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CHAPTER 2

Sodium currents in isolated rat CA1 pyramidal and dentate granule neurons in the post-Status Epilepticus model of epilepsy

S.O.M. Ketelaars, J.A. Gorter, E.A. van Vliet, F.H. Lopes da Silva and W.J. Wadman

ABSTRACT

Status epilepticus (SE) was induced in the rat by long-lasting electrical stimulation of the hippocampus. After a latent period of one week, spontaneous seizures occurred which increased in frequency and severity in the following weeks, finally culminating after three months in a chronic epileptic state. In these animals we determined the properties of voltage-dependent sodium currents in acutely isolated CA1 pyramidal neurons and dentate granule cells using the whole-cell voltage-clamp technique.

The conductance of the fast transient sodium current was smaller in SE rats (control: 84 ± 7 nS versus SE: 56 ± 6 nS) but related to a difference in cell size so that the neurons had a similar specific sodium conductance (control: 7.8 ± 0.8 nS/pF, SE: 6.7 ± 0.8 nS/pF). Current activation and inactivation were characterized by a Boltzmann function. After SE the voltage dependence of activation was shifted to more negative potentials (control: -45.1 ± 1.4 mV, SE: -51.5 ± 2.9 mV, P < 0.05). In combination with a small shift in the voltage dependence of inactivation to more depolarized potentials (control: -68.8 ± 2.3 mV, SE: -66.3 ± 2.3 mV), it resulted in a window current that was much increased in the SE neurons (median: 64 pA in control, 217 pA in SE, P < 0.05). The peak of this window current shifted to more hyperpolarized potentials (control: -44 mV, SE: -50 mV, P < 0.05). No differences were found in the sodium currents analyzed in dentate granule cells of control and SE animals.

The changes observed in CA1 neurons after SE contribute to enhanced excitability in particular when membrane potential is near firing threshold. They can, at least partly, explain the lower threshold for epileptic activity in SE animals. The comparison of CA1 with DG neurons in the same rats demonstrates a differential response in the two cell types that participated in very similar seizure activity.
INTRODUCTION

The hippocampus is strongly involved in the generation and maintenance of seizures underlying mesial temporal lobe epilepsy (mTLE) (Fountain et al., 1998; Gibbs III et al., 1997; Lothman et al., 1989). In the rat status epilepticus (SE) can be induced by long-lasting electrical stimulation of the hippocampus (Lothman et al., 1989; Lothman et al., 1991). This experimental animal model shares many features with human mTLE. Spontaneous recurrent seizures occur after a latent period of about 10 days. The most conspicuous histological changes consist of neuronal loss and gliosis in the CA regions of the hippocampus, the hilus and layer III of the entorhinal cortex (EC) (Lothman and Bertram III, 1993) and of mossy fiber sprouting in the dentate gyrus (DG) (Aronica et al., 2000). Physiologically, this electrically-induced post-SE model is typified by enhanced excitability and reduced inhibition in several (para)hippocampal regions such as the CA1 and the DG (Lothman et al., 1995; Mangan et al., 1995).

These physiological features can be due to synaptic changes as described by Lothman et al. in the hippocampal CA1 region and—to a lesser extent— in the DG region of SE rats (Lothman et al., 1995; Mangan et al., 1995). In addition, changes in excitability may be determined by modifications of intrinsic membrane properties (Hochman et al., 1999; Vreugdenhil, M. et al., 1998a; Vreugdenhil, M. and Wadman, 1995). Alterations in membrane ionic conductances can lead to an enhancement of cellular excitability and, ultimately, to the generation of epileptiform activity in the network (Davies, 1995; Dichter and Ayala, 1987; Lopes da Silva and Wadman, 1999; Vreugdenhil, M. and Wadman, 1995).

The main excitatory voltage dependent current is the sodium current, which controls the upstroke of the action potential (Hodgkin and Huxley, 1952). In excitable cells, voltage-gated sodium channels determine the duration and frequency of repetitive neuronal firing (Hille, 1992; Urenjak and Obrenovitch, 1996) and therefore constitute a prominent factor in the generation and/or spread of abnormal discharges and epileptic seizures (Sashihara et al., 1994). The importance of the sodium current for neuronal stability is also reflected in the fact that a large class of anti-epileptic drugs interact with it (Davies, 1995; Rogawski and Porter, 1990). In this study we investigated whether changes in voltage-gated sodium currents could be related to the epileptogenesis in the electrically-induced post-SE model of epilepsy. We analyzed the sodium currents of the principal neurons of both main networks that can be distinguished in the hippocampus, the CA1 and the DG. We used the preparation of acutely isolated neurons and employed the patch-clamp technique in whole-cell voltage-clamp mode.
Chapter 2

EXPERIMENTAL PROCEDURES

Animals and electrode implantation

Eleven adult male Sprague-Dawley rats (Harlan Zeist, The Netherlands), weighing 385 gram at the time of decapitation, were used in this study. The rats were housed in individual cages under a controlled environment (21 ± 1°C; humidity 60%; lights on 08.00–20.00 h). Food and water were available ad libitum. Rats were anesthetized with an intra-muscular injection of ketamine/xylazine (57/9 mg/kg) and placed in a stereotactic apparatus. For stimulation of the angular bundle, insulated stainless steel electrodes (60 μm diameter) were implanted (7.2 mm AP, 4.5 mm ML). In order to record hippocampal EEG and field potentials a second pair of electrodes was implanted into the left dentate gyrus of the hippocampus under electrophysiological control. The location of the electrodes was verified during the operation by recording field potentials evoked in the granule cell layer on stimulation of the angular bundle.

Seizure induction and EEG monitoring

Two weeks after recovery from the operation, each rat was transferred to a cage and connected to the recording and stimulation system via an electrical swivel (Air Precision). A week later the rats were given a long-lasting electrical stimulus (duration 0.5 ms, intensity maximal 500 μA) to the angular bundle. The protocol (10 s train of 50 Hz biphasic square pulses, delivered every 13 s, for up to 90 minutes) was comparable to the one that Lothman et al. used to induce limbic epilepsy (Lothman et al., 1989). A Status Epilepticus (SE) was considered to have been established, if electrographic recordings showed clear periodic epileptic discharges with a frequency of 1–2 Hz that lasted for at least one hour after stopping the electrical stimulation (n = 5). To prevent further intensification of the seizures that could lead to death of the animal, the rats were injected with pentobarbital (i.p. 60 mg/100 g body weight) at 4 hours after the stimuli. Rats were placed under continuous EEG monitoring for three months. Sham-operated control rats (n = 6) had electrodes implanted, they were handled in the same way but did not receive tetanic stimulation. The stimulated rats displayed spontaneous seizures accompanied by stage IV–V behavior (Racine, 1972). Their EEG signals were amplified and recorded by a PC, which used a seizure detection program (Harmony, Stellate Systems, Montreal, Canada) to monitor and quantify seizure activity during the rest of the experiment. The experimental animals were killed 3 months after the SE, when spontaneous seizures had long reached a steady-state. The animal experiments were performed in accordance with the regulations of the Animal Welfare Committee of the university. All efforts were made to minimize the number of animals used and their suffering.
**Cell preparation**

Each experimental day, a rat was decapitated after anesthesia with a mixture of 70% CO\(_2\)/30% O\(_2\). Both hippocampi were rapidly removed and cut into 300 μm thick transversal slices. The CA1 and DG region were dissected out of each hippocampal slice and incubated for 40 minutes at 32°C (Kay and Wong, 1986). The incubation chambers were filled with dissociation solution containing (in mM): 120 NaCl, 5 KCl, 1 CaCl\(_2\), 1 MgCl\(_2\), 20 PIPES, 25 D-glucose and a mixture of 0.5 mg/ml trypsin (from bovine pancreas, type XI), 0.5 mg/ml collagenase (from Clostridium histolyticum, type I) and 0.3 mg/ml pronase (from Streptomyces griseus, type XIV). pH was adjusted to 7.0 (NaOH). The tissue pieces were constantly stirred and kept in an O\(_2\) atmosphere. After the enzymatic treatment the tissue pieces were kept in the same dissociation solution without the enzymes now at room temperature (20°C). One CA1 or one DG piece of tissue was triturated in 0.5 ml extracellular solution using fire-polished Pasteur pipettes. It was then transferred to the recording chamber on the microscope. Neurons with a bright and smooth appearance and no visible organelles were selected for electrophysiological measurements (Vreugdenhil, M. and Wadman, 1992).

**Sodium current recordings**

During the experiments the cells were superfused with an extracellular solution designed to pharmacologically isolate voltage-activated sodium currents. It contained (in mM): 90 CholineCl, 20 NaCl, 10 HEPES, 2 CaCl\(_2\), 1 MgCl\(_2\), and 25 D-glucose. To block potassium currents the extracellular solution also contained (in mM): 25 tetraethylammonium chloride (TEACl), 5 4-aminopyridine (4-AP) and 5 CsCl; calcium currents were blocked by the addition of 100 μM CdCl\(_2\). The pH of this solution was set to 7.4 (HCl) and osmolarity was 285 mOsm. Patch pipettes (2–4 MΩ) were filled with an intracellular solution containing (in mM): 10 EGTA, 10 HEPES, 5 NaCl, 2 MgCl\(_2\), 0.5 CaCl\(_2\), 25 TEACl, 110 CsF, 20 phosphocreatine, 2 MgATP, 0.1 NaGTP, 0.1 leupeptin and 50 units/ml phosphocreatine kinase; pH was adjusted to 7.3 (CsOH) and osmolarity was 310 mOsm. Experiments were carried out at room temperature (20°C). All chemicals were purchased from Sigma, Zwijndrecht, The Netherlands.

After gigaseal formation and cell membrane rupturing, series resistance was compensated for at least 40%. We calculated that the worst-case error in comparing the voltage dependence of activation or inactivation between groups attained at most 0.5 mV. The membrane capacitance was read from the amplifier dials as a measure of membrane surface. The currents were sampled at a frequency of 5 kHz and analyzed with an Atari (TT030) computer-controlled Axopatch 200A amplifier using custom-made software. Holding membrane potential was set at -70 mV. The cells were allowed to stabilize for 5 minutes before current recordings started. In most cells the protocol series for sodium current recording was repeated six times at 5, 10, 15, 18, 21 and 24 minutes after entering the whole-cell configuration. This time series was used
to determine the stability of the recordings for each cell. Only electrotonically compact cells that did not escape voltage-clamp and showed little rundown within the 25 minutes recording time were incorporated in the analysis. Current traces were corrected off-line for linear leak using the conductance determined by a voltage step of $-5 \text{ mV}$ and $+5 \text{ mV}$ around the pre-pulse potential of $-150 \text{ mV}$ (see Results). The leak conductance was determined for all sweeps in a series of measurements and this value was used for correction; in this way voltage-dependent currents could never be subtracted out.

**Statistics**

Data are given as mean ± standard error of the mean (S.E.M.). Statistical comparisons were performed with Student's t-test unless stated otherwise. $P < 0.05$ was assumed to indicate a statistical difference.

**RESULTS**

**Epileptic state**

Long-lasting repetitive stimulation evoked in most rats Status Epilepticus (SE) that lasted for one hour or longer. Rats that did not show SE after stimulation were not incorporated in this study. After stimulation the animals were monitored for up to three months. From the second week onward, spontaneous seizures of increasing intensity and severity were recorded. The five chronic epileptic animals incorporated in this study experienced between 4-40 seizures per day of quite variable intensity and duration. All rats had stage IV-V behavioral seizures characterized by generalization and tonic-clonic convulsions.

**Sodium currents in CA1 pyramidal neurons**

Sodium currents were activated by depolarization (25 ms) to levels between $-70 \text{ mV}$ and $+10 \text{ mV}$ from a pre-pulse potential of $-150 \text{ mV}$ (Figure 2.1A: CA1 pyramidal neuron of a control rat (CA1-C), B: CA1 pyramidal neuron of a SE rat (CA1-SE); the voltage protocol is given as an inset). Tetrodotoxin (TTX, 0.5 $\mu$M) completely blocked the current (data not shown), confirming that it was a TTX-sensitive voltage-dependent sodium current. The depolarization activated a fast, transient inward sodium current that first increased in amplitude as the channels open and at higher potentials decreased due to the reduced driving force. We determined the peak amplitude of the current for each step and constructed a current-voltage relation. This IV-curve was fitted to the Goldman-Hodgkin-Katz current equation (Hille, 1992) using a Boltzmann function to describe the sodium permeability as a function of membrane voltage ($V$):
Figure 2.1: Sodium current activation in CA1 pyramidal neurons

(A) A set of sodium currents evoked in a neuron from the CA1-Control group (CA1-C). Sodium currents were activated by 25-ms depolarising voltage steps ranging from −70 mV to +10 mV, after a 500-ms hyperpolarizing pre-pulse to −150 mV (protocol given as inset). (B) A set of sodium currents evoked with the same protocol in a neuron from an epileptic rat (CA1-SE). (C) The peak amplitudes of the sodium currents in CA1-C neurons (circles, n = 17) and CA1-SE neurons (squares, n = 9) are plotted as a function of membrane potential. The smooth curves indicate the fit with the extended Goldman-Hodgkin-Katz current equation (see Results, Equation 2.1). The maximal conductance was smaller in the CA1-SE neurons and the voltage of half-maximal activation of the CA1-SE cells was shifted to more hyperpolarized potentials. Error bars indicate S.E.M.

\[
I(V) = g_{\text{max}} \times V \times \frac{1}{1 + \exp\left(\frac{V_h - V}{V_c}\right)} \times \frac{[Na^+]_{in}}{[Na^+]_{out}} - \exp(-\alpha V)
\]

(Equation 2.1)

with \( \alpha = \frac{F}{RT} \) and \( g_{\text{max}} = \alpha F [Na^+]_{out} P_0 \)

where \( P_0 \) is the maximal permeability, \( F \) is the Faraday constant, \( R \) the gas constant and \( T \) represents the absolute temperature. The maximal conductance of the current is \( g_{\text{max}} \) (nS), while the potential of half-maximal activation (\( V_h \)) and the slope parameter (\( V_c \)) characterize its voltage dependence. The sodium concentrations result in a reversal potential (\( E_{Na} \)) of +35 mV.

In CA1 neurons from control rats (CA1-C) the threshold for the sodium current was around −65 mV and the peak current amplitude increased with membrane potential to
reach a maximum around $-35 \text{ mV}$. The IV-curve for these neurons resulted in a $V_h$ of $-45.1 \pm 1.4 \text{ mV}$, a $V_c$ of $2.1 \pm 0.3 \text{ mV}$ and a $g_{\text{max}}$ (at reduced $\text{Na}^+ \text{ gradient}$) of $83.9 \pm 7.4 \text{ nS}$ ($n = 17$) (Figure 2.1C). At first inspection the currents in the control and the SE group were similar, showing fast activation followed by a slower inactivation (Figure 2.1A–B). In CA1 neurons from SE rats (CA1-SE) the sodium current threshold was also around $-65 \text{ mV}$ and the current increased with depolarization up to $-40 \text{ mV}$. The IV-curve for the CA1-SE neurons was characterized by a $V_h$ of $-51.5 \pm 2.9 \text{ mV}$, a $V_c$ of $1.9 \pm 0.5 \text{ mV}$ and a $g_{\text{max}}$ of $55.5 \pm 6.1 \text{ nS}$ ($n = 9$) (Figure 2.1C). $V_h$ in CA1-SE neurons was $6.4 \text{ mV}$ more negative than that of the CA1-C neurons ($P < 0.05$). $V_c$ of the CA1-SE neurons was not different from that of control neurons. The maximal conductance ($g_{\text{max}}$) in the SE neurons was significantly smaller than in the control neurons ($P < 0.05$). However, when the specific sodium conductance was calculated as $g_{\text{max}}$ divided by the cell capacitance as a measure of membrane surface, the difference did not reach significance (CA1-C neurons $7.8 \pm 0.8 \text{ nS/pF}$, CA1-SE neurons: $6.7 \pm 0.8 \text{ nS/pF}$), because of the smaller size of the neurons in the SE group ($11.6 \pm 0.8 \text{ pF}$ in CA1-C versus $8.8 \pm 1.1 \text{ pF}$ in CA1-SE).

**Steady-state inactivation in CA1 pyramidal neurons**

The voltage dependence of steady-state inactivation of the sodium current was measured by varying a 500 ms hyperpolarizing pre-pulse from $-150$ to $-35 \text{ mV}$ followed by a 25 ms depolarization to $-25 \text{ mV}$ (Fig. 2.2A, CA1-C and 2.2B, CA1-SE neuron). The peak amplitude of the current ($I$) evoked at $-25 \text{ mV}$ was normalized to $I_{\text{max}}$ and plotted as a function of pre-pulse potential ($V$). The curve was fitted with a Boltzmann equation:

$$I(V) = \frac{I_{\text{max}}}{1 + \exp \left( \frac{V_h - V}{V_c} \right)} \quad \text{(Equation 2.2)}$$

where $V_h$ is the potential of half-maximal inactivation and $V_c$ is proportional to the slope of the curve. For illustration purposes we constructed the Boltzmann curve for each experimental group using the mean values of $V_h$ and $V_c$ (Fig. 2.2C). For CA1-C cells the mean value of $V_h$ was $-68.8 \pm 2.3 \text{ mV}$ and $V_c$ was $-6.5 \pm 0.3 \text{ mV}$ ($n = 17$). The inactivation curve in CA1-SE neurons was characterized by a $V_h$ of $-66.3 \pm 2.3 \text{ mV}$ and a $V_c$ of $-6.1 \pm 0.3 \text{ mV}$. The differences did not reach significance.

**Window current in CA1 pyramidal neurons**

There is a narrow voltage range where the activation and inactivation curves of the fast sodium current overlap and where they are responsible for the appearance of a persistent current (Johnston and Wu, 1995). To illustrate this we give in Figure 2.3A
Figure 2.2: Sodium current inactivation in CA1 pyramidal neurons

(A) Typical set of sodium currents evoked in a CA1-C neuron to determine steady-state inactivation. Step depolarizations to -25 mV from a 500-ms hyperpolarizing pre-pulse between -150 mV and -35 mV provided the sodium current from different levels of steady-state inactivation (protocol given as inset). (B) A set of sodium currents evoked with the same protocol in a neuron from an epileptic rat (CA1-SE). (C) The level of steady-state inactivation ($I/I_{\text{max}}$) was plotted as a function of membrane potential for both CA1-C neurons (circles, $n = 17$) and CA1-SE neurons (squares, $n = 9$) and fitted with a Boltzmann function (see Results, Equation 2.2). Error bars indicate S.E.M.

The activation function as the normalized conductance (Boltzmann part of Equation 2.1) together with the normalized inactivation function. We calculated the window current for each cell as the product of Equations 2.1 and 2.2 using the parameters obtained from the fitted activation and inactivation function, which made it easier to isolate this current from persistent inward or outward currents. For illustration the absolute window current for both experimental groups was constructed using the mean values of the parameters ($V_c$, $V_h$, and $g_{\text{max}}$, Fig. 2.3B). For statistical comparisons, however, the window current was constructed for each individual neuron. The maximal amplitude of each window current and the membrane voltage where it occurred were determined. The amplitudes of the window currents did not obey a normal distribution ($P < 0.01$; Shapiro-Wilk test for normality), so that we used non-parametric statistics for comparisons. The maximal amplitude of the window current was larger in the CA1-SE neurons (median: 217 pA, $n = 9$) than in the CA1-C neurons (median: 64 pA, $n = 17$, $P < 0.05$, Mann-Whitney U-test). The optimal conditions for evoking the persistent sodium current ($I_{\text{NaP}}$) do not match those used in this study of the fast transient current and this prevented a quantitative comparison of the size of the window current with $I_{\text{NaP}}$. Because our window current is directly
Figure 2.3: Window current in CA1 pyramidal neurons

(A) The mean activation function as the normalized conductance and the inactivation function are shown for both the CA1-C and CA1-SE neurons. The overlap of these functions gives rise to a persistent “window” current that was increased in the SE neurons. (B) The absolute window current for each experimental group was constructed as the product of the fitted activation and inactivation function (Results, Equation 2.1 and 2.2) using the mean values of the parameters ($V_c$, $V_h$ and $g_{\text{max}}$). The maximal amplitude of the window current was larger in the CA1-SE neurons. The voltage at which maximal amplitude occurred was shifted to more hyperpolarized potentials in the CA1-SE neurons.

calculated from the curves fitted to the experimental data, it is not surprising that the amplitudes correspond with those observed in the current traces. The voltage at which maximal amplitude occurred was significantly shifted from $-44 \text{ mV}$ in controls to $-50 \text{ mV}$ in CA1-SE neurons ($P < 0.05$, Mann-Whitney U-test). For each cell we also determined the voltage range where the window current exceeded a level larger than the noise level estimated to be $10 \text{ pA}$. In the CA1-SE neurons this voltage range had a median of $26 \text{ mV}$, while it was only $20 \text{ mV}$ in controls. This range difference was qualitatively supported by the indirect measure of window size calculated as the difference in $V_h$ between activation and inactivation, which changed from $23.7 \pm 1.8 \text{ mV}$ in controls to $14.8 \pm 2.7 \text{ mV}$ in CA1-SE neurons ($P < 0.01$).

One could argue that in order to understand excitability it is more important to know the ratio between the window and the transient sodium current than to know the absolute size of the window current. Therefore we calculated for each cell the ratio between the peak amplitude of the window current and the peak amplitude of the fast transient current. In CA1-C cells the peak amplitude of the window current was 1.7% of the peak of the transient current, while in the CA1-SE cells its amplitude was 10.8% of the peak of the transient current ($P < 0.05$). A complicating factor in evaluating the consequences of this difference in window current is that the peak current does not occur at the same voltage.
Current kinetics in CA1 pyramidal neurons

To determine the kinetics of activation and inactivation, we fitted the time course of the sodium current during a 25-ms depolarization, with a single-exponential third-order rise time and a double-exponential decay of the form:

\[
I(t) = \left[1 - \exp\left(\frac{t_0 - t}{\tau_a}\right)\right]^3 \left[A_1 \exp\left(\frac{t_0 - t}{\tau_{i,1}}\right) + A_2 \exp\left(\frac{t_0 - t}{\tau_{i,2}}\right)\right]
\]

(Equation 2.3)

where \(t_0\) is the time of the depolarizing step, \(\tau_a\) is the time constant of activation and \(\tau_{i,1}\) and \(\tau_{i,2}\) are the time constants of a fast- and a slow-inactivating component with respectively \(A_1\) and \(A_2\) as their amplitude. We could not detect any significant differences in the time constants of activation and inactivation between neurons from the SE and the control animals (Table 2.1). The voltage dependence of both activation and inactivation kinetics is shown in Figure 2.4. Within the voltage range shown in this figure, both activation and inactivation were faster with increasing depolarization.

**Figure 2.4: Sodium current kinetics of CA1 pyramidal neurons**

(A–C) The voltage dependence of activation (A) and inactivation (B: \(\tau_{i,1}\), and C: \(\tau_{i,2}\)) kinetics of CA1-C (n = 17) and CA1-SE (n = 9) neurons is shown. The time course of the sodium current during a 25-ms depolarization was fitted with a single-exponential third-order rising phase and a double-exponential decay (Equation 2.3). Error bars indicate S.E.M.
Table 2.1: Sodium current kinetics

<table>
<thead>
<tr>
<th>Kinetics</th>
<th>CA1 Control (n=17) ms</th>
<th>SE (n=9) ms</th>
<th>DG Control (n=10) ms</th>
<th>SE (n=12) ms</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\tau_a$ (at $-25$ mV)</td>
<td>$0.4 \pm 0.1$</td>
<td>$0.5 \pm 0.1$</td>
<td>$0.4 \pm 0.1$</td>
<td>$0.6 \pm 0.2$</td>
</tr>
<tr>
<td>$\tau_{i,1}$ (at $-25$ mV)</td>
<td>$0.6 \pm 0.1$</td>
<td>$0.7 \pm 0.1$</td>
<td>$0.7 \pm 0.1$</td>
<td>$0.5 \pm 0.1$</td>
</tr>
<tr>
<td>$\tau_{i,2}$ (at $-25$ mV)</td>
<td>$4.8 \pm 0.6$</td>
<td>$5.0 \pm 0.7$</td>
<td>$5.7 \pm 0.7$</td>
<td>$4.0 \pm 0.7$</td>
</tr>
<tr>
<td>$\tau_{rec}$ (at $-70$ mV)</td>
<td>$33.0 \pm 4.6$</td>
<td>$36.1 \pm 4.9$</td>
<td>$49.9 \pm 11.2$</td>
<td>$37.6 \pm 3.8$</td>
</tr>
<tr>
<td>$\tau_{rec}$ (at $-80$ mV)</td>
<td>$15.4 \pm 2.1$</td>
<td>$16.2 \pm 2.3$</td>
<td>$23.4 \pm 4.8$</td>
<td>$17.6 \pm 1.6$</td>
</tr>
<tr>
<td>$\tau_{rec}$ (at $-90$ mV)</td>
<td>$9.1 \pm 1.3$</td>
<td>$8.8 \pm 1.0$</td>
<td>$13.5 \pm 3.1$</td>
<td>$10.3 \pm 1.0$</td>
</tr>
</tbody>
</table>

Time constants of activation ($\tau_a$) and inactivation ($\tau_{i,1}$ and $\tau_{i,2}$) determined at $-25$ mV for all experimental groups. Time constant of recovery from inactivation ($\tau_{rec}$) was determined at $-70$, $-80$, and $-90$ mV for all experimental groups.

Recovery from inactivation in CA1 pyramidal neurons

The time course of the recovery from inactivation was determined using a double-pulse protocol. The interval $\Delta t$ (during which the current was allowed to recover) between two depolarizations that lasted 25 ms was varied between 1 and 200 ms. The recovery time constant was determined at membrane voltages of $-70$, $-80$ and $-90$ mV (Fig. 2.5, protocol as inset). The ratio (R) between the amplitude of the sodium current activated by the second pulse and its maximal value at $\Delta t = 200$ ms is plotted as a function of $\Delta t$ (Fig. 2.5) and fitted with a single-exponential function to give the time constant of recovery from inactivation ($\tau_{rec}$):

![Figure 2.5: Recovery from inactivation of sodium currents in CA1 pyramidal neurons](image)

A double-pulse protocol with a variable interval ($\Delta t = 1, 2, 5, 10, 20, 50, 100$ or $200$ ms) between two depolarizations to $-25$ mV was used to investigate the recovery from inactivation at $-70$, $-80$ and $-90$ mV (protocol as inset). The ratio (R) between the amplitude of the sodium current activated by the second pulse and its maximal value at $\Delta t = 200$ ms is plotted as a function of $\Delta t$ and fitted with a single-exponential function (Equation 2.4) to give the time constant of recovery from inactivation ($\tau_{rec}$, Table 2.1) for the CA1-C ($n = 17$) and CA1-SE ($n = 9$) neurons. Error bars indicate S.E.M.
\[ R(\Delta t) = 1 - \exp\left( -\frac{\Delta t}{\tau_{\text{rec}}} \right) \]  

(Equation 2.4)

Recovery from inactivation was slightly faster at more hyperpolarized potentials (Fig. 2.5). Table 2.1 summarizes all kinetic data, clearly showing that SE neurons were not different from control neurons in any of the calculated aspects.

The persistent differences in CA1 pyramidal neurons after SE can be summarized as follows: while the maximal conductance of the sodium current was significantly smaller in SE cells, the specific sodium conductance was the same. The kinetics of current activation, inactivation and recovery from inactivation were not different from controls. We found a significant shift of the activation curve towards more negative potentials. In combination with the small shift of the inactivation function towards more positive potentials, this resulted in an increased window current (>300% in absolute current, >500% when considered as a fraction of the fast transient current). The peak of the window current was shifted 6 mV to more negative potentials.

Sodium current activation in DG granule neurons

From the same series of animals, DG granule cells were isolated and sodium currents were measured using the same procedures, solutions and protocols as described above for the CA1 neurons. The typical sodium currents in granule cells from the DG control group (DG-C) as well as the SE group (DG-SE) were comparable to the ones recorded in the CA1 pyramidal neurons. Figure 2.6 illustrates typical sets of sodium currents evoked by 25 ms step depolarizations from a holding potential of −150 mV to potentials between −70 mV and +10 mV for a DG-C (Fig. 2.6A) and a DG-SE neuron (Fig. 2.6B). In DG granule cells from control and SE rats sodium currents had a threshold for activation that was around −65 mV and the maximal current amplitude was reached at a membrane potential of −30 mV. The same Goldman-Hodgkin-Katz Boltzmann equation (Equation 2.1) that was used for the CA1 cells, was also used to characterize the IV relations in the DG neurons (Fig. 2.6C). The parameters obtained for DG-C neurons (n = 10) were for \( g_{\text{max}} \) 40 ± 7 nS; for \( V_h \) −47 ± 3 mV and for \( V_c \) 2.6 ± 0.5 mV. For the DG-SE neurons (n = 12) we found for \( g_{\text{max}} \) 33 ± 4 nS; for \( V_h \) −47 ± 2 mV and for \( V_c \) 2.0 ± 0.3 mV. None of these parameters were different between the two groups. Using cell capacitance as a measure for cell surface (DG-C neurons: 5.5 ± 0.7 pF and DG-SE neurons: 5.2 ± 0.5 pF) we calculated a specific sodium conductance of 9.1 ± 2.5 nS/pF in DG-C neurons and a similar one in DG-SE neurons: 7.0 ± 0.9 nS/pF. Although the DG cells had almost half the membrane surface area of the CA1 neurons, their specific conductance was about the same.
Figure 2.6: Sodium current activation in DG granule cells

(A) Set of sodium currents evoked in a granule cell from the DG-Control group (DG-C). Sodium currents were activated by 25-ms depolarising voltage steps ranging from −70 mV to +10 mV, after a 500-ms hyperpolarizing pre-pulse to −150 mV (protocol given as inset). (B) Set of sodium currents evoked with the same protocol in a granule cell from an epileptic rat (DG-SE). (C) The peak amplitudes of the sodium currents in DG-C neurons (circles, n = 10) and CA1-SE neurons (squares, n = 12) are plotted against the membrane potential. Smooth lines indicate the fit with the extended Goldman-Hodgkin-Katz current equation (Results, Equation 2.1). Error bars indicate S.E.M.

Steady-state inactivation in DG granule neurons

The voltage-dependence of steady-state inactivation of the sodium current was measured with the same protocols as described above for the CA1 pyramidal neurons. The normalized amplitude of the current evoked at −25 mV was plotted as a function of pre-pulse potential and fitted with a Boltzmann equation (Equation 2.2). The mean value of the parameters V_h for the DG-C neurons (n = 10) was −72.3 ± 3.0 mV and for the slope parameter V_c it was −6.5 ± 0.2 mV. The inactivation curve of DG-SE neurons (n = 12) was characterized by a V_h of −67.7 ± 1.5 mV and a V_c of −6.4 ± 0.2 mV. The difference in the V_h of inactivation between the two groups was similar, if not slightly larger than in CA1 neurons but also here it did not reach significance.

Window current in DG granule neurons

The activation and inactivation curves of the DG granule cells of both the control and the SE animals overlap, indicating the presence of a window current in this specific voltage range (Johnston and Wu, 1995) (Fig. 2.7A), although less than in the CA1 pyramidal neurons. Figure 2.7B compares the window current of both experimental
Figure 2.7: Window current in DG granule cells

(A) The activation function as the normalized conductance and the inactivation function are shown for both the DG-C and DG-SE neurons. The overlap of these functions gives rise to a persistent "window" current, which was hardly different in the SE neurons. (B) The absolute window currents for both experimental groups were constructed as the product of the fitted activation and inactivation function using the mean values of the parameters ($V_c$, $V_h$ and $g_{\text{max}}$; Results, Equation 2.1 and 2.2).

groups as calculated from their mean parameters. For statistics the window current of each neuron was calculated from the activation and inactivation parameters. The maximal amplitude of the window current changed from a median of 14 pA in the DG-C cells ($n = 9$) to 31 pA in DG-SE neurons ($n = 11$). The window current was smaller than in the CA1 group and not different between the DG-SE and the DG-control group. Also here we calculated the window current as a percentage of maximal peak of the transient current and found 2.0% for the DG-C neurons and 2.5% for the DG-SE neurons. These values are about the same as that obtained for the CA1 control group. The voltage where the peak of the window current occurred was not different between the two groups: a median of −49 mV was found in DG-C neurons and −47 mV for DG-SE neurons. This voltage lies in between the two values recorded for the CA1-C and CA1-SE group and was not significantly different from either one. Not surprisingly, the voltage range where a window current existed did also not differ between the two groups.

Current kinetics and recovery from inactivation in DG granule neurons

Activation and inactivation kinetics of DG sodium currents could also be fitted with Equation 2.3, to give three time constants as in the case of the CA1 neurons. No significant differences in the time constants of activation and inactivation were detected between the two groups (Table 2.1). The same conclusion was drawn for the time course of the recovery from inactivation (Equation 2.4) that was examined with the same double-pulse protocol as shown in Figure 2.5. In DG-SE neurons it was not different from DG-C values at any of the measured voltages (data in Table 2.1).
DISCUSSION

In this study we investigated sodium currents in neurons isolated from the hippocampal CA1 and DG areas of rats that displayed spontaneous seizures three months after an electrically-induced Status Epilepticus. In the chronic epileptic phase, the sodium conductance per cell was significantly reduced in the CA1 neurons from the SE group, but when expressed as specific sodium conductance this difference vanished due to a smaller cell size. The kinetics of current activation, inactivation and recovery from inactivation were not different between the control and the SE group. The values that we report for the voltage of half-maximal (in)activation are all lower than those reported in previous papers (Reckziegel et al., 1998; Vreugdenhil, M. et al., 1998a; Werkman et al., 1997), while the slope parameters for the Boltzmann activation curves are also smaller. It is not clear whether the differences relate to our experimental conditions. After SE the voltage dependence of activation in the CA1 pyramidal neurons was significantly shifted (6.5 mV) to more negative potentials. Such a shift will lower the spike threshold of the neuron and thus enhance its excitability. A small positive shift of the inactivation function will enlarge the number of sodium channels that can be recruited for activation. The shifts in voltage dependence have an additional important consequence: the overlap of the activation and the inactivation curve for the SE rats was substantially larger. This overlap constitutes a voltage-window in which a persistent current exists, because activation occurs at a voltage where the current will not completely inactivate. This so-called window current looks non-inactivating within the appropriate (narrow) voltage range. When it is of sufficient amplitude, it is important for cellular excitability (Johnston and Wu, 1995). Within the voltage range of the window this current cannot be distinguished from the classical persistent sodium current, $I_{\text{NaP}}$ (Crill, 1996; French et al., 1990), however, at voltages above $-30$ mV the window current will vanish while the $I_{\text{NaP}}$ will continue to exist. The optimal conditions for measuring $I_{\text{NaP}}$ do not match those necessary for reasonable control over the fast sodium current that is responsible for the window current. Therefore this study did not allow to quantitatively assess the relative importance of the window current with respect to $I_{\text{NaP}}$. In the CA1 neurons the maximal amplitude of the window current was significantly larger in SE rats when compared with controls. We expressed the size of the window current in two different ways: (1) as an absolute amplitude that is relevant to establish its maximal effect on membrane voltage of a specific cell, and (2) as the ratio between the maximal window current and the peak of the fast transient current. Both lead to the same conclusion. In a neuron with an input resistance as low as 50–100 MΩ, the window current in the SE animals will shift the membrane potential by several millivolts. It will drive the membrane voltage closer to firing threshold, and promote high-frequency firing (Johnston and Wu, 1995), thereby lowering the threshold for epileptic activity. The relevance of the window current for membrane potential is not only dependent on its
amplitude but also on the voltage where it occurs. The shift of the voltage dependence of the window current observed in the SE neurons brings it to a voltage range closer to resting membrane potential. Based on current-clamp data, Rempe et al. reported that after SE, CA1 pyramidal cells display prolonged depolarizations and multiple action potentials coinciding with multiple population spikes (Rempe et al., 1995); these data could be explained by our observations.

In DG cells no significant differences of the sodium current properties were detected after SE. The relative magnitude of the window current with respect to the transient sodium current in DG cells was the same in the SE and the control group, and similar to that observed in neurons of the CA1 control group.

The preparation of isolated neurons especially at room temperature and under reduced sodium concentration gradient allowed determination of the fast sodium current under reasonable voltage-clamp conditions (Vreugdenhil, M. et al., 1998a). However, the reduced morphology of isolated neurons imposes restrictions on our interpretations: they have only small dendritic remnants and we will miss differences in sodium current that specifically occur in the dendritic tree.

In previous studies using the same approach, we showed that capacitance correlated well with cell size determined from micro-photographs (Vreugdenhil, M. and Wadman, 1992). Whether absolute current or specific conductance is the most important factor for neuron excitability strongly depends on whether the assumption of uniform specific conductance (as we use in the specific sodium conductance calculations) is realistic. Further speculations about these aspects need to incorporate the complete neuron, but a clearly inadequate voltage-clamp in in situ measurements of sodium currents makes that approach inappropriate.

Since we can only compare groups of neurons in differently treated animals and not follow individual neurons during epileptogenesis, we can not determine whether our differences indicate changes in neuronal membranes, or whether they result from different subsets of CA1 pyramidal neurons. Many CA1 neurons die shortly after intense and prolonged seizure activity (Du et al., 1995) and preferential loss of certain populations of neurons in SE animals seems to occur (Gorter et al., 2003; Mathern et al., 1997). It is not clear why some neurons survive and others do not and it is even less clear whether there is a close relation between cell survival and the properties of the sodium current. Enhancement of firing activity could lead to an increase of calcium influx through voltage-activated channels or it could induce excitotoxicity by the release of toxic amounts of neurotransmitter such as glutamate. We also cannot exclude that we measured a class of neurons in SE rats that were able to survive epileptic seizures by way of a complicated compensatory reaction.
Basic mechanisms

Differences in the voltage dependence of the sodium current activation or inactivation can have various causes such as a molecular modification of the sodium channels or a different relative contribution of channel subunits/subtypes in SE rats (Catterall, W.A., 1992). Charge-neutralizing mutations at N-terminal positions in the voltage-sensing S4 domains of the α-subunit produce strong shifts of the voltage dependence of activation in hyperpolarizing direction (Catterall, W.A., 1992). Point mutations on the cytoplasmic linker joining domains III and IV of the α-subunit can selectively shift the voltage dependence of inactivation in depolarizing direction (Catterall, W.A., 1992). Changes in the expression ratios of sodium channel subtypes could contribute to the differences. Such changes have been reported during early development (Beckh et al., 1989) and in tissue resected from temporal lobe epilepsy patients (Lombardo et al., 1996). The mechanisms mentioned so far are particularly attractive to explain persistent and long-lasting effects as occur during epileptogenesis after SE. On a shorter time base, intracellular modulation by modulators like PKC is known to affect the inactivation function and leads to modulation of excitability (Cantrell et al., 1999; Numann et al., 1991). Modulation of the voltage dependence of the sodium current can be expected during the strong challenges as occur during spontaneous seizures. However, we do not know of a single modulator that produces exactly the set of modulations that we observed after SE. Combinations of acute modulation and changes in expression pattern are certainly possible and would complicate the pattern even further.

Whether the differences are the cause or the consequence of the epileptic activity is a hard question to answer. Activity alone could already be an important factor in modulating the sodium current. In cerebellar Purkinje cells acutely isolated from postnatal rats, Nam et al. found that spontaneous activity correlated with a negative shift of the activation curve of the sodium current, resulting in an increased window current. The voltage dependence of inactivation was not significantly changed in these cells (Nam and Hockberger, 1997). Baruscotti et al. found that in contrast to newborn cells, old rabbit sino-atrial cells did not exhibit spontaneous firing. This was due to a shift of the activation curve to more depolarized potentials (Baruscotti et al., 1996).

Comparison with kindling epileptogenesis

Previous research in our group has shown that the sodium current is affected by kindling epileptogenesis. In kindled rats, a shift of the sodium current inactivation curve to a more depolarized level was found, but in contrast with the study in SE rats the voltage-dependent activation was not affected at all (Vreugdenhil, M. et al., 1998a). The shift of the inactivation curve that was measured 6 weeks after the last kindled seizure (2.9 mV) was numerically comparable with the difference that we now determined after SE (2.6 mV). The smaller S.E.M. in the kindling experiments can
partly be explained by the larger number of observations, but could also reflect the much larger variability in time course of the spontaneous seizures in the post-SE model when compared with the highly systematic induction of seizures in the kindling model.

The most remarkable difference between kindling and the post-SE model is that post-SE rats exhibit spontaneous seizures, whereas kindled rats do not (Lothman et al., 1991) or only after an extremely long period of stimulation (Pinel and Rovner, 1978). The shift of the activation curve and the enhanced window current in CA1-SE neurons will both reduce the threshold for spontaneous seizures. If on the other hand the spontaneous seizures also contribute to the shift in the activation function, this forms a positive feedback loop that maintains the epileptic state and the spontaneous activity characteristic for this model. The difference in current inactivation in the kindling situation leads only to a small difference in window current that does not shift significantly in voltage. In the post-SE model the shift of the activation function contributes the most to the enhancement and the voltage shift of the window current, but the difference in inactivation curve is still responsible for about 20% of the increase in size.

**CA1 versus DG**

The CA1 region of the hippocampus has been implicated as a major site for seizure initiation in chronic temporal lobe epilepsy. The dentate gyrus normally operates to retard seizures, but under conditions of enhanced epileptogenesis, it may support or even amplify seizure activity (Rempe et al., 1995). There is a strong interaction between the various hippocampal regions in the initiation and spread of seizures in mTLE (Bertram, 1997), which implies that they participate to a large extent in the same seizure events during epileptogenesis (Bertram, 1997; Lothman et al., 1991; Rempe et al., 1995). In control conditions the sodium currents in neurons in CA1 and DG were comparable. Their conductance in the CA1 pyramidal neurons was twice as large as in dentate granule cells, but this effect can be completely attributed to a difference in cell size, as the specific sodium conductance was not different between the two groups. However, while sodium current properties of CA1 pyramidal neurons were significantly different after SE, we could not find differences in DG granule cells. The small positive shift of the inactivation function was numerically comparable in CA1 and DG neurons after SE. However, the combination with the strong shift in activation function leads to a larger window current in CA1, while in DG the enhancement was much smaller and will not affect excitability, because it did not shift into the right voltage range.

**Conclusions**

The most prominent difference observed after SE in CA1 was a large window current that had about three times the peak amplitude of controls and was shifted 6 mV to
more hyperpolarized potentials. The difference in sodium current could cause spontaneous seizures. It is not clear whether the epileptic activity also directly or indirectly affects the sodium current, thereby creating a feedback loop that maintains the epileptic state. Our observations do not exclude other factors like neurogenesis, sprouting or cell death to contribute to the pathology of the electrically-induced post-SE model. It is well known that they occur and affect neuronal network stability. They will, however, be additive to the changes in intrinsic membrane properties. Whether the differences in sodium current are related to specific changes in ion channel subunit composition or whether they result from modulation of the channels by intracellular substances needs to be determined.

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