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Effect of Drugs Used for Neuropathic Pain Management on Tetrodotoxin-resistant Na^+ Currents in Rat Sensory Neurons

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Background: **Tetrodotoxin-resistant Na^+ channels play an important role in generation and conduction of nociceptive discharges in peripheral endings of small-diameter axons of the peripheral nervous system. Pathophysiologically, these channels may produce ectopic discharges in damaged nociceptive fibers, leading to neuropathic pain syndromes. Systemically applied Na^+ channel–blocking drugs can alleviate pain, the mechanism of which is rather unresolved. The authors investigated the effects of some commonly used drugs, *i.e.*, lidocaine, mexiletine, carbamazepine, amitriptyline, memantine, and gabapentin, on tetrodotoxin-resistant Na^+ channels in rat dorsal root ganglia.**

Methods: **Tetrodotoxin-resistant Na^+ currents were recorded in the whole-cell configuration of the patch-clamp method in enzymatically dissociated dorsal root ganglion neurons of adult rats. Half-maximal blocking concentrations were derived from concentration–inhibition curves at different holding potentials (−90, −70, and −60 mV).**

Results: **Lidocaine, mexiletine, and amitriptyline reversibly blocked tetrodotoxin-resistant Na^+ currents in a concentration- and use-dependent manner. Block by carbamazepine and memantine was not use-dependent at 2 Hz. Gabapentin had no effect at concentrations of up to 3 mM. Depolarizing the membrane potential from −90 mV to −60 mV reduced the available Na^+ current only by 23% but increased the sensitivity of the channels to the use-dependent blockers approximately fivefold. The availability curve of the current was shifted by 5.3 mV to the left in 300 μM lidocaine.**

Conclusions: **Less negative membrane potential and repetitive firing have little effect on tetrodotoxin-resistant Na^+ current amplitude but increase their sensitivity to lidocaine, mexiletine, and amitriptyline so that concentrations after intravenous administration of these drugs can impair channel function. This may explain alleviation from pain by reducing firing frequency in ectopic sites without depressing central nervous or cardiac excitability.**

THE treatment of neuropathic pain still remains a major challenge in modern pain therapy. Na^+ channel blockers such as local anesthetics, antiarrhythmics, or anticonvulsants are among the drugs used clinically, and in some cases these drugs have beneficial effects when added to conventional analgesics or as the sole agents. In particular, lidocaine, mexiletine, and carbamazepine have been used successfully.[1] Lidocaine is a local anesthetic and a class Ib antiarrhythmic. Because of its high first-pass effect, it must be administered intravenously. Mexiletine is also a class I antiarrhythmic that can be administered orally, and carbamazepine is a tricyclic anticonvulsant. Other substances used include amitriptyline,[2] memantine,[3] and gabapentin.[4] Amitriptyline is a tricyclic antidepressant that centrally inhibits the reuptake of noradrenaline and serotonin and also blocks Na^+ channels. Memantine is an *N*-methyl-D-aspartate antagonist that is used as an antispastic in the treatment of Parkinson's disease. Gabapentin is a γ-aminobutyric-acid analog used as an anticonvulsant in refractory epilepsy.

The mechanisms underlying chronic pain syndromes are complicated. After peripheral nerve injury or destruction, ectopic sites of signal generation evolve in parts of the damaged nerve or in its sensory ganglion neurons.[5,6] These sites continuously initiate action potentials that are sensed as pain by the individual.[7] Furthermore, spinal sensitization and wind up processes in dorsal horn neurons augment the pain perception, leading to chronic pain syndromes.[8]

Recent evidence suggests a key role of neuronal tetrodotoxin-resistant Na^+ channels in the generation of nociceptive impulses in peripheral nerve fibers under both physiologic[9] and pathophysiologic conditions.[10] The accumulation of tetrodotoxin-resistant Na^+ channels in damaged nerves at the site of injury may lead to ectopic activity in nociceptive fibers,[10] and chronically damaged nerve fibers may have a less negative resting membrane potential at the injury site, triggering electric activity.[5] Because of their different voltage sensitivities of activation and inactivation, tetrodotoxin-resistant Na^+ channels are still capable of generating impulses at depolarized potentials, whereas tetrodotoxin-sensitive Na^+ channels are inactivated and cannot contribute to excitability.[11]

In this study, we investigated the effects of a variety of drugs more or less successfully used for chronic pain treatment, *i.e.*, lidocaine, mexiletine, carbamazepine, amitriptyline, memantine, and gabapentin, on neuronal tetrodotoxin-resistant Na^+ currents at different membrane potentials. Because these currents cannot be investigated in nociceptive fibers by the patch-clamp method, we used small- and medium-sized sensory ganglion neurons of adult rats, which are connected to

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nociceptive fibers and express tetrodotoxin-resistant Na^+ channels in their membrane.

Materials and Methods

Isolation of Dorsal Root Ganglion Neurons

Adult Wistar rats (200–300 g) were used for preparing the primary dorsal root ganglion–cell culture. Animals were killed by concussion and immediate cervical dislocation. The procedure has been approved by the local veterinarian authority. Dorsal root ganglia were removed from the full length of the vertebral column and placed into calcium- and magnesium-free phosphate-buffered saline. After cleaning the connective tissue from ganglia, they were incubated for 30 min at 37°C in 2 mg/ml collagenase Worthington type CLS II (Biochrom, Berlin, Germany) and 2 mg/ml trypsin type III-S (Sigma, Deisenhofen, Germany) dissolved in phosphate-buffered saline, in a shaking water bath. Afterward, the ganglia were washed three times with plating medium (as described in Solutions) and transferred into 80 μg/ml DNAse, type IV (Sigma, Deisenhofen, Germany) and 100 μg/ml trypsin inhibitor, type I-S (Sigma). Fire-polished pipettes with decreasing diameter were then used for mechanically dissociating the cells. After this procedure, the cells were plated out in 35-mm uncoated culture dishes and stored in plating medium under 95% O_2 and 5% CO_2 at room temperature until the start of the experiment. Cells were used for the experiments within 24–72 h after preparation. Because the culture dishes were not coated, cells did not adhere to the bottom and consequently did not generate processes. Space clamp problems were thus avoided. Significant changes in Na^+ current properties, *i.e.*, amplitude and time course of the currents, were not detected during this time period.

Electrophysiologic Techniques and Data Acquisition

Tetrodotoxin-resistant Na^+ currents were recorded using the whole-cell patch-clamp method.[12] A culture dish containing the cells was placed on the stage of an inverted microscope, and the plating medium was changed to low Na^+ Tyrode (Solutions). Experiments were conducted at 22°C.

Patch pipettes were pulled from glass capillaries (Type CEEBEE 101-PS; Chr. Bardram, Svendborg, Denmark) using a Flaming/Brown Micropuller (Sutter Instrument Company, Science Products GmbH, Hofheim, Germany). The pipettes were fire polished before use and, when filled with internal solution, had a resistance of 0.8–1.2 M.

Current recordings were performed with an Axopatch 200B patch-clamp amplifier (Axon Instruments, Burlingame, CA) in the voltage-clamp mode, and data were filtered at 5 kHz, digitized at 20 kHz using a 12-bit AD-converter (Labmaster TM-40 AD/DA board; Scientific Solutions, Solon, OH), and stored on the hard disk of a personal computer, which also served as the stimulus generator. All experiments were conducted with capacitance and series resistance compensation. PClamp 6.0 software (Axon Instruments) was used for acquisition and analysis of currents. To determine blocking potencies for tonic and use-dependent block, concentration–inhibition curves were constructed from relative peak current reduction by the drugs. For this, Na^+ currents were elicited by a 50-ms depolarizing pulse to −10 mV, preceded by a 50-ms hyperpolarizing prepulse to −110 mV. The impulse protocol was applied as a train of 10 pulses at a frequency of 2 Hz once in control solution, in different local anesthetic concentrations, and again in control solution to check reversibility. Fractional inhibition of the current was measured by dividing the peak current in the presence of drug by the peak current in the previous control solution during both the first (tonic inhibition) and the 10th pulse (use-dependent inhibition) of the train. The holding potential was set to either −90, −70, or −60 mV to evaluate its effect on blocking potencies of the drugs.

Availability of the current in dependence of prepulse potential was assessed by applying 50-ms prepulses (E_p) to different potentials before a 10-ms test pulse to 10 mV and plotting the peak Na^+ current elicited by the test pulse against E_p.

Solutions

Low Na^+ Tyrode used for the bath and control solution contained 35 mM NaCl, 110 mM choline-chloride, 5 mM KCl, 1 mM $CaCl_2$, 1 mM $MgCl_2$, 6 mM glucose, and 10 mM HEPES, with pH adjusted to 7.4 with tetraethylammonium-hydroxide. Tetrodotoxin 100 nM was added to suppress tetrodotoxin-sensitive Na^+ currents; 20 mM tetraethylammonium-chloride was added to block K^+ currents. The low Na^+ concentration was necessary to reduce the magnitude of Na^+ currents to improve voltage-clamp conditions, *i.e.*, minimize voltage errors caused by series resistance. For current-clamp experiments, Tyrode solution containing 145 mM NaCl, 5 mM KCl, 1 mM $CaCl_2$, 1 mM $MgCl_2$, 6 mM glucose, 10 mM HEPES adjusted to pH 7.4 with NaOH was used as the bath solution.

The internal solution (CsF_i) contained 140 mM CsF, 10 mM NaCl, 3 mM EGTA, 10 mM HEPES; pH was adjusted to 7.2 with CsOH. Internal cesium fluoride was used to suppress potassium and calcium currents. For current-clamp experiments, KCl_i containing 140 mM KCl, 10 mM NaCl, 3 mM EGTA, and 10 mM HEPES, with pH adjusted to 7.2 with KOH, was used as the internal solution.

Plating medium was freshly made up of 26 ml minimum essential medium, 3 ml fetal calf serum, 1,000 IU penicillin, 1 mg streptomycin, and 0.6 ml L-glutamine (200 mM). All chemicals were obtained from Sigma, Deisenhofen, Germany. Gabapentin was purchased from Parke Davis, Freiburg, Germany.

Solution exchanges were performed with a multiple-barrel perfusion system. The barrels of the perfusion system were directly connected to syringes containing the control and test solutions. The syringes were constantly driven by a perfusion pump, with a steady solution flow of 5 ml/h, which corresponds to a flow speed of 40 mm/min in each barrel. After formation of the whole-cell configuration, the cell was lifted up, still attached to the pipette tip, and placed into the outlet of the barrel containing the desired solution. The seal quality as well as the signal-to-noise ratio were not influenced by this procedure. Solution exchanges were completed within 1 s, and currents were elicited 3 min after each solution exchange.

Statistical Analysis

To evaluate blocking potencies, fractional inhibition (f_i) was plotted against blocker concentration (c) for tonic and use-dependent (2 Hz) block. Nonlinear least-squares fitting of

$$f_i = 1/(1 + [IC_{50}/c]^h)$$

to the data points was performed to evaluate half-maximal inhibiting concentrations (IC_{50}); h is the Hill coefficient.

The availability curves of the Na^+ current in dependence on prepulse potential (E_p) were fitted with a Boltzmann function

$$I_{Na} = I_{Na,max}/(1 + \exp\{[E_p - E_{50}]/k\})$$

where E_{50} is the potential for half-maximal availability, k the steepness factor, and $I_{Na,max}$ the maximal available Na^+ current.

Significance testing was performed with the Student *t* test for paired samples, and the calculated *P* values are reported in the text. Significance testing, fitting procedures, and the preparation of the figures were performed with Fig.P 5.0 software (Biosoft, Cambridge, United Kingdom). Data points indicate mean ± SEM, given parameters are fitted values ± SE, where the latter represents the 95% confidence interval for the estimated parameter.

Results

Na^+ currents were recorded mainly from small- to medium-sized dorsal root ganglion cells with a cell size of 32 ± 10 μm (128 cells) in which a mixture of varying proportions of tetrodotoxin- and tetrodotoxin-resistant Na^+ currents exist. Tetrodotoxin-sensitive Na^+ currents are blocked by 97% when adding 100 nM tetrodotoxin, which sufficiently isolates the tetrodotoxin-resistant current.[11] However, the tetrodotoxin-resistant channel of the SNS type is blocked half-maximal by 31 μM tetrodotoxin, and the SNS2 type already by 1 μM tetrodotoxin.[13] We therefore chose the addition of 100 nM tetrodotoxin to the external solution for our experiments to isolate tetrodotoxin-sensitive currents from tetrodotoxin-resistant ones. At a holding potential (E) of −90 mV, the mean peak amplitude of tetrodotoxin-resistant Na^+ currents elicited by 50-ms depolarizations to −10 mV was 6.35 ± 3.26 nA (27 cells). Changing E to more depolarized potentials, *i.e.*, −70 mV and −60 mV, reduced the current to 89 ± 23% ($P = 0.0766$) and 77 ± 24% ($P = 0.0003$), respectively.

At E of −90 mV, lidocaine, mexiletine, carbamazepine, memantine, and amitriptyline reversibly inhibited tetrodotoxin-resistant Na^+ currents in small dorsal root ganglion neurons (fig. 1). Current inhibition by the drugs was concentration-dependent and complete at high concentrations. Lidocaine, mexiletine, and amitriptyline produced intense use-dependent inhibition of the currents at 2-Hz stimulation; in contrast, carbamazepine and memantine showed only little use dependency. The use-dependent blockers also induced faster inactivation of the current traces during the first pulse. Gabapentin showed neither tonic nor use-dependent block at concentrations up to 3 mM and was thus excluded from further investigations. To quantify blocking potencies, concentration–inhibition curves were constructed from fractional block of the peak current during the first (tonic block) and the 10th pulse (use-dependent block) of a 2-Hz train at an E of −90 mV (fig. 2). Equation 1 was fitted to the data to give half-maximal inhibiting concentrations (IC_{50}) for tonic and use-dependent inhibition of the drugs, which are listed in table 1. Lowering E to −70 and −60 mV strongly enhanced the blocking effect of lidocaine (fig. 3A), mexiletine, and amitriptyline, but not of carbamazepine and memantine. IC_{50} values for tonic and use-dependent block were also evaluated at holding potentials (E) of −70 and −60 mV (fig. 3B and table 1). To depict the effect of holding potential on the potencies of the drugs, the IC_{50} values were plotted against E (fig. 4).

Na^+ channel blockers that induce use-dependent block are known to shift the availability curve of Na^+ currents in dependence of voltage in the hyperpolarizing direction.[14] We tested the effect on lidocaine on the availability curve (h_∞) of tetrodotoxin-resistant currents in five cells (fig. 5). Fitting equation 2 to the availability curves gave half-maximal availability potentials of −35.6 ± 6.1 mV for control, −37.3 ± 5.9 mV for 100 μM lidocaine, and −40.9 ± 6.4 mV for 300 μM lidocaine.

Discussion

During the last few years, a large body of evidence accumulated that neuronal tetrodotoxin-resistant Na^+ channels play an important role in peripheral nociception and in the development of chronic pain syndromes.

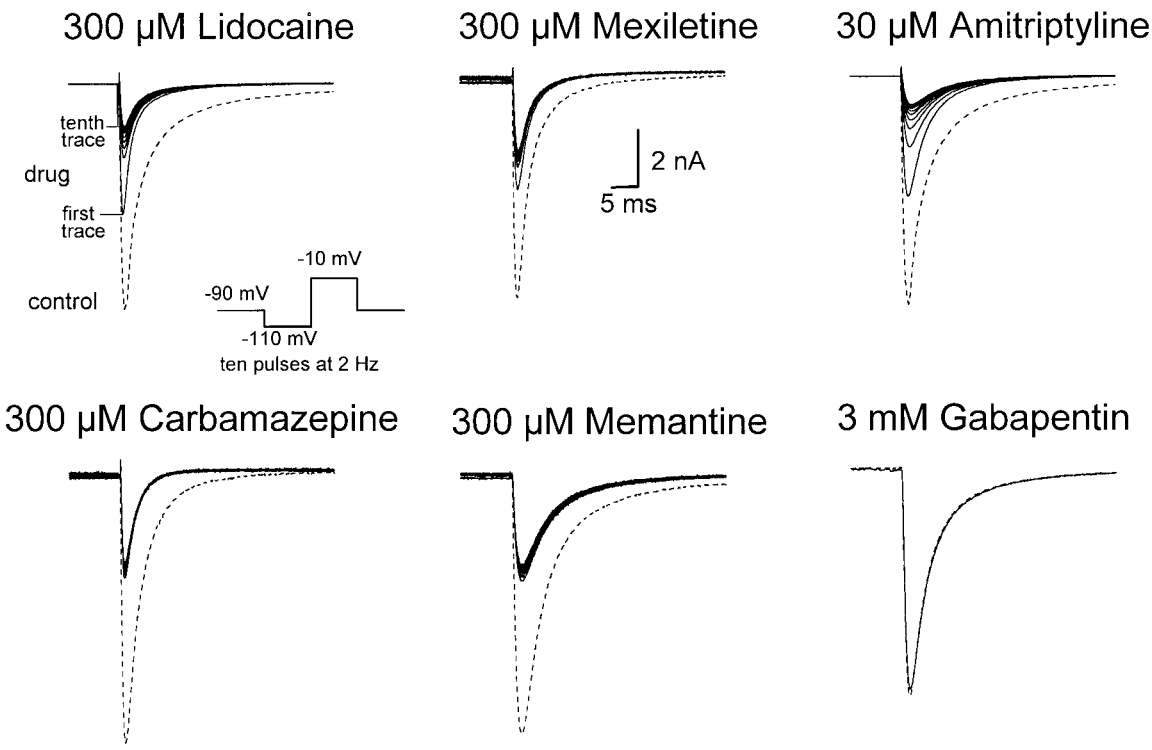

Fig. 1. Effects of the drugs on tetrodotoxin-resistant Na^+ currents in dorsal root ganglion cells. In each set of currents, dashed lines represent the current traces elicited by the first pulses in control solution; solid lines are 10 successive traces obtained at 2-Hz stimulation in drug. The inset shows the protocol of a single pulse sequence with a 50-ms hyperpolarizing prepulse and a 50-ms test pulse. Holding potential was −90 mV.

The channels are important for the impulse initiation process in peripheral nociceptive nerve endings[9] and can convey action potentials along C fibers.[15] Tetrodotoxin-resistant Na^+ channels are abundant in small sensory neurons that represent the somata of C-fiber axons, and despite down-regulation in the soma after axotomy or peripheral nerve damage,[10,16,17] channels accumulate at the site of injury on the axon.[10] The accumulation of Na^+ channels may result in repetitive activity as demonstrated from computational studies[18] and electrophysiologic experiments.[7] Although there is evidence for the involvement of tetrodotoxin-sensitive Na^+ channels in

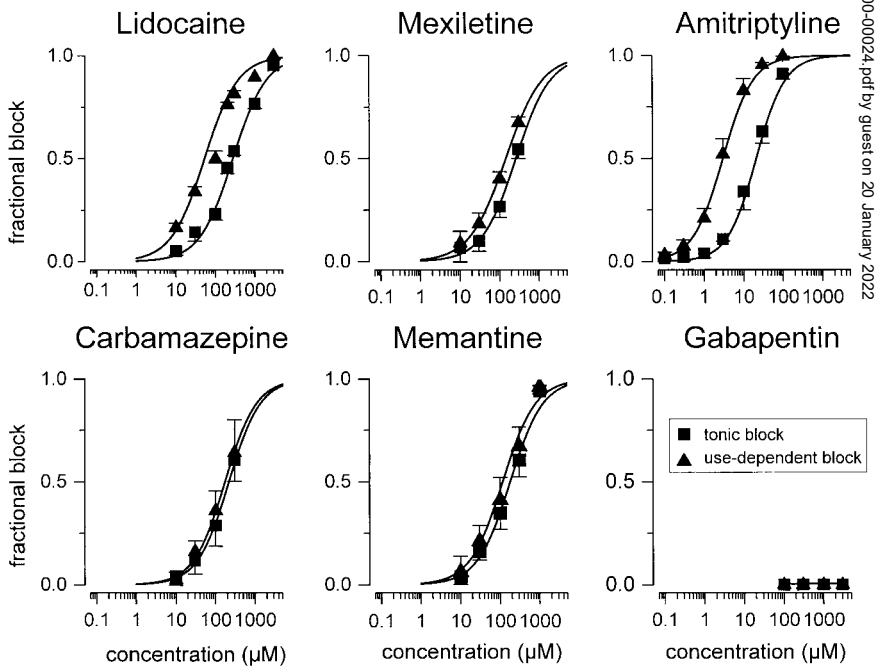

Fig. 2. Concentration–inhibition curves for tonic and use-dependent block. Fractional block of the current elicited by the first (squares) and tenth (triangles) pulse of the 2-Hz stimulus train is plotted against blocker concentration. Holding potential was −90 mV in all experiments shown. Data points are mean values, and error bars represent SEM. Curves are nonlinear least-squares fits of equation 1 to all data points at the first or the 10th pulse giving half-maximal blocking concentrations (IC_{50}), which are listed in table 1.

Table 1. Half-maximal Blocking Concentrations (IC_{50}) ± Standard Error of the Fit as Derived from Nonlinear Least-squares Fitting with Equation 1 for Tonic and 2-Hz Use-dependent Block at Different Holding Potentials

	−90 mV		−70 mV		−60 mV	
Agent	Tonic (μM)	Use-dependent (μM)	Tonic (μM)	Use-dependent (μM)	Tonic (μM)	Use-dependent (μM)
Lidocaine	277 ± 17 (21)	79 ± 6 (12)	128 ± 19 (6)	53 ± 5 (6)	53 ± 5 (5)	23 ± 3 (5)
Mexiletine	258 ± 18 (5)	142 ± 8 (5)	242 ± 22 (6)	100 ± 9 (6)	58 ± 9 (6)	28 ± 4 (6)
Amitriptyline	18.2 ± 1.3 (6)	2.7 ± 0.2 (6)			4.3 ± 1.1 (5)	1.6 ± 0.3 (5)
Carbamazepine	216 ± 22 (5)	172 ± 20 (5)	146 ± 17 (8)	118 ± 14 (8)	101 ± 11 (5)	94 ± 11 (5)
Memantine	178 ± 13 (5)	135 ± 12 (5)			115 ± 17 (4)	116 ± 16 (4)

Holding potential is given in the top row; number of experiments are given in parentheses.

ectopic firing,[7] the importance of tetrodotoxin-resistant Na^+ channels in the peripheral nociceptive system is intriguingly demonstrated on knockout mice, which do not possess these channels.[19] These animals apparently behaved normally but showed analgesia to noxious mechanical stimuli and delayed development of inflammatory hyperalgesia.

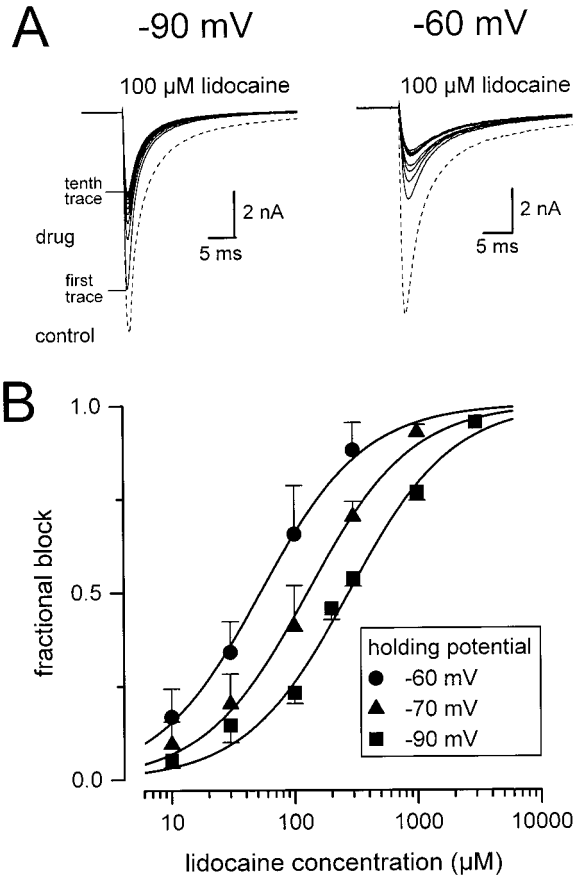

Fig. 3. Effect of holding potential on lidocaine block. (*A*) Traces of tetrodotoxin-resistant Na^+ currents. Dashed lines represent the current trace elicited by the first pulse in control, and solid lines are 10 successive traces at 2-Hz stimulation in 100 μM lidocaine. Holding potential was set either to −90 mV (*left*) or −60 mV (*right*). (*B*) Tonic block of the current as elicited by the first pulse of the stimulus train at different holding potentials is plotted against lidocaine concentration. Data points are mean values, and error bars represent SEM. IC_{50} values obtained by fitting equation 1 to all data points at each holding potential are listed in table 1.

Neuronal tetrodotoxin-resistant Na^+ currents in primary sensory neurons of the dorsal root ganglion have distinct properties different from tetrodotoxin-sensitive Na^+ currents. Activation and inactivation time courses are slower, and voltage dependence of activation and inactivation both lie in a more depolarized range compared with neuronal tetrodotoxin-sensitive Na^+ currents.[11] These features and its rapid repriming kinetics make this current ideal for impulse generation under the pathophysiologic conditions found in ectopic sites. The tetrodotoxin-resistant Na^+ channel is thus a significant target for a putative antinociceptive mechanism. Furthermore, a tetrodotoxin-resistant Na^+ channel has also been cloned from human sensory ganglia,[20] which underlines its importance for nociception and possible antinociceptive therapies in humans. Unfortunately, however, to our knowledge selective blockers for neuronal tetrodotoxin-resistant Na^+ channels, which could be of inestimable value for pain treatment, have not been found to date.

The drugs investigated in this study are nonselective and block tetrodotoxin-sensitive Na^+ channels even more potently than tetrodotoxin-resistant ones.[21] However, the dependence of blocking potency on membrane potential may be of relevance for the successful use of the drugs in pain management. In damaged peripheral nerves that spontaneously fire action potentials, the sustained depolarized potential alters the ratio of tetrodotoxin-sensitive and -resistant channels in favor of the latter, providing the grounds for spontaneous activity.[22] An important issue of our work is that without blocker the tetrodotoxin-resistant current is only slightly reduced by depolarization so that the channels may contribute to spontaneous activity under these conditions. Our work further shows that the depolarized membrane potential combined with repetitive firing has strong impact on the blocking potencies of the use-dependent blocking drugs. The half-maximal blocking concentration of lidocaine, for example, decreases more than 12-fold from 277 μM at −90 mV for tonic block to 23 μM at −60 mV for use-dependent block when stimulated at 2 Hz (table 1). Because, after intravenous administration, therapeutic plasma concentrations of lidocaine are close to the latter concentration,[23,24] current through tetrodotoxin-resistant Na^+ channels is reduced and herewith

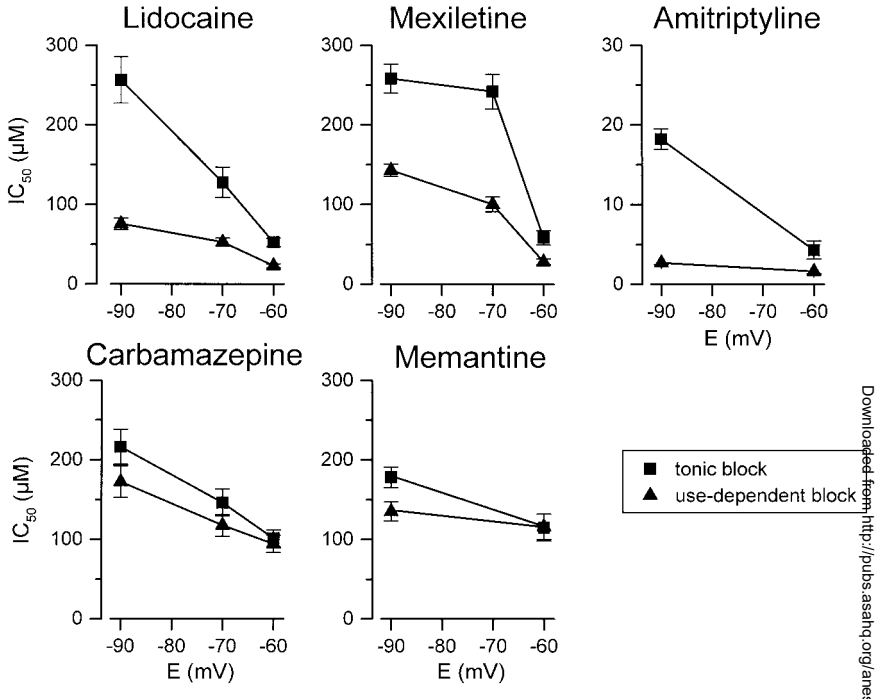

Fig. 4. Dependence of potency on holding potential. IC_{50} values of the drugs for the first pulse (tonic block) and the 10th pulse of a 2-Hz train (use-dependent block) are plotted against holding potential. Data points are fitted values, and error bars represent the SE.

spontaneous activity generated by these channels. As for lidocaine, depolarization and repetitive stimulation of the cell also increases affinity of mexiletine (ninefold) and amitriptyline (11-fold) but has little effect on carbamazepine and memantine (table 1).

The use-dependent blocking drugs may thus directly interfere with the impulse initiation process in an ectopic site of an injured nerve. Na^+ channels in the central nervous system and the heart are less susceptible to the drugs because of the intact negative membrane potential of their cells, so that almost normal excitability is maintained in these tissues.

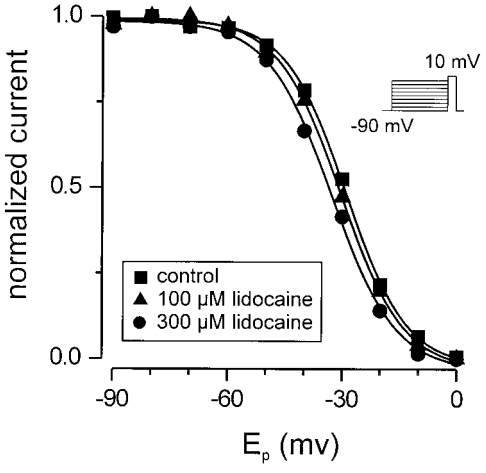

Fig. 5. Availability in dependence of prepulse potential of the tetrodotoxin-resistant Na^+ current in control and in 100 and 300 μM lidocaine. Holding potential was −90 mV; pulse protocol is given in the inset. The prepulse duration was 50 ms, and test pulse duration was 10 ms. Curves represent nonlinear least-squares fits of equation 2 to the data points; the fitted parameters are given in the text. To better demonstrate the influence of lidocaine on the potential dependence of availability, the curves were normalized to $I_{Na,max} = 1$.

The influence of holding potential on blocking potencies of local anesthetics is a well-known phenomenon that has been observed in other preparations with tetrodotoxin-sensitive Na^+ channels.[25] For instance, potencies of bupivacaine enantiomers increased fivefold when changing a 5-s prepulse from −120 mV to −70 mV, tetrodotoxin-sensitive Na^+ channels in rat pituitary GH3 cells.[26] Rat brain IIa channels increase their affinity to lidocaine, carbamazepine, and phenytoine when the membrane potential is depolarized,[27] but in contrast to tetrodotoxin-resistant Na^+ channels, tetrodotoxin-sensitive channels inactivate when depolarized to potentials at which an increase in affinity occurs. Depolarizing the membrane from −128 mV to −66 mV dramatically increased the affinity of rat brain IIa channels to the drugs but also reduced the current by 90%.[27] This is also apparent in the tetrodotoxin-insensitive (not resistant) heart Na^+ channels, as demonstrated by Bean *et al.*,[28] who found a 30-fold increase in affinity of rabbit Purkinje fiber Na^+ channels to lidocaine when depolarizing from −90 mV to −60 mV, and also a reduction of the Na^+ current by 99% already without the blocker. Clinically, increased affinity of Na^+ channels is important in heart muscle cells because they spent a considerable period in the depolarized state in which the drugs bind to the channel. Slow unbinding during repolarization may then counteract ventricular tachyarrhythmias (lidocaine and mexiletine), and very slow unbinding (bupivacaine) may induce ventricular arrhythmias. In contrast, in intact axons of the peripheral nervous system, the resting membrane potential is very negative to assure impulse

propagation so that sensitivity to local anesthetics is rather low.

The increase in potency in dependence of membrane potential is often explained with the modulated receptor hypothesis.[14,29] Essentially, this hypothesis is based on the assumption that Na^+ channels in the inactivated state have a much higher affinity compared with channels at rest. Because the amount of inactivated channels increases at depolarized potentials, the overall affinity to local anesthetics increases. In contrast, neuronal tetrodotoxin-resistant Na^+ currents do not inactivate in the potential range in which an increase of drug affinity was observed. This is demonstrated by the availability curve (fig. 5), which shows almost full availability of the current at −60 mV. It is therefore unlikely that the increased affinity results from an increase of inactivated Na^+ channels alone. This is further supported by availability curves, which were shifted in the hyperpolarized direction by only 5 mV with lidocaine compared with 20 mV for tetrodotoxin-sensitive currents.[14] Because the modulated receptor hypothesis requires large shifts of the availability curve, we believe that it does not apply for tetrodotoxin-resistant Na^+ current block by local anesthetics, and rather think that either activation to a preopen state of the channel, spontaneous openings of the channel, or enhanced slow inactivation with the drug may be responsible for voltage dependence of block. This hypothesis is strengthened by the observation that the use-dependent blockers accelerate the inactivation time course of the current, which may be caused by an open-channel block. Further analysis of current kinetics of whole-cell currents and on the single-channel level is needed to resolve molecular mechanisms underlying the observed phenomena.

Ectopic generation of nerve impulses may occur in peripheral sites[30,31] or in dorsal root ganglion cells.[5,6] Lidocaine[32,33] and mexiletine[32] suppress these discharges. The effective concentration of lidocaine to suppress tonic neural injury discharges half-maximal *in vitro* was found to be 24 μM (5.7 μg/ml).[11] The minimal plasma level of lidocaine necessary for pain suppression is 6 μM (1.5 μg/ml),[24] and plasma levels for carbamazepine were 21–72 μM (5–17 μg/ml)[34] when used for treating neuralgias. Plasma levels of mexiletine were found to be 4–12 μM (0.75–2.18 μg/ml)[35] when administered orally at 450-mg doses, and those of amitriptyline ranged between 0.3 and 0.5 μM (93–140 ng/ml).[36] However, because of the lipophilicity of the drugs, final concentrations in the nerve may be higher. From our observations, we conclude that only lidocaine, mexiletine, and amitriptyline may be able to silence ectopic discharges by blocking tetrodotoxin-resistant Na^+ currents. Carbamazepine and memantine do not affect the current in systemic concentrations, and gabapentin has no effect at all. Abdi et al.[37] showed that lidocaine and amitriptyline reduced peripheral nerve discharges in a chronic pain model of the rat, whereas gabapentin did not. In another work, carbamazepine silenced discharges in A-fibers from rat saphenous neuromas, which most probably do not contain tetrodotoxin-resistant Na^+ channels with 33 μM (7.9 μg/ml),[38] but effects on C-fibers, which do contain tetrodotoxin-resistant Na^+ channels, were not investigated.

In the present study, we measured currents in cells obtained from healthy rats. However, tetrodotoxin-resistant currents recorded in cells from rat model of neuropathic pain (chronic constriction injury) demonstrate different electrophysiologic properties, *i.e.*, shift of the activation and inactivation voltage dependence to more negative potentials.[39] Under these conditions, the cells may be even more sensitive to the use-dependent blockers, because if the voltage dependence of drug affinity is connected to the voltage sensitivity of the channel, the former will also be shifted to more negative potentials. Further studies on currents of cells from injured axons are needed to clarify this.

The tetrodotoxin-resistant current in peripheral nervous system is heterogeneous. At least two different Na^+ channels underlie the current, as demonstrated in whole-cell patch-clamp investigations,[40,41] single-channel analysis,[40] and molecular biology (SNS/PN3 and SNS2/NaN).[13] However, it appears that only SNS/PN3 but not SNS2/NaN are important for the development of neuropathic pain syndromes.[42] In our study, we focused only on the whole-cell tetrodotoxin-resistant Na^+ current. It was beyond the scope of this study to investigate differential effects of the drugs on the different types of tetrodotoxin-resistant channels. Furthermore, their physiologic and pathophysiologic function in peripheral sensory processing must be clarified in detail before such investigations could be interpreted.

In summary, the analgesic properties of the use-dependent blockers lidocaine, mexiletine, and amitriptyline may be a result of their selectivity for tetrodotoxin-resistant Na^+ channels at depolarized membrane potential over tetrodotoxin-sensitive Na^+ channels in intact cells. However, interaction with the latter channels at higher concentrations limits their use because adverse effects on excitability do occur. For the near future, more selective blockers of tetrodotoxin-resistant Na^+ channels might be available, and it is believed that by this we shall hold new powerful analgesics in our hands.

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