Mexiletine and Lidocaine Suppress the Excitability of Dorsal Horn Neurons

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BACKGROUND: Spinal sensitization and facilitatory processes in dorsal horn neurons after nerve injury alter spinal outflow leading to enhanced pain perception and chronic pain syndromes. Clinically used Na^+ channel blockers at doses which do not block conduction can relieve such chronic pain. Although much attention has been paid to their effect upon afferents, less work has been done with their effect on the excitability of central sensory neurons. Thus, we investigated the effects of the Na^+ channel blockers mexiletine and lidocaine on sensory spinal dorsal horn neurons.

METHODS: Patch-clamp recordings were directly performed in visualized neurons of the substantia gelatinosa in the spinal cord of young rats to investigate the effect of mexiletine and lidocaine in different types of dorsal horn neurons (tonically firing, adapting-firing, and single spike neurons).

RESULTS: All three different types of neurons responded dose-dependently to mexiletine and lidocaine. Both local anesthetics reversibly inhibited Na^+ and K^+ currents. The half-maximal inhibitory concentration for Na^+ conductance block was 89 ± 2 or 54 ± 6 μM and for delayed-rectifier K^+ conductance block was 582 ± 36 or 398 ± 14 μM for lidocaine and mexiletine, respectively. The inhibition of Na^+ and K^+ currents consecutively altered the properties of single action potentials and reduced the firing rate of tonically firing and adapting-firing neurons.

CONCLUSIONS: In clinically relevant concentrations, lidocaine and mexiletine reduced the excitability of sensory dorsal horn neurons via a blockade of Na^+ and K^+ channels. Our work confirms that, in addition to the peripheral effects of lidocaine and mexiletine, modulation of voltage-gated ion channels in the central nervous system contributes to the antinociceptive effects of these drugs used in pain therapy.

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The mechanisms of pain secondary to peripheral nerve injury are complex. Experimental evidence collected thus far indicates that peripheral nerve injury causes signal generation in the damaged nerves and in their sensory neurons.[1] The initiated action potentials (AP) lead to spinal activation sensed as painful by humans and animals.[2] In addition to the altered afferent traffic, spinal sensitization and facilitatory processes in dorsal horn neurons may contribute to the aberrant encoding of the afferent traffic leading to spinal outflow interpreted by supraspinal centers as noxious.[3]

Voltage-gated sodium channel blockers may play crucial roles in nociception.[4] Clinically used Na^+ channel blockers, such as lidocaine and mexiletine, show beneficial effects in treating neuropathic pain when added systemically as a sole drug or as adjuvants.[5–7] Importantly, these effects may be observed at plasma concentrations which do not alter the conducted potential.

Because of the high first-pass effect, lidocaine can only be administered IV. The drawbacks of continuous IV therapy, cost, and invasiveness of the treatment usually preclude its use in long-term treatment. Mexiletine, the oral congener of lidocaine, extends the use of IV lidocaine therapy and provides an alternative long-term approach in neuropathic pain treatment. The analgetic actions of lidocaine and mexiletine might be generated through central, peripheral, or mixed mechanisms.[8] Although some evidence suggests the central nervous system as the main target of

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these two drugs,[9,10] the related mechanisms must still be described in detail.

Based on behavioral, anatomical, and electrophysiological data, substantia gelatinosa neurons of the spinal cord form the first relay for a variety of different fiber types, particularly for those conveying nociceptive information via small diameter afferent fibers.[11] Thus, they play a pivotal role in the maintenance of aberrant somatosensory transmissions associated with nerve injury.[12,13] Regarding their firing patterns as a response to a long depolarizing pulse, we classified spinal dorsal horn neurons into three major physiological groups: tonically firing neurons (TFNs) or category 1 neurons, adapting-firing (AFNs) or category 2 neurons, and single spike neurons (SSNs) or category 3 neurons, supported by investigations showing that the substantia gelatinosa is formed by neurons with diverse intrinsic firing properties.[11,14–17] TFNs are characterized by maintained firing to intracellular depolarizing current pulses and by little spike frequency adaptation during sustained depolarization. They respond to cutaneous and visceral nociceptive stimulation and to nociceptive thermal stimuli.[18] AFNs generate adapted series of APs dependent on the received information from the same nocireceptors.[18] Stimulation of the excitatory field results in depolarization of TNFs and AFNs and increased AP firing. SSNs generate only up to two APs and can act as coincidence detectors encoding information by detecting the occurrence of simultaneous yet separate input signals.[19] Thus, TFNs and AFNs neurons represent an important pharmacological site for the antinociceptive action of different drugs in the central nervous system.

Thus, this study focuses on the underlying mechanisms of how lidocaine and mexiletine affect the excitability of different types of dorsal horn neurons involved in pain transmission.

METHODS

Preparation of Dorsal Horn Neurons

Experiments were performed by means of the patch-clamp technique[20] on 200 μm slices, prepared from the lumbar spinal cord (L3-6) of young rats (2–7 wk old) of both sexes.[21]

All animals were killed by concussion and rapid decapitation according to the standards of the German guidelines. The procedure was approved by the Institutional Animal Care Committee and reported to the Local Veterinarian Authority in Giessen (Regierungspräsidium Giessen, Deutschland). The spinal cord was carefully removed and put into ice-cold preparation solution enriched with O_2–CO_2 (95%–5%). The pial membrane of the spinal cord was removed and the spinal cord was embedded in 2% agar. The spinal cord was sliced and then incubated for 1 h at 32°C. The standard procedure of cell cleaning by repetitive blowing and suction of the bath solution via a broken patch pipette was not applied because each slice contained numerous dorsal horn neurons with clean surfaces.

Chemicals and Solutions

Detailed description is given in Online Supplement (available at www.anesthesia-analgesia.org).

Electrophysiology

Identification of Dorsal Horn Neurons

In spinal cord slices, dorsal horn neurons were identified as multipolar cells with a soma (8–12 μm diameter) located in the substantia gelatinosa.[22] Details are given in the Online Supplement (available at www.anesthesia-analgesia.org). Resting potentials in intact neurons were measured between −78 and −50 mV and the input resistance was 1.2 ± 0.4 GΩ.

Entire Soma Isolation (ESI) Method

Experiments in voltage clamp mode were performed using the method of ESI to reduce series resistance. Identification of a neuron in the spinal cord slice was followed by the isolation procedure monitored under infrared optics (Hamamatsu Photonics, Japan). A detailed description of the ESI method has been given in the Online Supplement (available at www.anesthesia-analgesia.org).

Current Recording

Whole-cell recordings were performed as previously described.[21,23] For a detailed description see the Online Supplement (available at www.anesthesia-analgesia.org).

Statistical Analysis and Fitting

Numerical values are expressed as mean ± SE of the mean. The normalized current amplitudes in the concentration-effect curves were fitted using a nonlinear least-squares method with the equation: $f(C) = 1 \times (1 + C(IC_{50})^{-1})^{n})^{-1}$. C is the blocker concentration, half-maximal inhibitory concentration (IC_{50}) the half-maximal inhibiting concentration, and n the Hill coefficient.[21]

For each individual recording, the firing frequency was determined as $f = (N - 1) \times (\Delta T)^{-1}$, whereas N is the number of spikes and ΔT the time interval between the first and the last spike.

Intergroup differences were assessed by a factorial analysis of variance with *post hoc* analysis using Fisher's least significant difference test. Student's paired t-test was used to compare the frequency of five repetitive current pulses before and after mexiletine or lidocaine application. Significance is assumed at the value $P < 0.05$.

RESULTS

The effects of the Na^+ channel blockers mexiletine and lidocaine were evaluated in voltage-clamp and current-clamp experiments. Na^+ currents were recorded in external TEA-solution. Pipettes were filled

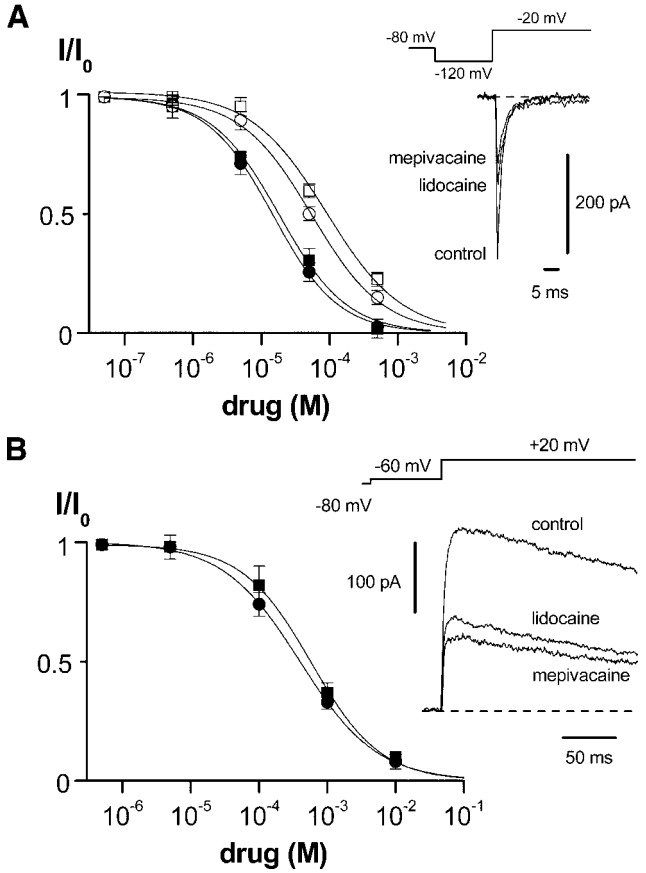

Figure 1. Concentration dependence of current suppression by mexiletine and lidocaine. (A) Concentration–inhibition curves for tonic (□ lidocaine; ○ mexiletine) and use-dependent (■ lidocaine; ● mexiletine) block. Current amplitudes (I) were normalized by the amplitude of the corresponding current recorded in control solution (I_0). Data points were fitted using the Hill equation. The IC_{50} value was 89 ± 2 μM (n = 5) for lidocaine and 54 ± 6 μM (n = 5) for mexiletine for tonic inhibition and 32 ± 3 μM (n = 5) for lidocaine and 18 ± 2 μM (n = 5) for mexiletine for use-dependent block. The Hill coefficient was 0.9. (B) Concentration dependence of K_{DR} current suppression by lidocaine and mexiletine (n = 5 for each concentration). The IC_{50} value was 582 ± 36 μM (n = 5) for lidocaine and 398 ± 14 μM (n = 5) for mexiletine. The Hill coefficients were 0.9 and 0.8.

with high-Cs_{in} solution. At a holding potential (E) of −80 mV, lidocaine and mexiletine reversibly inhibited tetrodotoxin-sensitive (TTXs) Na^+ currents (Fig. 1A). The current inhibition induced by these two drugs was concentration-dependent and complete at high concentrations. The concentration-effect curves of lidocaine or mexiletine revealed tonic inhibition IC_{50} of 89 ± 2 μM (n = 5) or 54 ± 6 μM (n = 5), respectively (Fig. 1A). Lidocaine and mexiletine produced a use-dependent block of the currents at 10-Hz stimulation. The half-maximum inhibiting concentration for the use-dependent inhibition was 18 ± 3 μM (n = 5) for lidocaine and 15 ± 2 μM (n = 5) for mexiletine. Delayed-rectifier K^+ (K_{DR}) currents were recorded in external choline-Cl solution supplemented with TTX using pipettes filled with high-K_{in} solution. Figure 1B demonstrates the inhibitory effect of lidocaine and mexiletine showing that mexiletine and lidocaine reduced the K_{DR} current. The concentration-effect curves of lidocaine or mexiletine revealed tonic inhibition IC_{50} of 582 ± 36 μM (n = 5) or 398 ± 14 μM (n = 5), respectively (Fig. 1B). The K_{DR} channels inhibition was reversible.

To investigate the impact of current inhibition on the excitability of the neurons involved in pain transmission, intact TFNs, AFNs, and SSNs dorsal horn neurons were examined by current clamp. The SSNs, AFNs, and TNFs had resting membrane potentials of −55 ± 2 mV (n = 50), −54 ± 2 mV (n = 31), or −58 ± 2 mV (n = 27), respectively. Application of lidocaine or mexiletine had no effect on resting membrane potential or input resistance of the neurons at clinically relevant concentrations. First, we compared the single APs after application of lidocaine or mexiletine using 1 ms depolarizing current pulses. All three types of neurons responded to mexiletine and lidocaine in a dose-dependent manner. The peak amplitude of the single AP was decreased, the duration of the APs (measured at half-maximal amplitude) was increased, and the maximum positive slope and negative slope decreased, indicating a Na^+ or K_{DR} current blockade, respectively (Tables 1 and 2).

The changes in repetitive firing behavior after application of mexiletine and lidocaine were investigated on TFNs and AFNs dorsal horn neurons. Figure 2 illustrates the effects of mexiletine and lidocaine in clinically relevant concentrations on a series of APs in TFNs. The lowest concentration of mexiletine (0.5 μM) reduced the number of APs from 9.6 ± 1.9 to 4.2 ± 1 (n = 5). It is noteworthy that 60% of the neurons were still able to generate a series of APs. The maximum firing frequency was reduced to 44%. In contrast, 5 and 50 μM mexiletine virtually abolished the repetitive firing neuronal activity. Mexiletine (5 μM) decreased the number of APs from 9.2 ± 1.6 to 1.8 ± 0.3 (n = 6; Fig. 2) and 50 μM decreased from 7.2 ± 1.6 to 1.0 ± 0.6 (n = 5; not shown). The effects of lidocaine in clinically relevant concentrations corresponded to that observed with mexiletine in low concentrations. Lidocaine (5 μM) significantly reduced the generation of APs (from 7.2 ± 1.3 to 1.6 ± 0.2; n = 5). After lidocaine application, a series of APs was detected only in one neuron. Lidocaine applied in a higher concentration (30 μM) completely abolished the AP series generated during the long depolarization pulses (number of APs from 8.2 ± 0.9 to 1 ± 0; n = 6; not shown). Mexiletine and lidocaine were also tested in AFNs (not shown). Mexiletine (0.5, 5, and 50 μM) reduced the maximum number of APs from 3 ± 0 to 1.2 ± 0.2 (n = 5; $P <$ 0.05), 5.3 ± 1.9 to 1.3 ± 0.3 (n = 4; $P < 0.05$), or 4 ± 0.4 to 1 ± 0 (n = 7; $P < 0.05$), respectively. Similar results were obtained with lidocaine. The decrease in the maximum number of APs after application of 5 μM lidocaine was 63% (from 5.3 ± 0.6 to 2 ± 0.3; n = 4).

Table 1. The Effects of Increasing Mexiletine Concentrations on Single Action Potentials

	Overshoot (mV)	Duration (ms)	Max. positive slope (Vs^{-1})	Max. negative slope (Vs^{-1})	*n*
SSNs					
Control (μM)	25.2 ± 1.8	2.8 ± 0.1	81.1 ± 5.7	−37.2 ± 1.6	9
0.5	23.5 ± 3.2	3.1 ± 0.3	67.8 ± 8.4	−34.6 ± 2.3	9
5	9.1 ± 1.9*	3.9 ± 0.3*	32.6 ± 5.6*	−19.3 ± 2.5*	11
50	7.2 ± 3.5*	5.4 ± 0.6*	18.8 ± 7.6*	−13.3 ± 3.6*	6
TFNs					
Control (μM)	39.3 ± 0.4	2.2 ± 0.1	131.6 ± 1.5	−57.8 ± 2.8	7
0.5	38.3 ± 2.3	2.2 ± 0.1	119.7 ± 6.6	−54.7 ± 5.2	7
5	28.3 ± 3.1†	3.0 ± 0.1	74.5 ± 8.2*	−40.3 ± 4.1†	6
50	11.5 ± 7.1*	5.7 ± 1.3*	41.3 ± 23.6*	−16.8 ± 6.7*	5
AFNs					
Control (μM)	34.5 ± 1.7	2.9 ± 0.2	106.7 ± 6.5	−40.6 ± 3.2	5
0.5	30.1 ± 5.8	3.3 ± 0.5	85.0 ± 18.5	−36.6 ± 7.9	5
5	21.7 ± 4.8*	4.1 ± 0.7	63.4 ± 17.7†	−28.4 ± 7.9	5
50	14.9 ± 4*	5.2 ± 0.9*	42.1 ± 11.2*	−17.3 ± 3.8*	5

The duration of the action potentials is measured at the half-maximum potential. Positive and negative slope are the maximum values measured at the rising and the falling phase.
SSNs = single spike neurons; TFNs = tonically firing neurons; AFNs = adapting-firing neurons.
Significance levels are expressed as * $P < 0.001$, † $P < 0.01$, and ‡ $P < 0.05$ compared with controls.

Table 2. The Effect of Increasing Lidocaine Concentrations on Single Action Potentials

	Overshoot (mV)	Duration (ms)	Max. positive slope (Vs^{-1})	Max. negative slope (Vs^{-1})	*n*
SSNs					
Control (μM)	24.2 ± 1.8	3.0 ± 0.2	81.0 ± 7.6	−37.5 ± 3.0	7
5	13.5 ± 1.8*	4.4 ± 0.5†	44.3 ± 8.9*	−23.4 ± 4.0*	7
30	5.0 ± 0.9*	5.4 ± 0.7*	20.2 ± 2.5*	−12.2 ± 1.3*	6
TFNs					
Control (μM)	38.0 ± 3.1	2.5 ± 0.2	122 ± 9.6	−52.4 ± 3.3	5
5	28.0 ± 5.9	2.8 ± 0.3	93.2 ± 10.7	−39.6 ± 3.8†	5
30	21.9 ± 3.1*	3.9 ± 0.3*	63.7 ± 10.4*	−27.3 ± 3.4*	7
AFNs					
Control (μM)	28.3 ± 1.8	2.6 ± 0.3	96.5 ± 7.2	−46.2 ± 3.1	7
5	18.9 ± 2.4*	3.6 ± 0.5	68.3 ± 6.1†	−32.6 ± 4.2†	7
30	6.4 ± 2.3*	4.8 ± 0.5*	23.4 ± 6.8*	−14.2 ± 3.0*	8

The duration of the action potentials is measured at the half-maximum potential. Positive and negative slope are the maximum values measured at the rising and the falling phase.
SSNs = single spike neurons; TFNs = tonically firing neurons; AFNs = adapting-firing neurons.
Significance levels are expressed as * $P < 0.001$, † $P < 0.05$, and ‡ $P < 0.01$ compared with controls.

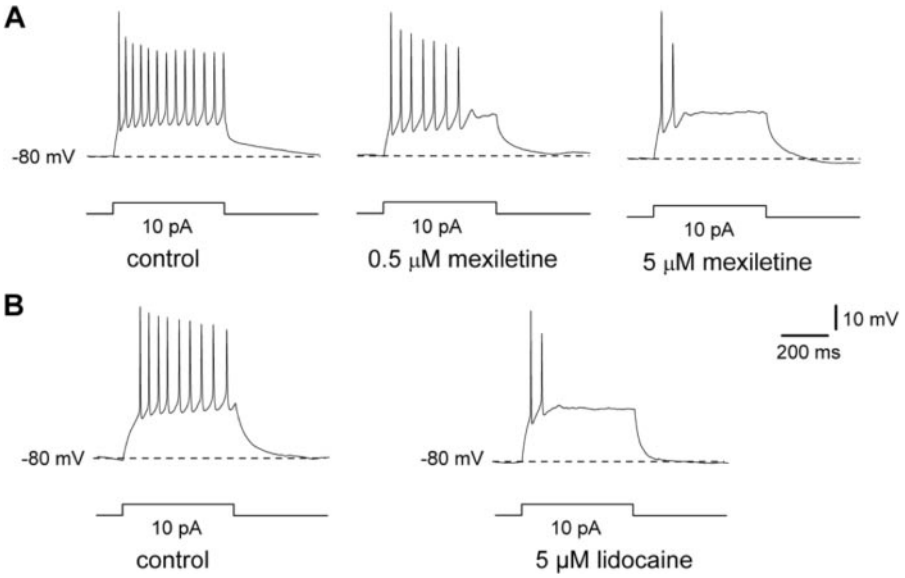

Figure 2. Decrease of firing frequency by mexiletine and lidocaine. (A) Series of action potentials are shown under control conditions (left) and after application of 0.5 (middle) and 5 μM (right) mexiletine. Dotted lines present holding potential of −80 mV. (B) The maximum firing frequency in control solutions was compared with the frequency after application of 5 μM lidocaine. Action potentials were evoked by 500 ms current pulses. Dotted lines indicate holding potential of −80 mV.

After administration of 30 μM lidocaine, the maximum number of APs was reduced to 20% (from 4.9 ± 0.6 to 1.3 ± 0.4; $n = 8$).

Voltage-dependent K^+ channels and Na^+ channels have been shown to influence interspike intervals and firing rates in neurons of the substantia gelatinosa.[24,25]

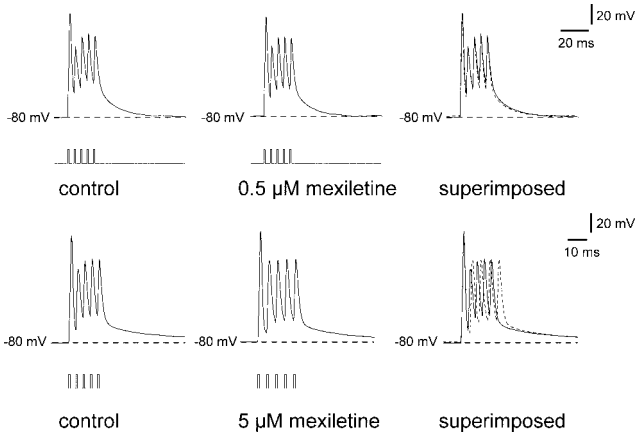

Figure 3. Mexiletine reduces the maximum action potential frequency after repetitive pulses with different interspike intervals. Figures show the effects under control conditions (left), after application of 0.5 (middle, above) and 5 μM (middle, below) mexiletine and superimposed images (right). Impulse protocol is presented below the registration.

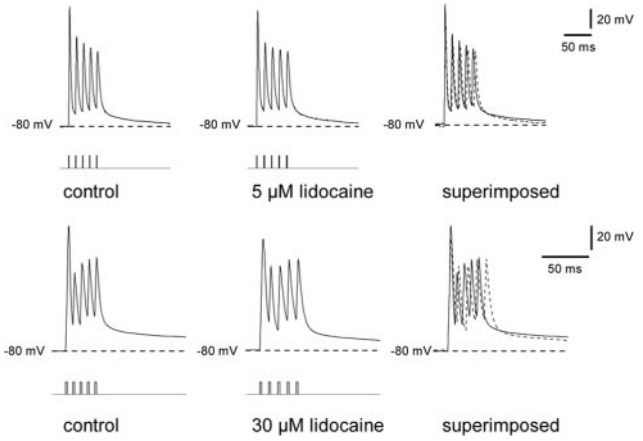

Figure 4. Lidocaine reduces the maximum action potential frequency after repetitive pulses with different interspike intervals. The maximum action potential frequency was decreased with increasing concentrations of lidocaine. Figures show currents in control solutions (left) and after applications of 5 (middle, above) and 30 μM (middle, below) lidocaine and superimposed images (right). Membrane potential was adjusted to −80 mV. Impulse protocol is shown below the registration.

Therefore, we examined the effects of mexiletine and lidocaine on interspike intervals in response to five repetitive pulses in dorsal horn neurons. The mean frequency of the dorsal horn neurons was 231 ± 9 Hz (n = 18), 207 ± 10 Hz (n = 29), 204 ± 10 Hz (n = 19), in SSNs, AFNs, or TFNs, respectively. Analysis of the action of mexiletine and lidocaine on all types of dorsal horn neurons showed a dose-dependent increase of the interspike intervals and a dose-dependent decrease of the maximum possible frequency (Figs. 3 and 4 and Table 3).

DISCUSSION

Despite the increasing number of drugs tested for the treatment of neuropathic pain conditions during the last decade, Na^+ channel blockers, such as local anesthetics, still remain an important and effective therapeutic tool in modern pain therapy.[5,26,27] In chronic pain therapy, lidocaine infusions are frequently used to help to identify those patients most likely to benefit from an oral treatment of Na^+ channel blockers. Patients, who have once achieved therapeutic lidocaine levels, usually show a good response to orally administered mexiletine.[28,29] Although the clinical responses to both local anesthetics are relatively well described, it is not characterized in detail how lidocaine and mexiletine affect the excitability of central sensory neurons. Therefore, we have compared the effects of lidocaine and mexiletine on the repetitive firing behavior of substantia gelatinosa neurons via direct patch-clamp recording from visualized intact neurons of the spinal cord and found that lidocaine and mexiletine exert similar effects on the neuron excitability by blocking both, the TTXs Na^+ (only TTXs Na channels are expressed in dorsal horn neurons)[30] and the delayed-rectifier K^+ conductances, at clinically relevant concentrations.

Substantia gelatinosa neurons of the spinal cord receive primary afferent input that encodes nociceptive information conducted by C- and/or Aδ-fibers.[31–33] In the chronic pain state, abnormal activities are produced in the periphery. The dorsal horn neurons become sensitized and respond more vigorously to peripheral input, indicating that they represent a key element in the transmission of pain-related information and thus are of particular interest for pain therapy. During the last decades, different cell types, like TFNs, AFNs, and SSNs were described in the substantia gelatinosa of the spinal cord. Our investigations show that lidocaine and mexiletine both administered in clinically relevant concentrations significantly affect the single action potential properties in all three types of neurons. Furthermore, lidocaine and mexiletine decreased the frequency and stability of neuron firing involved in nociceptive pain processing. The lidocaine and mexiletine concentrations that evoke these cellular effects correspond to the concentrations necessary for antinociceptive action during systemic administration in clinical studies. Plasma concentrations of lidocaine after IV administration of 2–2.5 mg/kg generally reach 10–60 μM.[5,34] The steady state of the plasma mexiletine level after an oral dose of 450 mg/d ranged from 4 to 7 μM.[35] In this study, both local anesthetics decreased the Na^+ current, reduced the amplitude, decreased the maximum rate of rise, and increased the width of a single AP in a concentration-dependent manner. Inhibition of Na^+ channels by lidocaine or mexiletine have been also shown in peripheral nerve and dorsal ganglion neurons[36–38] and in neurons of the central nervous system.[22,39] Our results support the assumption that reduced numbers of voltage-gated Na^+ channels affect single impulse generation, which is also in good agreement with previous findings from electrophysiological studies.[16,38]

Table 3. Mexiletine and Lidocaine Decreases the theoretical Maximum Possible Frequency in Dorsal Horn Neurones

Group	Drug	Concentration (μM)	Reduction in maximum firing frequency	n
SSNs	Mexiletine	0.5	10 ± 4%	$n = 7$
		5	21 ± 7%*	$n = 9$
		50	41 ± 6%†	$n = 6$
	Lidocaine	5	23 ± 11%	$n = 6$
		30	27 ± 4%*	$n = 6$
TFNs	Mexiletine	0.5	3 ± 2%	$n = 7$
		5	23 ± 8%*	$n = 6$
		50	44 ± 9%*	$n = 5$
	Lidocaine	5	8 ± 4%	$n = 5$
		30	20 ± 2%*	$n = 5$
AFNs	Mexiletine	0.5	9 ± 4%	$n = 5$
		5	16 ± 4%*	$n = 5$
		50	40 ± 9%‡	$n = 5$
	Lidocaine	5	10 ± 4%	$n = 8$
		30	22 ± 6%*	$n = 5$

For a detailed description of the frequency calculation after application of repetitive short current pulses see Methods. The theoretical maximum firing frequency is the maximum frequency the neuron is able to follow stimulation.

SSNs = single spike neurons; TFNs = tonically firing neurons; AFNs = adapting-firing neurons.

Significance levels are expressed as * $P < 0.05$, † $P < 0.001$, and ‡ $P < 0.01$ compared with control.

The delayed-rectifier K^+ current underlies the major K^+ conductance in AFNs and TFNs.[16,17] In clinically relevant concentrations both lidocaine and mexiletine caused a significant decrease in the delayed-rectifier K^+ current. Until recently, only a few studies could show that both drugs are able to block neuronal voltage-gated K^+ channels,[40–42] whereas two-pore domain K^+ channels[43,44] or ATP-dependent K^+ channels in other cell types[45] are sensitive to local anesthetics. The reduction of delayed-rectifier K^+ channels in dorsal horn neurons induced by lidocaine and mexiletine could account for the significant reduction of the firing frequency as shown in studies by Melnik et al.[16,17] Although the inhibitory effects of lidocaine and mexiletine on the delayed-rectifier K^+ current was less pronounced than on the Na^+ current, the blockade of the delayed-rectifier K^+ current could become important if these neurons had small resources of K^+-currents, because the safety factor for K^+-channels seems to be lower than two.[24]

Progress in understanding the role of ion channels in repetitive firing behavior of subtantia gelatinosa neurons has demonstrated a complex interaction between Na^+ and delayed-rectifier K^+ conductances.[17,24] In TFNs the voltage-gated Na^+ and delayed-rectifier K^+ channels were shown to generate the basic pattern of tonic firing, whereas Ca^{2+}-dependent conductances stabilized firing and regulated discharge frequency.[17] In AFNs Ca^{2+}-dependent conductances do not contribute to adapting firing but Na^+ channels seem to be critical for determining the appearance of spike frequency adaptation.[16] Because of the finding that AFNs and TFNs are key elements for nociception in the central nervous system, the modulation of their ion channels generating APs provides an important therapeutic approach for the treatment of neuropathic pain. In this study, lidocaine and mexiletine changed the single AP properties through a blockade of the Na^+ and delayed-rectifier K^+ channels. Consecutively, the frequency of APs is decreased. Furthermore, both drugs show similar effectiveness for reducing the excitability of all types of dorsal horn neurons at lower concentrations. Thus, our work clearly confirms that, in addition to their peripheral effects, the modulation of voltage-gated ion channels in the central nervous system contributes to the antinociceptive effects of lidocaine and mexiletine used in clinical pain therapy.

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