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Lidocaine partially depolarizes the S4 segment in domain IV of the sodium channel

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Abstract

Previous studies have shown that lidocaine and other local anesthetic drugs (LAs) cause use-dependent block of sodium current (I_{Na}), i.e., block that increases with membrane depolarization by allosteric coupling between drug binding in the inner pore and the S4s in domains III and IV. MTSET protection experiments have established that LAs stabilize DIIIS4 in an outward, depolarized position. Similar tests have not been reported for the DIVS4, although LAs have been shown to reduce DIV's contribution to total gating charge by about one third and to alter its movement such that it contributes more gating charge at negative potentials around -100 mV compared to non-drug-bound sodium (Na) channels. To investigate whether lidocaine reduces the gating charge of DIVS4 by causing it to adopt either a depolarized position at rest or by restricting its outward movement upon depolarization, we performed MTSET protection experiments on I_{Na} of the mutant Na channel, R1628C (R3C-DIV), in the presence and absence of 10 mM lidocaine. The results indicate that lidocaine causes the DIVS4 to assume a more depolarized position, which facilitates its movement upon depolarization leading to the excess gating charge at potentials near -100 mV.

Keywords

Gating current; $Na_v1.5$; Heart; Mutation; Local anesthetic drug

Introduction

Sodium (Na) channels remain an important target for therapy of cardiac arrhythmias by lidocaine and other local anesthetic drugs (LAs) because of the presence of use-dependent block of ionic current, a property that results from the variable affinity for the drug based upon different voltage-dependent conformations of the channel [11,12]. The cardiac Na

(Na_v1.5) channel is a protein of about 2,000 amino acids, and it is responsible for the rapid conduction of the cardiac impulse throughout heart muscle. Voltage dependence arises from its four voltage sensors formed by the fourth (S4) of six transmembrane segments in each of four domains of the α subunit of Na channels [4]. The movements of the voltage sensors are coupled to changes in the channel's three-dimensional conformation to precisely control the channels' kinetic states. Because use-dependent block of I_{Na} results from changes in membrane potential consistent with the modulated receptor hypothesis [11,12], it would be anticipated that local anesthetic drugs such as lidocaine would also affect the gating charge–voltage (Q – V) relationship of cardiac Na channels. Such is the case, and studies have shown a dramatic effect on the Q – V relationship characterized by a large reduction in Q_{max} (typically 38–40%) accompanied by an excess of gating charge at more negative test potentials producing a shift of the $V_{1/2}$ to more negative potentials associated with a reduction in its voltage-dependent slope (see Fig. 1) [7,9]. Reductions of Q_{max} by LA drugs have also been demonstrated for other Na channel isoforms, e.g., see [2,5,13,18]. Recently, it has been shown that the reduction in Q_{max} arises from altered movement of the voltage sensors in domains III and IV with little or no apparent involvement by the S4s in domains I and II [18,23]. Lidocaine completely inhibits the movement of DIIIS4 by stabilizing it in an outward, depolarized position while the charge contribution of DIVS4 was reduced by about one third. Although the movement of the DIVS4 was only partially affected, the remaining gating charge moves with an altered voltage dependence, and it is responsible for excess gating charge at negative potentials near –100 mV and a leftward shift in the $V_{1/2}$ of the Q – V relationship (see Fig. 1).

Even though lidocaine has a greater effect on DIIIS4 than on DIVS4, when individual S4s were artificially stabilized in an outward conformation using externally applied MTSEA–biotin [25], stabilization of only the DIVS4 resulted in a comparable increase in the affinity of lidocaine block compared to stabilization of DIIIS4 alone. These results suggested that partial stabilization of the DIVS4 may promote lidocaine block as much as the complete stabilization of the DIIIS4. To better define the effects of lidocaine on the position of the DIVS4, we undertook protection experiments using mutant cardiac Na channels containing a cysteine substitution in the DIVS4 (R1628C or DIV-R3C) and extracellular MTSET (MTSET_O), and found that lidocaine allowed MTSET_O to modify DIVS4 even when the holding membrane potential was –150 mV, thus indicating that lidocaine stabilizes the DIVS4 in a depolarized position.

Methods

cDNA clones and cell preparation

In hH1a Na channels (Na_v1.5, kindly provided by H. Hartmann and A. Brown [10]), the third outermost arginine residue (R1628 based upon hH1a numbering or R1629 based upon hH1 numbering, [6]) in the S4 segment of domain IV was mutated by four-primer PCR [3] to a cysteine. This mutation was made in the background Na channel in which the cysteine in the outer pore at position 373 was mutated to a tyrosine (C373Y) [21] in order to avoid reaction with MTS reagents. All cDNA inserts were confirmed by sequencing, and the cDNAs were subcloned directionally into the mammalian expression vector pcDNA 5/FRT (Invitrogen, Carlsbad, CA, USA). Multiple single tsA201 cells were fused using polyethylene glycol to form large, single fused cells, cultured for several days to allow for membrane remodeling, and then transiently transfected with channel constructs using calcium phosphate (Invitrogen) [25]. Cells were maintained in Dulbecco's Eagle Medium (DMEM) (Invitrogen) supplemented with 10% fetal bovine serum, 1% penicillin–streptomycin, and selection antibiotic in 60–100-mm Corning (Acton, MA, USA) culture dishes.

Solutions, recording technique, experimental protocols, and analysis

Control extracellular solution contained (in millimolar) 15 NaMES, 185 TMA-MES, 2 Ca(OH)₂, 2 CaCl₂, and 10 HEPES (pH 7.2). Intracellular solution contained 200 TMA⁺, 200 F⁻, 10 EGTA, and 10 HEPES (pH 7.2). TMA⁺, MES⁺, and 4 mM Ca²⁺ were used because they minimized leak currents. [2-(Trimethylammonium)ethyl] methanethiosulfonate bromide (MTSET; Toronto Research Chemicals, ON, Canada) at 200 μM was added to the extracellular solution just prior to use. Changes in bath solution were achieved by placing the cell adjacent to the inlet of one of four parallel experimental chambers containing the desired extracellular solution. Typically, the cell membrane potential was set to -150 mV, and the cell was moved to MTSET-containing solution for 5–7 min. while the V_{hp} was maintained at -150 mV, then it was moved back to control solution and washed for 1–2 min before repeating the voltage-clamp protocols. When the V_{hp} was changed to 0 mV, the cell was moved to a test chamber with MTSET_O and depolarized to 0 mV for 1–2 min, and then repolarized to -150 mV before moving the cell back to control solution. It has previously been shown that similar concentrations of MTSET_O have no effect on Na_V1.4 [28] or Na_V1.5 with the C373Y pore mutation [24]. For gating current (I_g) experiments, the intracellular solution contained 200 TMA⁺, 75 F⁻, 125 MES⁻, 10 EGTA, and 10 HEPES (pH 7.2), and 1.0 μM saxitoxin (Calbiochem Corp., San Diego, CA, USA) added to the extracellular solution. Lidocaine (Sigma-Aldrich, St. Louis, MO, USA) was added to the experimental solutions at a high concentration of 10 mM to ensure a uniform population of Na channels bound to drug.

Data were obtained using a National Instruments PXI-1002 chassis with a PXI-6052 multi-function 16-bit converter and LabView 7.0 (National Instruments Corp., Austin, TX, USA), filtered by the headstage (~100 kHz), with data digitized at either 100 kHz or 200 kHz. For all recordings and labeling, the temperature was set to 13°C using a SensorTEK (Physiotemp Instruments, Inc., Clifton, NJ, USA) TS-4 thermoelectric stage mounted beneath the bath chambers, which typically allowed temperature to vary less than 0.5°C during an experimental set. Data were analyzed utilizing Matlab (The Mathworks, Inc., Natick, MA, USA) and Origin (OriginLab Corp, Northampton, MA, USA). Peak I_{Na} was taken as the mean of four data samples clustered around the maximal value of current that had been digitally filtered at 5 kHz, and leak corrected by the amount of the extrapolated time-independent linear leak calculated from the linear conductance calculated from currents between -190 mV and -110 mV. Traces were capacity corrected using four to eight scaled current responses to steps typically between -150 mV and -180 mV recorded before each pulse protocol.

Voltage-dependent Na channel availability data were fit with a Boltzmann relationship:

$$I_{Na}/I_{max}=1/(1+\exp(V_c - V_{1/2}/dx)) \quad (1)$$

where I_{Na} is the peak current during a 50-ms test depolarization to 0 mV after a 500-ms conditioning pulse to various conditioning potentials (V_c). The fitted parameters were the maximal current (I_{max}), the half-point of the relationship ($V_{1/2}$), and the slope factor of the relationship (dx). There were 1.5 s between each sweep. For comparison between cells, data were normalized to I_{max} for each cell in control.

Peak I_{Na} from step depolarizations were fit with a modified Boltzmann equation:

$$I_{\text{Na}} = (V_t - V_{\text{rev}})G_{\text{max}} / (1 + \exp(V_t - V_{1/2}/dx)) \quad (2)$$

where I_{Na} is the peak current in response to a 50-ms step depolarization to the test potential (V_t) running at 1 Hz. The fitted parameters were G_{max} , the maximal conductance; $V_{1/2}$, the half-point of the relationship; dx , the slope factor in millivolts; and V_{rev} , the reversal potential. For comparison between cells, data were normalized to G_{max} for each cell in control.

Gating current protocols contained four replicates offset by one fourth of 60-Hz cycle to aid in rejection of this frequency. For I_g measurements, the membrane potential was held at -150 mV and stepped to test potentials for 26.5 ms at 1 Hz, and were leak corrected by the mean of 2 to 4 ms of data usually beginning 8 ms after the change in test potential and capacity corrected using four to eight scaled current responses to steps between -150 mV and -180 mV.

Differences between parameters were assessed using paired t tests, and parameters were considered significant when $p < 0.05$. Data in text are expressed as means $\pm$ SD, and data in figures are graphed as means $\pm$ SEM.

Results

The effects of LAs on the Q - V relationship of cardiac Na channels have been previously published, e.g., [5,7,8,18], and are illustrated in Fig. 1a. Note the large decrease in Q_{max} of nearly 40% and the presence of additional gating charge at test potentials between -120 and -80 mV, which accounts for the shift in the $V_{1/2}$ and the change in slope factor. Previous studies have shown that about three fourths of the decrease in Q_{max} by LAs results from the complete stabilization of the DIIS4 in an outward, depolarized position [18,23] while the remaining one fourth of the decrease in Q_{max} arises from the partial inhibition of the DIVS4. Also, it has been determined that the total contribution of the DIVS4's gating charge is approximately 30% of Q_{max} [26], and, therefore, LAs inhibit about one third of the DIVS4 gating charge. The reduction in the gating charge of DIVS4 could result either from LAs (1) biasing DIVS4 in a partially depolarized position normally associated with a more positive membrane potential or (2) restricting/altering the outward movement of DIVS4 when the membrane potential is depolarized. Either possibility would decrease the contribution of DIVS4 to total gating charge when the channel is bound by LAs. To determine which is the case, we carried out MTSET protection experiments of a channel in which a cysteine replaced the third outermost arginine in DIVS4 (DIV-R3C).

Earlier studies on the MTS accessibility of cysteine substitutions of arginine residues in the DIVS4 have shown voltage-dependent modification of DIV-R2C and DIV-R3C with both residues accessible to extracellular MTS reagents only when the membrane potential is depolarized [28]. We selected DIV-R3C for study rather than R2C for three reasons: (1) The decay kinetics of I_{Na} in response to step depolarizations of DIV-R3C more closely approximates WT channels whereas DIV-R2C displays markedly slowed current decay [26,28] consistent with the third outermost arginine in the S4-DIV making only a small contribution to the gating charge from that domain. (2) Because R3C is one turn closer to the intracellular end of S4, it should provide a stronger test of LA's effect on the DIVS4 position. (3) MTSET_O modification produces much more dramatic changes in kinetics in DIV-R3C than it does in DIV-R2C [28]. In the presence of 10 mM lidocaine, changes in the Q - V relationship of R3C-DIV were similar to WT (Fig. 1b), although the mutant channel exhibited a somewhat smaller decrease in Q_{max} ($33 \pm 3\%$) and a only a modest increase in

gating charge at test potentials near -100 mV as would be expected if the gating charge contribution from the S4-DIV were decreased. Despite this, however, the voltage dependence of gating charge (the slope factor of the Q - V relationship) was reduced as in WT channels.

MTSET_O modification of R3C-DIV was voltage dependent as seen in the peak I_{Na} voltage (I - V) relationships for DIV-R3C (Fig. 2). Consistent with the previously reported studies of Na_v1.4, DIV-R3C in Na_v1.5 was modified after exposure to 200 μ M MTSET_O when the membrane potential was depolarized to 0 mV for 1 – 2 min. The most apparent change in the peak I - V relationship after modification was the increase in maximum conductance, G_{max} , ($\sim 30\%$) secondary to marked slowing of I_{Na} decay (Fig. 2b, lower). In contrast, DIV-R3C was protected from modification by MTSET_O when the cell membrane potential was held at -150 mV; there was no change in G_{max} or on I_{Na} time course (Fig. 2b, middle).

Voltage-dependent Na channel availability relationships also demonstrated voltage-dependent changes by MTSET_O. When V_{hp} was held at -150 mV during exposure to MTSET_O, there was no change in the voltage-dependent availability curves after washout of MTSET_O (Fig. 3). In contrast, when the membrane was held at 0 mV while the cell was exposed to MTSET_O, the availability relationship shifted rightward by nearly $+15$ mV, and I_{max} increased by almost 20% . Because of the easily recognized change in the $V_{1/2}$ of channel availability, we used this as the indicator of DIV-R3C modification by MTSET_O.

If lidocaine were to move the DIVS4 into a depolarized position even though the membrane potential remained hyperpolarized, then MTSET_O should be able to modify R3C-DIV at a holding the membrane potential of -150 mV in the presence of lidocaine. If, on the other hand, lidocaine stabilizes DIVS4 at or near its resting position, then in the presence of lidocaine MTSET_O would not be expected to modify the channel when the holding potential is maintained at -150 mV. We repeated the MTSET_O protection experiments in the presence and absence of lidocaine while the membrane potential was held constant at -150 mV. Figures 4 and 5 show the results for four cells. In the absence of lidocaine, Na channel availability relationships obtained in control and after the exposure to 200 μ M MTSET_O while the V_{hp} was -150 mV showed almost no change. However, in the presence of lidocaine, even though the membrane potential was held at -150 mV, the cysteine in DIV-R3C was no longer protected from modification by MTSET_O. Similar results for lidocaine-enhanced modification of DIV-R2C by MTSES_O was found for two cells (data not shown). We conclude, therefore, that lidocaine stabilizes the DIVS4 in a depolarized position when lidocaine is bound.

Discussion

Even though the LA binding site has been shown to be within the inner pore of the Na channel, drug binding greatly affects the movement of the S4s in both domains III and IV. Presumably, the coupling between the drug-binding site and the S4s impart the voltage-dependent change in the binding site affinity for LA drug leading to use-dependent block of I_{Na} as suggested by the modulated receptor hypothesis [11,12]. The experiments reported here further define the action of LAs on the DIVS4 by demonstrating that drug binding causes the DIVS4 to assume a depolarized position. Given that previously it has been estimated that lidocaine affects movement of only one third of the gating charge contributed by DIVS4 [23,26], we conclude that LAs only partially depolarize this voltage sensor (Fig. 5). It appears that the partial depolarization of the S4 by lidocaine predisposes it to move at more negative potentials than it would otherwise, thus producing an “excess” of gating charge observed at negative potentials compared to WT (see Fig. 1). Recently, it has been shown that the charged residues in the S4 voltage sensor interact with a “charge transfer

center” formed by the rigid cyclic cap from a Phe and a negatively charged Glu (one turn down in the corresponding S2) in combination with a third negatively charged residue, Asp, in the adjacent S3a segment of the chimera K_V1.2–K_V2.1 channel [27]. If such a charge transfer center also exists in Na channels, then lidocaine binding in the pore may ultimately cause the DIVS4 to become reset where one of its more outward positioned charged residues interacts with the charge transfer center at negative holding membrane potentials instead of one of the more inward charged residues in non-drug-bound channels. Although the cysteine substitution for R3 in DIV would be expected to reduce the amount of excess charge in lidocaine at negative potentials, the mutation itself could possibly bias the S4 to a slightly outward position, further contributing to a reduction in the excess gating charge at negative potentials in lidocaine. This possibility is suggested by the somewhat larger reduction in excess charge recorded at negative test potentials in lidocaine than would be expected if R3C-DIV only reduced $Q_{\max}$ by about 4% as suggested by previous studies [26]. Even if this were the case, R3C-DIV was not available for modification by MTSET_O at a holding potential of –150 mV (see Figs. 2 and 3). We speculate that the increase in peak the peak I – V relationship by MTSET_O modification of R3C-DIV results from the slower decay rate of I_{Na} reflecting impaired fast activation of the modified channels.

The details of the mechanism of coupling between lidocaine binding in the inner pore and the S4s in domains III and IV of Na channels remain unknown, but it is useful to consider a model based on the crystal structures of voltage-gated K channels, e.g., [16], in which depolarization causes the S4s to move outward thereby permitting the S4–S5 amphipathic helical linkers to move away from the pore domain allowing the four intracellular ends of the S6 helices to hinge outward and open the pore. The inner vestibule of the Na channel pore, formed by the four S6 segments, has been shown to contain the high-affinity binding site for LAs; in particular, there is a cluster of residues in both the DIII and DIV S6s including DIVS6-Phe1759 and DIVS6-Tyr1766, and DIIS6-Leu1461 (numbering based on hH1a) [14,19,20]. Of these, Phe1759 in the DIVS6 is clearly the most important because its mutation to a non-aromatic residue abolishes use-dependent block [1,9,17,20]. Based upon the structure of the voltage-gated K_V1.2 channel [16], the S4–S5 linkers in domains III and IV would be expected to couple to their respective voltage-sensor domains. If bulk water fills the large inner vestibule of the open Na channel pore then protonation of the alkylamino head of lidocaine would allow its charged amino group to make an electrostatic π -electron interaction with the critical aromatic ring of Phe1759 in the DIVS6 [9,15] placing lidocaine in a narrow cavity (about 6 Å in width) between DIIS6 and DIVS6. This may brace the DIIS4 in an outward position, thereby decreasing its contribution to gating charge [18,23] and cause the DIVS4 to assume a partially depolarized position that facilitates its movement at negative potentials while also decreasing its contribution to channel charge.

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Fig. 1.

Gating charge–voltage (Q – V) relationships for WT (**a**) and R3C-DIV (**b**) channels before and after exposure to 10 mM lidocaine. Each Q_{max} from the Boltzmann fits to the individual cell's Q – V relationships in control solution (*open circles*) was used to normalize the Q_{max} in lidocaine (*closed circles*). **a** In WT, the Q_{max} after lidocaine was decreased by 0.38, $V_{1/2}$ shifted from -56 mV to -65 mV, and the slope factor flattened (from -11 mV to -18 mV). **b** In R3C-DIV ($n=4$ cells), the Q_{max} after lidocaine was decreased by 0.33 ± 0.03 and the slope factor flattened (from -17 ± 1 mV to -24 ± 2 mV) (both changes were significant at $p < 0.05$). The $V_{1/2}$ was not significantly shifted (-55 ± 4 mV in control and -55 ± 6 mV in lidocaine). Panel (**a**) was adapted from [23].

Fig. 2.

The effect of extracellular MTSET on the peak $I-V$ relationships of DIV-R3C as a function of membrane potential. Panel (a) shows the mean peak $I-V$ relationship for four cells in control solution (closed circles), after exposure to 200 μ M extracellular MTSET at a V_{hp} of -150 mV for 5–7 min (closed squares), and after exposure to 200 μ M extracellular MTSET at a V_{hp} of 0 mV for 1–2 min. After each exposure to MTSET_O, the cell was moved back to control solution before the voltage-clamp protocols were performed. All current traces were normalized to the maximum inward current in control. The lines represent the best fits to Eq. (2), where G_{max} decreased to 0.97 ± 0.05 after exposure at V_{hp} -150 mV (ns) but it increased to 1.3 ± 0.13 after exposure at 0 mV ($p < 0.05$). The $V_{1/2}$ was -48 ± 5 mV in control, -51 ± 5 mV at V_{hp} -150 mV ($p < 0.05$), and -53 ± 5 mV at V_{hp} 0 mV ($p < 0.05$). The slope factors were -6.6 ± 0.8 mV in control, -6.5 ± 0.9 mV at V_{hp} -150 mV (ns), and -5.4 ± 1.1 mV at V_{hp} 0 mV ($p < 0.05$). Panel (b) shows families of I_{Na} traces in control (top), after exposure to 200 μ M MTSET_O at a V_{hp} of -150 mV (middle), and after exposure to 200 μ M extracellular MTSET_O at a V_{hp} of 0 mV (bottom).

Fig. 3.

The effect of extracellular MTSET on the voltage-dependent Na channel availability relationships of DIV-R3C. Panel (a) shows the mean availability relationships for four cells in control solution (*closed circles*), after exposure to 200 μ M extracellular MTSET at a V_{hp} of -150 mV for 5–7 min (*closed squares*), and after exposure to 200 μ M extracellular MTSET at a V_{hp} of 0 mV for 1–2 min. All current traces were normalized to $I_{\max}$ in control. The *lines* represent the best fits to Eq. (1), where $I_{\max}$ was unchanged at 1.0 ± 0.1 after exposure to MTSET₀ with a V_{hp} of -150 mV (ns), but it was increased to 1.2 ± 0.1 after exposure at 0 mV ($p < 0.05$). The $V_{1/2}$ was -117 ± 1 mV in control and was -120 ± 1 mV after exposure to MTSET₀ at V_{hp} -150 mV. This was an amount consistent with the background shift in kinetics seen under these experimental conditions [22]. In contrast, the $V_{1/2}$ shifted rightward by 15 mV to -105 ± 2 mV after exposure to MTSET₀ at V_{hp} 0 mV ($p < 0.05$). The slope factors were 11.3 ± 2.2 mV in control, 11.4 ± 2.1 mV at V_{hp} -150 mV (ns), and 13.7 ± 2.2 mV at V_{hp} of 0 mV ($p < 0.05$). Panel (b) shows families of current responses at test steps to 0 mV from 500-ms conditioning steps to potentials between -180 and -40 mV in control (*top*), after exposure to 200 μ M MTSET₀ at a V_{hp} of -150 mV (*middle*), and after exposure to 200 μ M extracellular MTSET₀ at a V_{hp} of 0 mV (*bottom*)

Fig. 4.

Lidocaine removes protection of DIV-R3C by MTSET₀ during hyperpolarization. (a) shows the mean Na channel availability relationships for the same four cells in control solution (closed circles), after exposure to 200 μ M extracellular MTSET at a V_{hp} of -150 mV for 5–7 min (closed squares), and after exposure to 200 μ M extracellular MTSET in 10 mM lidocaine for 5–7 min also at a V_{hp} of -150 mV. After the control I_{Na} measurements had been obtained, the cells were moved to a bath chamber containing MSTET₀ for 5–7 min while the V_{hp} was maintained at -150 mV, and then returned back to the control solution and washed for 1–2 min before repeating the voltage-clamp protocols. The four cells were then moved to another bath chamber containing MSTET₀ in 10 mM lidocaine for 5–7 min while the V_{hp} was still maintained at -150 mV before returning back to the control solution, washed for 1–2 min, and then repeating the voltage-clamp protocols. All current traces were normalized to I_{max} in control solution. The lines represent the best fits to Eq. (1), where I_{max} was unchanged at 0.99 ± 0.1 (ns) after exposure to MTSET₀, but it was increased to 1.1 ± 0.1 ($p < 0.05$) after exposure to 10 mM lidocaine even while holding the membrane potential at -150 mV. The $V_{1/2}$ was -118 ± 4 mV in control, and it shifted slightly leftward to -120 ± 1 mV (ns) after exposure to only MTSET₀ at $V_{hp} -150$ mV. In contrast, it shifted rightward to -111 ± 4 mV ($p < 0.05$) after exposure to both lidocaine and MTSET₀. The slope factors were 10.4 ± 1.2 mV in control, 10.9 ± 1.2 mV (ns) in MTSET₀, and 13.3 ± 0.6 mV ($p < 0.05$) in both lidocaine and MTSET₀. Panel (b) shows families of current responses at test steps to 0 mV following 500-ms conditioning steps to potentials between -180 and -40 mV in control (top), after exposure to MTSET₀ (middle), and after exposure to both lidocaine and extracellular MTSET₀.

Fig. 5.

Schematic diagram showing the position of the four voltage sensors at rest with a hyperpolarized membrane potential. In control solutions, all the S4s are in a fully inward, rested state configuration. In the presence of lidocaine, the DIIIS4 is fully extended in a depolarized position [23] as illustrated with the entirety of its S4 moved upward and outside the cell membrane. The DIVS4 is in a partially depolarized position (about one third extended) with the remainder able to move upon a subsequent step depolarization