Overexpression of the nNaVs 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 (NaV1.5).

Recent findings indicate that expression of neuronal sodium channel isoforms (nNaVs) in the rat hippocampus increases during epilepsy. This enhanced expression of TTX-sensitive NaVs is thought to potentiate seizures by increasing INaL and by also increasing excitability in brain cells. Missense mutations in NaV1.1 and NaV1.2 that increase INaL amplitude in hereditary epilepsy seem to confirm this hypothesis. However, epilepsy patients display prolonged QT interval and enhanced expression of NaV1.1 even in absence of mutations in neuronal NaVs. These observations raise the possibility that, as in the brain, epilepsy enhances expression of TTX-sensitive NaVs in cardiac cells thereby modifying their excitability and increasing INaL. In the heart, we showed that an increase in INaL is associated with long-QT syndrome and sudden death. In this study, we wanted to test the hypothesis that upregulation of nNaVs 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 INaL amplitude. Epilepsy also resulted in an increased contribution of TTX-sensitive channels to peak INa,L a reduced activation threshold, and a slower recovery from inactivation. All of these effects on INa,L 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 NaV1.1 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 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.

Electrophysiology

APs were measured using the perforated patch technique with amphotericin (Sigma; 6 mmol/L) as the ionophore. For AP measurements, cardiomyocytes were superfused at room temperature with a solution containing (in mmol/L): 126 NaCl, 5.4 KCl, 2.0 CaCl2, 1.0 MgCl2, 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 MgCl2, 10 ethylene glycol tetraacetic acid (EGTA), 4 Na2-ATP, 10 HEPES (pH 7.2 with KOH). The INa 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.

INa was measured at room temperature using the patch clamp technique in voltage-clamp mode as previously described. The extracellular solution contained (in mmol/L): 125 choline-Cl, 2.5 NaCl, 2.5 NaOH, 2.8 Na acetate, 4 KOH, 0.5 CaCl2, 1.5 MgCl2, 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 INa measurements. Tetraethyl ammonium (5 mmol/L), CoCl2 (1 mmol/L), and BaCl2 (5 mmol/L) were used to block Ito, ICaL, and INa currents, respectively. For INa recordings, perfusion solution contained (in mmol/L): 125 NaCl, 5 NaOH, 2.8 Na acetate, 4 KOH, 0.5 CaCl2, 1.5 MgCl2, 20 HEPES, and 10 glucose (pH 7.4 with NaOH). For both INa and INa,L recordings, the patch pipette (1–3 MΩ) solution contained (in mmol/L): 15 NaCl, 5 KCl, 120 CsF, 1.0 MgCl2, 4 Na2-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: 

$$I_{Na,TTX} = I_{Na,Ctrl} \times \frac{1}{1 + \frac{[TTX]}{IC_{50,TTX}}} + \frac{1}{1 + \frac{[TTX]}{IC_{50,TTX}}}$$

where $I_{Na,TTX}$ is the sodium current in the presence of TTX, $I_{Na,Ctrl}$ is the sodium current in control solution, [TTX] is the TTX concentration, and IC50,TTX is the half-maximal inhibitory concentration for TTX.
Quantitative Real-Time Reverse Transcription Polymerase Chain Reaction

Sodium channel cDNA was measured as previously published.\textsuperscript{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\textsubscript{i} isoforms were tested to use 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 (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 amplification product 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.\textsuperscript{27,31} Proteins from the plasma membrane, cytosolic, and endosomal fractions were separated by centrifugation at 5000 to 10000 g, 20000 g, and 100000 g, respectively, as previously described by us\textsuperscript{27,31} and others.\textsuperscript{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\textsubscript{i},1.5 (ASC-013, Alomone), Na\textsubscript{i},1.1 (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\textsuperscript{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\textsubscript{o}) 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\textsubscript{o} 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\textsubscript{i,10} (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\textsubscript{i,50} 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.

We next compared properties of I\textsubscript{o} in sham and epileptic animals (Figure 3). The maximum amplitude of I\textsubscript{o} 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\textsubscript{o} activation threshold (Figure 3B). The mid-activation potentials

\[
\frac{1}{\text{TTXs}} \left(\frac{1}{1+(\text{TTX})/\text{IC}_{\text{50/TTXs}}}\right)
\]

was determined by fitting data points between 0.1 and 100 nmol/L TTX with the absorption isotherm: I\textsubscript{Na,TTX}/I\textsubscript{Na,Ctrl} = \frac{f_{\text{TTXs}}}{(1+1/(\text{TTX}/\text{IC}_{\text{50/TTXs}}))}, with f\textsubscript{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\textsubscript{TTXs} and IC\textsubscript{50/TTXs} 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.
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(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 INa 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 INa amplitude associated with epilepsy. However, this does not seem to be the case because the availability of INa 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).
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 (\( f_{\text{TTXs}} \)) and TTX resistant (\( f_{\text{TTXr}} \)) receptors. The TTX concentrations of half-maximal block (IC50/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 \( f_{\text{TTXs}} \) during epilepsy. The increase in the fraction of INa blocked by low concentration of TTX in epileptic animals is consistent with an augmentation of TTX-sensitive channels (IC50 ≈ 2–5 nmol/L)33 without significant effect of the cardiac sodium channel isoform NaV1.5 (IC50 ≈ 2–5 μmol/L). This result therefore indicates an increased contribution of TTX-sensitive channels to INa.

The late component of INa (INaL) is known to modulate APD. As seen in Figure 6, INaL 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 INa) from that measured in the absence of TTX. INaL density increased from 1.5±0.2 pA/pF in sham animals to 2.3±0.3 pA/pF during epilepsy. Application of 1 mmol/L TTX to block TTX-sensitive channels reduced the amplitude of INa 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 INa in cells from sham and epileptic animals were reduced to the same level. However, because INa amplitude was larger in epileptic rats, TTX reduced INa 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 (NaV1.5) are largely activated between −60 and −30 mV, whereas TTX-sensitive channels are not fully activated in that range of potentials.29–36 However, at −10 mV, all channels are activated. Therefore, a contribution of TTX-sensitive channels to INa 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 mmol/L and 1 μmol/L TTX blocked a larger portion of INa 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 INa 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 INaL in rat brains37–39 by enhancing expression of TTX-sensitive channels, including NaV1.1 in rat hippocampus.17,18,40 To verify if epilepsy was also associated with an
increased expression of NaV1.1 in the heart, we measured the amount of SCN1A and SCN5A cDNA, respectively, coding for the α-subunit of NaV1.1 and NaV1.5. Quantitative real-time RT-PCR experiments (Figure 8A) using specific primers to each gene show a significant increase (49%) in expression of the NaV1.1 cDNA in hearts from epileptic animals. Surprisingly, cDNA for NaV1.5 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 NaV1.2, NaV1.5, and NaV1.1.17,40 Results in this article combined with our earlier study showing the presence of neuronal TTX-sensitive sodium channels within the heart41,42 led to our hypothesis that a common mechanism is responsible for increasing the expression of nNaVs in both brain and heart during epilepsy.

Our RT-PCR and Western blot analysis confirmed that both cDNA and protein expression of NaV1.1 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 NaV1.5 mRNA but a doubling of the expressed protein in cells from epileptic animals. One potential explanation is that reduction in NaV1.5 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 NaV1.5 half-life at the plasma membrane. NaV1.2 is another neuronal channel involved in epilepsy, however, it does not seem to be expressed in the cardiac ventricle of rodents.33-44 Consistent with these observations, we could not detect NaV1.2 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 NaVs 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
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-NaVs 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-NaVs compared with NaV1.5; ranging from 0.05 to 0.13 for TTXs-NaVs and 0.002 to 0.005 for NaV1.5.10,41,45–48

Our study revealed that $I_{NaL}$ increased by 30±2% during epilepsy. Consistent with the contribution of TTXs-NaVs to $I_{NaL}$ peak, application of 1 nmol/L TTX (a concentration $≈$1000-fold less than the Kd for NaV1.5 channels) blocked 49±3% of $I_{Na}$ 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 $I_{Na}$ after application of 1 μmol/L TTX. Further support for an important contribution of TTXs-NaVs to $I_{NaL}$ during epilepsy comes from the high sensitivity of $I_{NaL}$ to TTX in a range of membrane potentials where TTX-s NaVs are maximally activated ($V_m > -30 mV$, Figure 7). In summary, the $I_{NaL}$ measurements are consistent with an enhanced expression of TTX-sensitive NaVs and no change in TTX-resistant NaVs.

The important role of $I_{NaL}$ in regulating repolarization time and QT interval is exemplified by studies of long-QT syndrome10–12 or the use of ranolazine (a $I_{NaL}$ blocker) to treat arrhythmias in myocardial ischemia and atrial fibrillation13–15,49 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 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 7.** Tetrodotoxin (TTX)-sensitive channels increase the amplitude of $I_{Na}$ 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 mask 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.
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.1,5–7 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 \( I_{Na,L} \) in a manner consistent with QT prolongation and conduction anomalies observed clinically. Previous studies have shown that epileptic conditions enhance expression of NaV1.1 and its contribution to \( I_{Na,L} \) in the brain hippocampus. A new finding also reported here is that overexpression of NaV1.1 in epileptic cardiomyocytes may also play a role in these effects on \( I_{Na} \). 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-NaVs 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.

References


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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$_2$PO$_4$, 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. $I_{Na}$ recovery from inactivation is slower during epilepsy. A) Standard double pulse protocol (S1-S2) to -20 mV with incremental recovery time interval ($\Delta t=5$ ms). B) Recovery from inactivation curves obtained from the ratio of $I_{Na}$ amplitudes (S2/S1) plotted against $\Delta t$. 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).