Antiepileptic drugs targeting sodium channels: subunit and neuron-type specific interactions

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Chapter 2

Biophysical Properties and Pharmacological interaction with Carbamazepine, Phenytoin and Lamotrigine of Human Brain Sodium Channel α-subunits Expressed in HEK293 Cells

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Abstract

Voltage-gated Na$^+$ channels are the therapeutic targets of the antiepileptic drugs (AEDs) carbamazepine (CBZ), phenytoin (DPH) and lamotrigine (LTG). Neuronal Na$^+$ channels in the brain contain one out of four distinct $\alpha$-subunits: Na$_{\alpha}$1.1, Na$_{\alpha}$1.2, Na$_{\alpha}$1.3 and Na$_{\alpha}$1.6. This study provides a systematic comparison of the biophysical properties of these four $\alpha$-subunits and it characterizes the interaction of CBZ, DPH and LTG with these four $\alpha$-subunits. Na$^+$ currents were recorded in voltage-clamp mode in HEK293 cells stably expressing one of the four $\alpha$-subunits. CBZ, DPH and LTG were bath applied. Na$_{\alpha}$1.2 and Na$_{\alpha}$1.3 subunits have a relatively slow recovery from inactivation as compared to the Na$_{\alpha}$1.1 and Na$_{\alpha}$1.6 subunits. Of the four subunits the Na$_{\alpha}$1.1 subunit generates the largest window current. LTG evokes a larger maximal shift of the steady-state inactivation relationship than CBZ and DPH and CBZ shows a faster binding rate to the $\alpha$-subunits than LTG and DPH. LTG binding to the Na$_{\alpha}$1.1 subunit is faster than to the other $\alpha$-subunits and LTG unbinding from the $\alpha$-subunits is approximately two times slower than that of CBZ and DPH. The four $\alpha$-subunits show subtle differences in their biophysical properties. This can contribute to differences in membrane excitability, depending on the (sub)cellular expression patterns of Na$^+$ channel $\alpha$-subunits. The difference in LTG binding to the four $\alpha$-subunits suggests a subunit-specific response to LTG. This can have relevance for AED effects on neuronal excitability, depending on $\alpha$-subunit (sub)cellular expression patterns.
Chapter 2

Introduction

Voltage-gated Na\(^+\) channels play an important role in cellular excitability and are responsible for the rising phase of the action potential. The Na\(^+\) channel protein consists of a pore-forming \(\alpha\)-subunit associated with auxiliary \(\beta\)-subunits (Catterall, 2000; Catterall et al., 2005). Expression of the \(\alpha\)-subunit alone is sufficient for the formation of a functional Na\(^+\) channel, but \(\beta\)-subunits (so far four types have been identified: \(\beta_1\) through \(\beta_4\)) can modulate the kinetics and trafficking of the channel (Isom, 2001; Patino & Isom, 2010). Of the ten known \(\alpha\)-subunits, only Na\(_\text{V}1.1\), Na\(_\text{V}1.2\), Na\(_\text{V}1.3\) and Na\(_\text{V}1.6\) are found in the brain (Yu & Catterall, 2003; Vacher et al., 2008).

The four brain Na\(^+\) channel \(\alpha\)-subunits have different cellular and subcellular expression patterns which crucially determine their functional role. Na\(_\text{V}1.1\) has recently been shown to be primarily expressed in GABAergic interneurons in the hippocampus (Yu et al., 2006; Ogiwara et al., 2007; Lorincz & Nusser, 2010) and cortex (Ogiwara et al., 2007; Martin et al., 2010). Mutations of Na\(_\text{V}1.1\) cause the epileptic phenotype due to the decreased inhibition of GABAergic interneurons (Yu et al., 2006; Ogiwara et al., 2007; Tang et al., 2009; Martin et al., 2010). Na\(_\text{V}1.2\) is primarily expressed along axons and on nerve terminals (Gong et al., 1999; Lorincz & Nusser, 2010). This localization suggests that Na\(_\text{V}1.2\) may be involved in axonal propagation of action potentials and relevant for neurotransmitter release. In rodents, Na\(_\text{V}1.3\) mRNAs have the highest levels in the embryonic and early postnatal brain, whereas Na\(_\text{V}1.3\) mRNAs (Whitaker et al., 2000) and proteins (Whitaker et al., 2001b) are extensively expressed in adult human brain. Na\(_\text{V}1.6\) is highly expressed in axon initial segments and in nodes of Ranvier along axons (Debanne et al., 2011) where it has a key role.
in action potential initiation and propagation. \(Na_V\text{1.6}\) is also moderately expressed in the somata and the dendrites of CA1 pyramidal neurons (Lorincz & Nusser, 2010) and can play an essential role in dendritic excitability.

Voltage-gated \(Na^+\) channels are key players in cellular excitability and they are the therapeutic target of the antiepileptic drugs: carbamazepine (CBZ), phenytoin (DPH) and lamotrigine (LTG). These drugs modulate voltage-gated \(Na^+\) channels in a use- and voltage-dependent manner which allows them to selectively prevent high frequency firing, with little effect on single action potential firing. CBZ, LTG and DPH all have a much higher affinity for the inactivated state than for the closed and open states of the \(Na^+\) channel and therefore stabilize the inactivated state, effectively blocking the \(Na^+\) conductance (Ragsdale & Avoli, 1998; Rogawski & Loscher, 2004).

In the majority of epileptic patients this is an effective mechanism to reduce or even prevent epileptic seizures, however, for unknown reasons it also fails in a substantial fraction of them. One possible cause could lie in subtle differences in the interactions of the drugs with specific sodium channel subtypes in combination with their regional and subcellular distribution. The interactions between the AEDs and \(Na^+\) channels or different \(Na^+\) channel \(\alpha\)-subunits have been described in several studies (Kuo & Bean, 1994; Kuo et al., 1997; Kuo & Lu, 1997; Goldin, 2001; Catterall et al., 2005), but a systematic comparison of the interactions of CBZ, DPH and LTG with the four major brain \(Na^+\) channel \(\alpha\)-subunits \(Na_V\text{1.1}\), \(Na_V\text{1.2}\), \(Na_V\text{1.3}\) and \(Na_V\text{1.6}\) using the same expression system and identical recording conditions is lacking.

In this study we will first compare the biophysical properties of the \(Na_V\text{1.1}\), \(Na_V\text{1.2}\), \(Na_V\text{1.3}\) and \(Na_V\text{1.6}\) pore forming \(\alpha\)-subunits, stably expressed in
HEK293 cells. Next we will determine their interactions with CBZ, DPH and LTG in a comparative way.

Methods

Stably transfected HEK293 cell lines

All experiments were performed in HEK293 cell lines stably expressing human Na\textsubscript{V}1.1, Na\textsubscript{V}1.2, Na\textsubscript{V}1.3 or Na\textsubscript{V}1.6 \(\alpha\)-subunits (a kind gift of GlaxoSmithKline, UK) that have previously been described (Chen et al., 2000; Burbidge et al., 2002; Mantegazza et al., 2005). The cell lines were generated using the pCIN5 vector (Chen et al., 2000; Burbidge et al., 2002).

Cell culture

The HEK293 cell lines were cultured in minimum essential medium (Gibco), containing 10% fetal calf serum (Gibco), 1% L-glutamine (200 mM, Gibco) and 1% penicillin/streptomycin (Gibco). Cells were grown in a 95% O\textsubscript{2}/5% CO\textsubscript{2} atmosphere at 37°C and with 95% humidity. One to two days prior to electrophysiological recordings, the cells were plated on glass coverslips.

Whole-cell voltage-clamp recordings

Cells grown on glass coverslips were placed in a recording chamber with 0.5 ml extracellular solution containing (in mM): NaCl 140, KCl 5, CaCl\textsubscript{2} 2, MgCl\textsubscript{2} 1, HEPES 10, and glucose 11; pH was adjusted to 7.4. The patch electrodes had resistances of 2-3 M\(\Omega\) and were filled with pipette solution containing (in mM): CsF 140, EGTA 10, HEPES 10, NaCl 5, MgCl\textsubscript{2} 2; the pH was adjusted to 7.3. Voltage-gated Na\textsuperscript{+} currents were recorded in whole-cell voltage-clamp mode at room temperature (20-22°C). After the whole-cell configuration was established, the cell was perfused with extracellular solution for \(~10\) minutes allowing Na\textsuperscript{+} currents to stabilize, and then moved into either control or drug-containing extracellular solution emitted from the
application pipette using the Fast-Step Perfusion system (SF-77B, Warner Instrument Corporation, Hamden, USA). Voltage-step protocols were applied by a personal computer-controlled Axopatch 200A amplifier. The membrane capacitance was read from the amplifier dials and used to indicate membrane surface. Compensation circuitry was used to reduce the series resistance error by at least 75%. The calculated liquid junction potential was 8.5 mV but no corrections were undertaken. The holding membrane potential was set at –70 mV and currents were sampled at a frequency of 5 kHz and analyzed using custom-made software. Each protocol (lasting 2-2.5 min) was performed at least twice in each extracellular solution (control or drug-containing). The control extracellular solution was applied before and after the drug-containing solution to detect possible slow run-down. Only cells that showed little current rundown over the recording time were incorporated in the analysis. Preferably, more than one concentration per cell was tested (with a maximum of three concentrations per cell). The currents were corrected off-line for linear non-specific leak and residual capacitive current.

**Drugs and reagents**

CBZ (Sigma), DPH (Sigma) and LTG (GlaxoSmithKline, UK) were dissolved in dimethylsulfoxide (DMSO, Sigma) to make stock solutions of 400 mM, 100 mM and 333 mM, respectively. They were then diluted in extracellular solutions to reach their final concentrations. DMSO concentrations in CBZ-, DPH- and LTG-containing extracellular solutions were respectively 0.05%, 0.2% and 0.3%; no DMSO effects on Na⁺ currents were observed.
Data analysis

Data are given as the mean ± standard error of the mean (S.E.M). Multiple groups were compared using an (one or two factor) ANOVA followed by a post-hoc Fisher Least Significant Difference (LSD) test. For comparison of the binding data a test for homogeneity of regression coefficients was applied. Unless otherwise stated, Student’s *t*-test was used for the direct comparison of two groups of parameters. *P*<0.05 was considered to indicate a significant difference.

Results

**Biophysical properties of Na*⁺* currents carried by Na*⁺*1.1, Na*⁺*1.2, Na*⁺*1.3 and Na*⁺*1.6 α-subunits**

**Voltage-dependent activation** Na*⁺* currents were activated by a voltage-step protocol that depolarized the cell to different voltages after complete removal of inactivation at -120 mV (Fig. 1Aa, inset). The peak amplitude of the Na*⁺* current was determined for each step and the current-voltage relationship (I-V curve) was constructed for each cell. The mean data points (I(V)) as a function of voltage (V) were fitted using a modified Goldman-Hodgkin-Katz current equation (Hille, 2001) in which a Boltzmann function is used to describe the voltage dependence of the Na*⁺* permeability (Fig. 1Ab):

\[
I(V) = \frac{P_0 \times F \times \alpha V \times [Na^+]_{out} \times [Na^+]_{in} / [Na^+]_{out} - \exp(-\alpha V)}{1 + \exp\left(\frac{V_{rev} - V}{\kappa}\right)}
\]

Eq. 1

Where \( \alpha = F/RT \) with \( F \) the Faraday constant, \( R \) the gas constant and \( T \) the absolute temperature. \([Na^+]_{out}\) and \([Na^+]_{in}\) are the extracellular and intracellular sodium concentrations. \( P_0 \) is the maximal Na*⁺* permeability and the voltage dependence of the conductance is described with a Boltzmann
function characterized by the potential of half-maximal activation ($V_h$) and a slope parameter ($V_c$). For practical measurements we prefer to substitute $P_0 F a [Na^+]_{out} = G_{max}$ where $G_{max}$ is the maximal conductance. The I-V curve for each cell was fitted to Eq 1 and the resulting values for $V_h$, $V_c$ and $G_{max}$ for the four $\alpha$-subunits are given in Table 1.

**Fig. 1** Voltage-dependent activation and steady-state inactivation of $Na^+$ currents through the $Na_v1.1$, $Na_v1.2$, $Na_v1.3$ and $Na_v1.6$ $\alpha$-subunits stably expressed in HEK293 cells. (A) Voltage-dependent activation. (a) Example traces of $Na_v1.3$ currents. $Na^+$ currents were activated by 25-ms depolarizing voltage steps ranging from $-70 \text{ mV}$ to $+10 \text{ mV}$, following a 500-ms hyperpolarizing pre-pulse to $-120 \text{ mV}$. (b) Available fraction. (a) Example traces of $Na_v1.6$. $Na^+$ currents were activated by 25-ms depolarizing voltage steps ranging from $-10 \text{ mV}$ to $+10 \text{ mV}$, following a 2500-ms hyperpolarizing pre-pulse to $-150 \text{ mV}$. (b) Available fraction.
Voltage-dependent steady-state inactivation

Na⁺ currents were activated by a voltage-step protocol where the same depolarization to –10 mV followed different prepotential steps (Fig. 1Ba, inset). The peak amplitude of the Na⁺ current evoked by the standard depolarization was determined for each pre-pulse voltage. The mean data points (I(V)) as a function of prepotential V were fitted with a Boltzmann function:

\[
I(V) = \frac{I_{\text{max}}}{1 + \exp\left(\frac{V_{h} - V}{V_{c}}\right)}
\]

Eq. 2

The available fraction is given as I(V)/I_{\text{max}} (Fig. 1Bb). \(V_{h}\) is the potential of half-maximal inactivation and \(V_{c}\) is the slope parameter. The data points of each cell were fitted with Eq. 2 and the resulting values for \(V_{h}\) and \(V_{c}\) for the four α-subunits are also given in Table 1.

Window currents The voltage range where activation and inactivation overlap defines the so-called window current: in this range the activated Na⁺ current will not completely inactivate and presents itself as a small voltage-dependent persistent current (Patlak, 1991; Johnston, 1995). Using the mean values for \(V_{h}\) and \(V_{c}\) for activation as well as inactivation (Table 1) we constructed for \(\text{NaV}_{1.1}\) the inactivation curve (available fraction) and the
activation curve (open fraction) as a function of membrane voltage (Fig. 2A). From these two curves the window current for \( \text{Na}_V 1.1 \) can be constructed analytically and the procedure was repeated for the other subunits (Fig. 2B). For statistical comparison of the magnitude of the window currents we calculated the area under the curves between -100 mV and 0 mV: \( \text{Na}_V 1.1: 371 \pm 46 \text{ pA} \cdot \text{mV} \) (n=34), \( \text{Na}_V 1.2: 91 \pm 10 \text{ pA} \cdot \text{mV} \) (n=34), \( \text{Na}_V 1.3: 223 \pm 19 \text{ pA} \cdot \text{mV} \) (n=30) and \( \text{Na}_V 1.6: 146 \pm 37 \text{ pA} \cdot \text{mV} \) (n=37). \( \text{Na}_V 1.1 \) is capable of generating a larger window current than the other three subunits (p<0.01; ANOVA, Fisher’s LSD post-hoc test). This is consistent with the observation that the difference between \( V_h \) for activation and inactivation is smallest in \( \text{Na}_V 1.1 \) (Table 1). Furthermore, the \( \text{Na}_V 1.3 \) window current had a larger magnitude than that of the \( \text{Na}_V 1.2 \) (p<0.01) and \( \text{Na}_V 1.6 \) (p<0.05) \( \alpha \)-subunits. We determined at which membrane voltage the window currents peaked: \( \text{Na}_V 1.1: -47.6 \pm 1.1 \text{ mV}, \text{Na}_V 1.2: -44.1 \pm 1.5 \text{ mV}, \text{Na}_V 1.3: -35.0 \pm 1.5 \text{ mV} \) and \( \text{Na}_V 1.6: -47.8 \pm 1.2 \text{ mV} \), where \( \text{Na}_V 1.3 \) peaked at a significantly higher value than all the others (p<0.01; ANOVA, Fisher’s LSD post-hoc test). The peak of the \( \text{Na}_V 1.2 \) window current was at a slightly more depolarized potential than those of the \( \text{Na}_V 1.1 \) and \( \text{Na}_V 1.6 \) subunits (p<0.05).
Fig. 2 Construction of window currents carried by the four α-subunits. (A) The mean normalized activation curve (Boltzman term of eq. 1) and the mean normalized inactivation curve (same as in Fig. 1Bb) in NaV1.1-expressing cells (n=34). (B) The window currents of the four α-subunits were constructed (as the product of the activation and inactivation functions; see A) for each cell by using the V_h and V_c parameters of the activation and inactivation curves and the G_max value. The average window currents carried by the four α-subunits NaV1.1 (n=34), NaV1.2 (n=34), NaV1.3 (n=30), NaV1.6 (n=37) are shown. Error bars indicate S.E.M.

Table 1 Activation and steady-state inactivation properties of Na⁺ currents carried by NaV1.1, NaV1.2, NaV1.3 and NaV1.6 α-subunits.

<table>
<thead>
<tr>
<th>Type</th>
<th>V_h (mV)</th>
<th>V_c (mV)</th>
<th>G_max (nS)</th>
<th>V_h (mV)</th>
<th>V_c (mV)</th>
<th>ΔV_h (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.1</td>
<td>–27.1±0.8aa,bb</td>
<td>5.4±0.2bb,bb</td>
<td>74.1±4.2bb,bb</td>
<td>–59.4±0.9aa</td>
<td>–5.5±0.3aa</td>
<td>32.4±1.0bb,cc</td>
</tr>
<tr>
<td>1.2</td>
<td>–24.3±1.0aa</td>
<td>4.7±0.2bb,cc</td>
<td>76.1±4.4bc,dd</td>
<td>–60.5±0.8bb</td>
<td>–4.9±0.1aa,bb,cc</td>
<td>36.2±0.9aa</td>
</tr>
<tr>
<td>1.3</td>
<td>–23.0±0.7bb,cc</td>
<td>4.6±0.2bb,dd</td>
<td>98.4±5.5bc,cc,ee</td>
<td>–58.6±0.5cc</td>
<td>–5.9±0.1bb</td>
<td>35.6±0.7bb</td>
</tr>
<tr>
<td>1.6</td>
<td>–25.9±0.4cc</td>
<td>5.6±0.1bc,dd</td>
<td>55.8±2.7bb,dd,ee</td>
<td>–64.3±0.5ab,bb,cc</td>
<td>–5.7±0.1cc</td>
<td>38.4±0.5bc,cc</td>
</tr>
</tbody>
</table>

ΔV_h indicates the difference between the activation V_h and inactivation V_h values. Cell numbers are given in brackets. a, b indicate p<0.05. aa, bb, cc, dd, ee indicate p<0.01. (ANOVA followed by Fisher’s LSD post-hoc test).

Recovery from inactivation Na⁺ currents were activated by a double-pulse protocol (Fig. 3A, inset). The amplitude of the Na⁺ current activated by the second depolarization and normalized to the first one is a function of the
time interval ($\Delta t$) between them and can be fit with a single-exponential function (Fig. 3Ad):

$$R(\Delta t) = 1 - \exp\left(-\frac{\Delta t}{\tau_V}\right)$$

Eq. 3

Where $\tau_V$ is the time constant that depends on the voltage during the removal of inactivation. The mean values for $\tau_V$ for the four $\alpha$-subunits are given in Fig. 3B where it can be seen that the recovery from inactivation is voltage-dependent (two-factor ANOVA, p<0.001).
Fig. 3 Voltage-dependent recovery from inactivation of Na⁺ currents. (A) The time course of recovery from inactivation was determined by a double-pulse protocol (protocol given as inset). The variable pulse interval (Δt = 3.4, 6.8, 12.6, 25, 50, 100 or 200 ms, during which the current was allowed to recover) between two 25-ms depolarizing voltage steps to −25 mV, was used to determine the recovery from inactivation at the membrane voltages −80 mV, −90 mV, and −100 mV. Example traces of Na⁺ currents are shown in (a) (Δt = 100 ms), (b) (Δt = 25 ms) and (c) (Δt = 3.4 ms). (d) The ratio of the peak amplitudes activated by the first and second pulses was plotted as a function of Δt and fitted with a mono-exponential function (Eq. 3). (B) Time constant values of NaV1.1 (n = 32), NaV1.2 (n = 34), NaV1.3 (n = 32) and NaV1.6 (n = 38) currents at the three membrane voltages. The recovery from inactivation was voltage- and subunit-dependent (two-factor ANOVA, p<0.001). Error bars indicate S.E.M. For comparison between different α-subunits at the same membrane potential, * and ** respectively indicate p<0.05 and p<0.01. For comparison of the same α-subunit at different membrane voltages, aa, bb, cc indicate p<0.01 (Fisher’s LSD post hoc test is performed for both comparisons). Due to non-homogeneity of variance, the statistical analysis was performed on the log-transformed data.

Pharmacology of NaV1.1, NaV1.2, NaV1.3 and NaV1.6 α-subunits

Frequency-dependent inhibition by CBZ. CBZ modulates voltage-gated Na⁺ currents in a frequency- and use-dependent manner due to its high affinity specific for the inactivated state (Rogawski & Loscher, 2004). The functional consequence of this phenomenon is that the current gives a strongly frequency-dependent response to repetitive depolarizations (Fig. 4). Na⁺ current was activated by 3-ms depolarizations at either 10 Hz or 50 Hz depolarization steps. Even without any drug the evoked current slowly decays due to incomplete recovery from inactivation at such frequencies (Fig. 4Aa,c). In the presence of 50 μM CBZ that reduction is larger as binding of CBZ to the inactivated state will refrain a fraction of the channels from responding to the depolarization (Fig. 4Ab). The development of the CBZ block was isolated by subtracting the two responses; the resulting
curve (Fig. 4Ad) can be fit with a single exponential function (Eq. 3) to give the time constant of the process (164 ms).

CBZ block developed faster at 50 Hz than at 10 Hz and it was also faster for the higher CBZ concentration (200 μM). Although the overall trend is similar for all α-subunits, there are subtle differences in the time course of their responses (Fig. 4B, multi-factor ANOVA for frequency, concentration and subunit; \( P<0.001 \)). Using the ratio of the time constants at 10 and 50 Hz we could also demonstrate that the frequency sensitivity of the block is independent of the CBZ concentration and consistent for the four α-subunits (Fig. 4C). In the following we will analyze in detail the interactions between the four α-subunits and CBZ, DPH and LTG.

**Fig. 4 Frequency-dependent block of Na⁺ currents by CBZ and LTG.** (A) Examples of Na⁺ current traces, evoked with 20 3-ms voltage steps to \(-10 \text{ mV}\);
pulse interval at –80 mV was 20 ms) before (a), during (b) and after (c) CBZ (50 μM) application. Protocol given as inset: pulse interval was 20 ms (“50 Hz”) or 100 ms (“10 Hz”). These frequencies reflect the pulse interval frequencies and not the actual pulse frequencies (which were ~44 Hz and ~8 Hz, respectively. (d) The blocked current amplitudes were plotted as a function of time and fitted with a mono-exponential function (Eq. 3) to yield the time constant describing the development of drug block. (B) Average time constants describing the development of CBZ (50 and 200 μM) block of Na⁺ currents activated with the 10 Hz or 50 Hz stimulation protocol (n=5-10). (C) Frequency sensitivity of CBZ block (ratio of τ₂₀ and τ₅₀ values from B) for 50 and 200 μM CBZ (n=5-10). CBZ showed a frequency- and concentration-dependent block (multi-factor ANOVA, p<0.001). Error bars indicate S.E.M., * and ** indicate p<0.05 and p<0.01 respectively for comparison of different α-subunits; “a” and “b” indicate p<0.01 and p<0.05 respectively for comparison of the same α-subunit at different stimulation frequencies but at the same concentration; “c” indicates p<0.01 for comparison of the same α-subunit at different concentrations but at the same stimulation frequency (all Fisher’s LSD post-hoc test).

AED effects on the inactivation properties of the Na⁺ currents The voltage-dependent preferential block of LTG (300 μM) to the inactivated state is demonstrated using Na⁺ currents evoked by a depolarization to -10 mV after a pre-pulse of either –130 mV or –80 mV (Fig. 5Aa). It is clear that the LTG binding at -80 mV is much more effective than the one at -130 mV (Fig. 5Aa).

The steady-state inactivation protocol (details in Fig. 1B) was used to systematically investigate the concentration dependence of this phenomenon. Increasing LTG concentrations (10-1000 μM) shift the inactivation curve of the Na⁺ current carried by Naᵥ1.2 to more hyperpolarizing potentials (Fig. 5Ab). The major effect is on the Vₗ parameter of the Boltzmann fit. The shift in respect to the control situation without LTG (ΔVₗ) was determined as a function of the applied LTG concentration ([LTG]) and this relation was well fit by a first order logistic function:
\[ \Delta V_h([LTG]) = \frac{\Delta V_{h_{\text{max}}}^h}{1 + EC_{50}/[LTG]} \]  
Eq. 4

where \( \Delta V_{h_{\text{max}}}^h \) is the shift of \( V_h \) for saturating concentration and \( EC_{50} \) is the concentration of half-maximal \( \Delta V_h \) (Fig. 5Ac; Na\( _V \)1.2, \( n=36 \)). The analysis was repeated for all subunits and the three AEDs; the associated concentration response curves are given in Fig. 5B and the data are summarized in Table 2. These results confirmed the suggestions drawn from Fig. 4: the overall profile of the interaction between these drugs and the Na\( ^+ \) channel subunits was similar, but for this property \( (V_h) \) LTG had a higher efficacy than CBZ or DPH and there were subtle subunit-specific differences. The latter were evaluated using the parameters given in Table 2, but as the \( EC_{50} \) and the \( \Delta V_{h_{\text{max}}}^h \) are not independent, we only accepted the conclusions if they were supported statistically by a model-free comparison of the responses at one or two single concentrations. Na\( _V \)1.6 is strongest modulated by CBZ, while Na\( _V \)1.3 is the weakest. For CBZ none of the differences between \( EC_{50} \) values reached significance. LTG also has its strongest effect on Na\( _V \)1.6, while Na\( _V \)1.2 is most sensitive to LTG (it has a lower \( EC_{50} \) values than all others). For DPH the strongest modulation is via the affinity (\( EC_{50} \)), where Na\( _V \)1.2 is more sensitive to DPH than all other subunits.

The direct consequence of the change in rate constants that is responsible for the shift in the inactivation curve is that it most likely also leads to a change in the time constant for the removal of inactivation. This is illustrated for CBZ at -80 mV (Fig. 6A) and -90 mV (Fig. 6B), based on experiments using the protocols given in Fig. 3A. CBZ concentration-dependently and subunit specific slowed the recovery from inactivation at -80 mV and -90 mV (multifactor ANOVA, \( p<0.01 \)). DPH and LTG have much slower
binding rates than CBZ (see below), so that it was impossible to detect and compare their effect on the recovery from inactivation using the same protocol with brief (25 ms) depolarization steps.

Fig. 5 AED effects on the inactivation properties of Na⁺ currents. (A) (a) Example traces of Na⁺ currents evoked at the testing potential of −10 mV following a 2500-ms hyperpolarizing pre-pulse at −130 mV or −80 mV in control, the presence of 300 µM LTG and wash (protocol given as inset). (b) The mean normalized steady-state inactivation curve was shifted to more hyperpolarizing direction with increasing concentrations of LTG (10-1000 µM). Na⁺ currents were evoked and analyzed in the same way as in Fig. 1B. (c) The absolute ΔVₘ values were plotted against the concentration of LTG and the individual data points were fitted with a logistic function (Eq. 4). (B) The average data points of the absolute ΔVₘ values induced by CBZ (10, 20, 50, 100 and 200 µM) (left panel), DPH (10, 20, 50, 100 and 200 µM) (middle panel) and LTG (10, 30, 100, 300 and 1000 µM) (right panel) were fitted with the logistic function. Error bars indicate S.E.M.
Table 2 AED effects on the inactivation properties of Na\(^+\) currents carried by the Na\(_{\alpha 1.1}\), Na\(_{\alpha 1.2}\), Na\(_{\alpha 1.3}\) and Na\(_{\alpha 1.6}\) α-subunits.

<table>
<thead>
<tr>
<th>Type</th>
<th>DPH</th>
<th>LTG</th>
<th>CBZ</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>EC(_{50}) (μM)</td>
<td>ΔV(_{h\text{-max}}) (mV)</td>
<td>EC(_{50}) (μM)</td>
</tr>
<tr>
<td>Na(_{\alpha 1.1})</td>
<td>35.4±9.5 (25)</td>
<td>16.6±1.4</td>
<td>245.0±58.7(^{cd}) (24)</td>
</tr>
<tr>
<td>Na(_{\alpha 1.2})</td>
<td>16.7±3.7(^{ab, a}) (24)</td>
<td>13.7±0.9(^{ab, a})</td>
<td>123.8±16.8(^{ab, ic}) (36)</td>
</tr>
<tr>
<td>Na(_{\alpha 1.3})</td>
<td>54.9±6.9(^{ab, b}) (40)</td>
<td>17.3±0.8(^{ab})</td>
<td>217.2±39.8(^{b}) (36)</td>
</tr>
<tr>
<td>Na(_{\alpha 1.6})</td>
<td>31.3±4.9(^{ab, b}) (28)</td>
<td>16.6±0.8(^{a})</td>
<td>230.8±31.9(^{cc}) (36)</td>
</tr>
</tbody>
</table>

Numbers of individual data points are indicated in brackets. a, b indicate P < 0.05; aa, bb, cc, dd indicate P < 0.01 for comparison between effects of the same AED on different α-subunits (Student’s t-test).

**Fig. 6** Concentration-dependent CBZ effects on the recovery from inactivation of Na\(^+\) currents. (A) Time constants describing the recovery from inactivation at the recovery potential of –80 mV (see Fig. 3) in the absence and presence of CBZ (10, 50 and 100 μM). (B) Time constants describing the recovery of the inactivation process at the recovery potential of –90 mV (note different Y-axis scales in A and
B). Error bars indicate S.E.M. At both potentials CBZ evoked a concentration-dependent slowing of the recovery from inactivation for all four α-subunits (two-factor ANOVA, p<0.01; n=4-12). Error bars indicate S.E.M.

**AED effects on the window currents** Changes in the activation and inactivation function affect the window current. The AED effects described here mainly shift the inactivation in hyperpolarizing direction, which implies that they always reduce the window current, and often also change its shape as illustrated in Fig. 7A for the NaV1.1 subunit and the three drugs at relevant concentrations. We quantified the effect using the same "area under the curve" measure (between -100 and 0 mV) as in Fig. 2B. This may underestimate the consequences of strong changes in the shape of the I-V curve, but it allows to relate the percentage block to the drug concentration. Quantification was attained as in Fig. 5B, using Eq. 4 in a slightly modified form (Fig. 7B). The EC$_{50}$ values are given in Table 3. Not surprisingly the effects have a tendency to follow the results obtained in Table 2, as the inactivation curve forms an essential part of the window-current. The window-current block by DPH is quite comparable to the modulation of the inactivation curve. LTG is a stronger modulator of the inactivation than DPH and the larger shifts imply that complete block of the window current is reached at much lower concentrations than the maximum shift, which explains the substantial higher sensitivity for LTG.
Fig. 7 Effects of CBZ, DPH and LTG on the window currents carried by Na\textsubscript{v}1.1, Na\textsubscript{v}1.2, Na\textsubscript{v}1.3 and Na\textsubscript{v}1.6 \(\alpha\)-subunits. (A) Examples of partly blocked window current carried by Na\textsubscript{v}1.1 by 20 \(\mu\)M CBZ (left panel; \(n=7\)), 20 \(\mu\)M DPH (middle panel; \(n=3\)) and 100 \(\mu\)M LTG (right panel; \(n=4\)). (B) Concentration-response relationships of CBZ (left panel), DPH (middle panel) and LTG (right panel) for blocking the window currents carried by the four subunits. The average data points were fitted with a logistic function (Eq. 5). Error bars indicate S.E.M.

Table 3 AED effects on the window currents carried by the Na\textsubscript{v}1.1, Na\textsubscript{v}1.2, Na\textsubscript{v}1.3 and Na\textsubscript{v}1.6 \(\alpha\)-subunits.

<table>
<thead>
<tr>
<th>Types</th>
<th>DPH</th>
<th>LTG</th>
<th>CBZ</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>EC\textsubscript{50} ((\mu)M)</td>
<td>EC\textsubscript{50} ((\mu)M)</td>
<td>EC\textsubscript{50} ((\mu)M)</td>
</tr>
<tr>
<td>Na\textsubscript{v}1.1</td>
<td>19.9 ± 2.0(^{aa,*}) (22)</td>
<td>83.4 ± 27.5(^{*}) (21)</td>
<td>80.2 ± 30.0 (31)</td>
</tr>
<tr>
<td>Na\textsubscript{v}1.2</td>
<td>10.4 ± 4.0(^{bc,**}) (21)</td>
<td>39.1 ± 16.9(^{*}) (26)</td>
<td>91.8 ± 12.8(^{**}) (31)</td>
</tr>
<tr>
<td>Na\textsubscript{v}1.3</td>
<td>52.5 ± 6.2(^{aa,bb,cc,*}) (31)</td>
<td>96.4 ± 35.6 (20)</td>
<td>84.7 ± 17.8(^*) (16)</td>
</tr>
<tr>
<td>Na\textsubscript{v}1.6</td>
<td>20.0 ± 3.5(^{cc}) (26)</td>
<td>81.5 ± 36.1 (34)</td>
<td>54.6 ± 23.6 (16)</td>
</tr>
</tbody>
</table>

Numbers of individual data points are indicated in brackets. \(a\) indicates \(P<0.05\) and \(aa, bb, cc\) indicate \(P<0.01\) for comparison between effects of the same AED on
different α-subunits (Student’s t-test). * indicates P<0.05 and ** indicate P<0.01 for comparison between effects of different AEDs on the same α-subunit (Student’s t-test).

Binding rates of AEDs to the inactivated Na\(^+\) channel α-subunits The pharmacological profiles reported above all depend on the binding rates of the AEDs to the inactivated Na\(^+\) channel, which can be determined using a voltage-step protocol (Fig. 8A, inset) (Kuo & Lu, 1997). The Na\(^+\) channel was exposed to a depolarizing voltage step at –40 mV of varying duration (30–2500 ms), which determined, given a specific binding rate, which fraction of the channels in the high affinity inactivated state would be bound by the AED (Fig. 8A). Next this depolarization was followed by a 5 ms hyperpolarization at –120 mV sufficient to remove inactivation from all unbound channels. Finally the latter fraction was exposed to a 25-ms depolarization to -10 mV. Subtracting the amplitude of this current from the one evoked in the absence of the AED yielded the blocked current. This procedure also corrected for a reduction in current amplitude due to a slow inactivation process (Fig. 8B). For first order blocking kinetics the relation between blocked current amplitude and pre-pulse duration should follow a single exponential function (comparable to Eq. 3) (Fig. 8B). Then, the reciprocal time constant (i.e. rate) is a linear function of the concentration and its slope represents the binding rate constant (Fig. 8C); this procedure was repeated to provide the binding rate constants of DPH, CBZ and LTG for all four α-subunits (Fig. 8D, Table 4). The CBZ binding rate constant was approximately three times larger than that of LTG or DPH (Table 4, \(p<0.01\)), while the latter two were not distinguishable. The Na\(_{V1.1}\) channel subtype had the largest binding rate constant with all three AEDs, although
the difference with the other three subunit types only reached significance for LTG.

Fig. 8 AED binding to the inactivated state of the four Na$^+$ channel α-subunits. (A) Example traces of Na$^+$ currents evoked with the test potential step to –10 mV following a 30-, 250-, and 2500-ms pre-pulse to –40 mV in (a) the absence of and (b) the presence of 50 μM DPH. Pre-pulse durations (Δt) were 30, 62.5, 125, 250, 500, 1000, 1500 and 2500 ms. Before the test potential step to –10 mV the voltage was stepped back for 5 ms to –120 mV to allow the drug-free channels to recover from inactivation (protocol given as inset). (B) The blocked Na$^+$ current amplitude was determined by subtracting the current recorded in the presence of DPH from
Chapter 2

The control current and was plotted against \( \Delta t \). The data points were fitted with a mono-exponential function to determine the time constant (\( \text{Tau} \)) for development of block in the presence of 10, 20, 50, 100 and 200 \( \mu \text{M} \) DPH. (C) The binding rates (1/\( \text{Tau} \), \( \text{s}^{-1} \)) in individual Na\(_\text{V1.1}\)-expressing cells are plotted against the DPH concentration. The slope of the linear regression gives the binding rate constant of 14200 \( \text{M}^{-1}\text{s}^{-1} \) to Na\(_\text{V1.1}\) for DPH (\( n=27 \)). The binding rate constants of CBZ, DPH and LTG for the four \( \alpha \)-subunits are given and compared in Table 4. (D) The mean binding rates of CBZ (left panel), DPH (middle panel) and LTG (right panel) to the four \( \alpha \)-subunits, fitted with a linear equation. Error bars indicate S.E.M.

Table 4 Binding rate constants of AEDs to the Na\(_{\text{V1.1}}\), Na\(_{\text{V1.2}}\), Na\(_{\text{V1.3}}\) and Na\(_{\text{V1.6}}\) \( \alpha \)-subunits.

<table>
<thead>
<tr>
<th>Types</th>
<th>Binding rate constant (x 10(^3) M(^{-1}) s(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DPH</td>
</tr>
<tr>
<td>Na(_{\text{V1.1}})</td>
<td>14.0 (\pm) 2.0(^*) (27)</td>
</tr>
<tr>
<td>Na(_{\text{V1.2}})</td>
<td>8.0 (\pm) 1.2(^*) (18)</td>
</tr>
<tr>
<td>Na(_{\text{V1.3}})</td>
<td>10.5 (\pm) 1.2(^*) (29)</td>
</tr>
<tr>
<td>Na(_{\text{V1.6}})</td>
<td>8.5 (\pm) 1.9(^*) (23)</td>
</tr>
</tbody>
</table>

Numbers of individual data points are indicated in brackets. \( a \) indicates \( p<0.05 \); \( aa, bb, cc \) indicate \( p<0.01 \) for comparison between subunits and \( ** \) indicates \( p<0.01 \) for comparison with CBZ (test for homogeneity of regression coefficients).

Unbinding rates of AEDs from the Na\(^+\) channel \( \alpha \)-subunits The affinity of an AED is a combination of the binding rate of the drug and the rate at which the drug dissociates from its binding site. The latter was determined with a voltage-step protocol that resembled the one used in Fig. 8: a depolarization from –120 mV to –40 mV lasted 2 seconds and allowed complete binding of the AED to the inactivated channels (Fig. 9A, inset). In the following hyperpolarization to –120 mV the unbinding of the drug is rate limiting as it is much slower than the removal of voltage dependent
inactivation. Varying the duration of the hyperpolarization (5-500 ms), testing the result with a standard depolarization to –10 mV and subtracting the current evoked in the absence of the drug, allowed to determine the single exponential that relates blocked current to pulse duration (Fig. 9Ac). The reciprocal of the time constant (i.e. the off-rate) is independent of drug concentration and defines the subunit-specific unbinding rate for CBZ, LTG and DPH (at –120 mV, Fig. 9B). The off rates were determined for each drug using two concentrations which always gave the same result (DPH: 20 & 100 μM, CBZ: 20 & 100 μM, LTG: 30 & 300 μM). The off rate of LTG is much lower than that of DPH and CBZ (Fig. 9B; p<0.01). The off-rates for all AEDs were always the lowest for the Na\textsubscript{V}1.6 α-subunit. For LTG the off-rate for Na\textsubscript{V}1.3 was also lower than that for the Na\textsubscript{V}1.1 and Na\textsubscript{V}1.2 α-subunit.

**Discussion**

In the present study we performed a detailed comparison of the biophysical and pharmacological properties of human Na\textsubscript{V}1.1, Na\textsubscript{V}1.2, Na\textsubscript{V}1.3 and Na\textsubscript{V}1.6 α-subunits stably expressed in HEK293 cells. Although these have previously been characterized individually (Goldin, 2001; Catterall et al., 2005), only subtle functional differences have been described and it is unclear from these studies whether these are tangible differences or whether they can be attributed to differences in cell host systems (e.g. CHO cells vs. HEK cells), transfection methods (transient, vs. stable) and/or recording solution compositions. Our study therefore represents the first systematic comparison of the four major brain Na\textsuperscript{+} channel α-subunits using the same expression system and identical recording conditions. We compared the biophysical properties of the four α-subunits and subtle differences were observed (Table 1).
Fig. 9 Unbinding rates of AEDs from the inactivated Na_{1.1}, Na_{1.2}, Na_{1.3} and Na_{1.6}. (A) Example traces of Na_{1.3} currents evoked with a testing step potential to −10 mV, following 5-, 30-, and 500-ms pre-pulses to −120 mV in the absence (a) and presence (b) of 50 μM CBZ. The membrane was held at −40 mV for 2000 ms to permit drug binding to the inactivated channels, followed by a step to a recovery potential at −120 mV with a variable time duration Δt to facilitate channels to recover from inactivation and to allow drug dissociation (protocol shown as inset). The blocked current was determined by subtracting the current recorded in the presence of CBZ from the control current (measured in the absence of CBZ) and plotted against the time duration of the recovery Δt (c). The data points were fitted with a mono-exponential equation to determine the time constant (Tau) of CBZ unbinding, showing that the unbinding rate is concentration-independent (tau...
~18 ms for 50 and 200 µM CBZ). (B) CBZ, LTG and DPH unbinding rates from all four α-subunits. These off rate values were determined by calculating the reciprocal of the time constants. For all four α-subunit subtypes, LTG showed a slower off rate than CBZ and DPH (multi-factor ANOVA followed by Fisher’s LSD post-hoc test). * indicates p<0.05 and ** indicates p<0.01 for comparison between different drugs dissociating from the same α-subunit. a indicates p<0.05 and aa, bb and cc indicate p<0.01 for comparison between different α-subunits from which the same drug dissociates. Error bars indicate S.E.M. Due to non-homogeneity of variance, the statistical analysis was performed on the log-transformed data.

The recovery from inactivation for the NaV1.1 and NaV1.6 subunits were relatively fast. The faster recovery from inactivation for the NaV1.1 subunit may also contribute to the fast-spiking feature of NaV1.1-expressing interneurons. NaV1.6 is present at high densities in axon initial segments and nodes of Ranvier (Boiko et al., 2001; Ogiwara et al., 2007; Lorincz & Nusser, 2008). The faster recovery from inactivation may facilitate action potential initiation and propagation along axons. The NaV1.2 and NaV1.3 subunits displayed a slower recovery from inactivation, which could result in a reduced excitability at the (sub-cellular) site where these subunits are present. For example, the presence of the NaV1.3 subunit in neonatal neurons may help provide new-born mammals with a neuroprotective mechanism against hypoxic conditions (Park & Ahmed, 1991; Cummins et al., 1994). Alternatively, this property may balance the increased excitability due to the presence of a window current and a persistent Na⁺ current through the NaV1.3 subunit at relatively depolarized membrane potentials (see above). Together with knowledge of precise distribution patterns of the four Na⁺ channel α-subunits, our data can provide more insight into neural computation and signal processing of single neurons.
The smaller difference between $V_h$ for activation and $V_h$ for inactivation for the NaV1.1 subunit results in a larger window current (Patlak, 1991; Johnston, 1995). The window current for the NaV1.1 subunit is indeed larger than those of the other three subunits. This may indicate that the cellular or subcellular locations where the NaV1.1 subunit is densely expressed are more excitable. Recent studies showed that the NaV1.1 subunit is primarily expressed in GABAergic interneurons and largely co-expressed in parvalbumin- and Kv3.1b-positive interneurons (Yu et al., 2006; Ogiwara et al., 2007; Lorincz & Nusser, 2010; Martin et al., 2010). The larger window current for the NaV1.1 subunit might contribute to the fast-spiking feature of these interneurons. Although not as large as the NaV1.1 window current, the NaV1.3 subunit was also found to generate a considerable window current. The peak of the NaV1.3 subunit was at a relatively depolarized membrane potential (~ -35 mV, whereas the peaks of the window currents through the other subunits were at ~ -45 mV). In addition to the window current that is present at relatively depolarized membrane potentials, the NaV1.3 subunit is also capable of generating a persistent Na$^+$ current, which peaks at even more depolarized membrane potentials (Sun et al., 2007). Therefore, in cells expressing the NaV1.3 subunit, the presences of a window current and a persistent Na$^+$ current through this channel that are active at depolarized membrane potentials, will have profound consequences for neuronal excitability.

We further compared the interactions of CBZ, DPH and LTG with the four α-subunits. The AED binding sites are located on the α-subunits of the Na$^+$ channels (Rogawski & Loscher, 2004). However, it has to be kept in mind that β-subunits can influence AED efficacy (Lucas et al., 2005; Uebachs et al., 2010).
The AEDs we studied block voltage-gated Na\(^+\) channels in a frequency- or use-dependent manner. Higher frequency activity causes Na\(^+\) channels to accumulate in the inactivated state and these AEDs have much higher affinity for the inactivated state than for the closed and open states (Macdonald & Kelly, 1995; Rogawski & Loscher, 2004). This was illustrated by the observation that CBZ blocked Na\(^+\) currents faster with higher frequency depolarization steps (50 Hz vs. 10 Hz) in a concentration-dependent manner. The increase in the degree of block from 10 Hz to 50 Hz was not influenced by the concentration of CBZ, suggesting that within the concentration range used (50 µM and 200 µM), CBZ binding rate is fast enough to effectively block Na\(^+\) currents with either protocol (50 Hz vs. 10 Hz). Therefore the development of CBZ block depends solely on the fraction of available (i.e. inactivated) channels. No differences in the frequency-dependent increase in the degree of CBZ block were found between the four \(\alpha\)-subunits.

The frequency- or use-dependent block of these AEDs is attributed to their preferential affinity for inactivated Na\(^+\) channels. They are able to concentration-dependently shift the steady-state inactivation curves of the Na\(^+\) currents to more hyperpolarizing potentials and to slow the recovery from inactivation process. When comparing the effects of the three AEDs, we observed that the maximal shift of the \(V_h (\Delta V_{h_{\text{max}}})\) value for steady-state inactivation evoked by LTG was larger than that of CBZ and DPH (~30 mV vs. ~15 mV). When comparing AED affinities for the four subunits subtle differences were observed. It appeared that the affinity of CBZ for the \(\text{Na}V_{1.3}\) subunit was somewhat higher (lower \(EC_{50}\) value) than that for the other three subunits. Furthermore, the affinities of DPH and LTG for the \(\text{Na}V_{1.2}\) subunit were found to be higher (lower \(EC_{50}\) values). We also
observed that the efficacy of CBZ and DPH in blocking the window current was in the same range as the effects on the steady-state inactivation. However, it appeared that for LTG the EC\textsubscript{50} values were smaller than those obtained for shifting the steady-state inactivation relationship (Tables 2 and 3). This could be explained by a relatively slow unbinding of LTG (as compared to CBZ and DPH), which could induce a more efficient block of the window current. This indicates that the sensitivity of neurons of different brain areas for AEDs could be distinctly different, dependent on the variant Na\textsuperscript{+} channel make-up of neurons present in these brain areas (Clare \textit{et al.}, 2000; Trimmer & Rhodes, 2004).

In order to test the hypothesis that differences in binding rates to the $\alpha$-subunits may underlie the different frequency sensitivity of AED block we measured binding rates experimentally. As expected we found that CBZ had a (much) higher binding rate to inactivated Na\textsuperscript{+} channels than DPH and LTG and the magnitude of these rates (CBZ: $25\sim30\times10^3$ M\textsuperscript{-1}sec\textsuperscript{-1}, DPH: $8\sim14\times10^3$ M\textsuperscript{-1}sec\textsuperscript{-1}, LTG: $8\sim15\times10^3$ M\textsuperscript{-1}sec\textsuperscript{-1}) are in the range previously described for native voltage-gated Na\textsuperscript{+} channels (Kuo & Bean, 1994; Kuo \textit{et al.}, 1997; Kuo & Lu, 1997). Thus the high CBZ binding rate can explain why the development of CBZ block (for all four $\alpha$-subunits) has a relative small frequency and concentration dependency. The difference in the binding rate constants of these three AEDs may underlie differences in their efficacy to control epileptic discharges. The therapeutic concentrations in the cerebrospinal fluid are $\sim8$ µM for DPH (Sherwin \textit{et al.}, 1973; Richens, 1979; Kuo \textit{et al.}, 1997), $5 \sim 13$ µM for CBZ (Kuo \textit{et al.}, 1997) and $\sim20$ µM for LTG (Kuo & Lu, 1997). So the macroscopic binding rates are $0.06\sim0.12$ sec\textsuperscript{-1} for DPH, $0.33\sim0.39$ sec\textsuperscript{-1} for CBZ and $0.16\sim0.30$ sec\textsuperscript{-1} for LTG (the product of drug concentration and the binding rate constant). With the
slowest binding rate, DPH would require a prolonged depolarization for at least a few hundred milliseconds in clinical situations to exert its antiepileptic action. Such a pharmacological property may ensure that DPH inhibits ictal discharges on sustained depolarization without affecting normal action potential firing, but it may also make DPH relatively ineffective in prohibiting shorter ictal depolarizations. In contrast to DPH, CBZ has the fastest binding rate, which may make it more effective than DPH against ictal discharges with relatively short depolarizations. The patients who respond well to CBZ, but not to DPH might have bursts of discharges with shorter depolarization phases.

Another parameter relevant for AED efficacy is the dissociation rate. The lower LTG off rate may explain why LTG is able to induce a larger shift of the steady-state inactivation curve as compared to the effects of CBZ and DPH since it occupies its binding site for a longer period, allowing it to evoke a larger larger shift of $V_h$ and consequently a larger block. This may also explain the more efficient block by LTG of the window current (see above).

The use-dependent manner of these AEDs suggests that they preferentially inhibit high-frequency discharges. Based on the necessity of high-frequency discharges for $Na^+$ current block to occur, one may reason that the drugs could also exert their blocking effect on $Na^+$ currents when high-frequency discharges occur in inhibitory interneurons. During a seizure onset of 20 to 30 Hz oscillations, principal neurons in deep and superficial layers of the entorhinal cortex did not generate action potentials during fast activity at ictal onset, whereas sustained firing was observed in putative interneurons (Gnatkovsky et al., 2008). It is possible that the drugs also inhibit sustained firing in these interneurons, which might have an important consequence on
the whole network excitability at the crucial time point of interictal-ictal transition. Our results show that LTG has a faster binding rate to the Na\textsubscript{V}1.1 subunit than to other three subtypes. Some recent studies indicate that Na\textsubscript{V}1.1 is a predominant sodium channel subtype in certain subpopulations of inhibitory interneurons (Yu et al., 2006; Ogiwara et al., 2007; Lorincz & Nusser, 2008). Combining these two findings, we suggest that the drugs might have different binding kinetics to distinct neuronal types. Therefore it would be of interest to investigate if a neuron-type dependent sensitivity for AEDs exists.

Our study shows that subtle differences exist between the biophysical properties of the four major Na\textsuperscript{+} channel α-subunits, which – depending on the channel make-up of neurons and/or subcellular compartments – can be important for differences in neuronal excitability. Furthermore, the also subtle differences between AED-Na\textsuperscript{+} channel interaction may – again in the light of α-subunit composition of neurons and/or subcellular compartments – have relevance for AED efficacy.

Acknowledgements

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