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The Role of Sodium Channels in Chronic Inflammatory and Neuropathic Pain

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Abstract: Clinical and experimental data indicate that changes in the expression of voltage-gated sodium channels play a key role in the pathogenesis of neuropathic pain and that drugs that block these channels are potentially therapeutic. Clinical and experimental data also suggest that changes in voltage-gated sodium channels may play a role in inflammatory pain, and here too sodium-channel blockers may have therapeutic potential. The sodium-channel blockers of interest include local anesthetics, used at doses far below those that block nerve impulse propagation, and tricyclic antidepressants, whose analgesic effects may at least partly be due to blockade of sodium channels. Recent data show that local anesthetics may have pain-relieving actions via targets other than sodium channels, including neuronal G protein-coupled receptors and binding sites on immune cells. Some of these actions occur with nanomolar drug concentrations, and some are detected only with relatively long-term drug exposure. There are 9 isoforms of the voltage-gated sodium channel α -subunit, and several of the isoforms that are implicated in neuropathic and inflammatory pain states are expressed by somatosensory primary afferent neurons but not by skeletal or cardiovascular muscle. This restricted expression raises the possibility that isoform-specific drugs might be analgesic and lacking the cardiotoxicity and neurotoxicity that limit the use of current sodium-channel blockers.

Perspective: Changes in the expression of neuronal voltage-gated sodium channels may play a key role in the pathogenesis of both chronic neuropathic and chronic inflammatory pain conditions. Drugs that block these channels may have therapeutic efficacy with doses that are far below those that impair nerve impulse propagation or cardiovascular function.

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Drugs that block voltage-gated sodium channels have been a mainstay of pain medicine since Koller and Freud introduced the use of cocaine as an ophthalmic anesthetic 120 years ago.²⁰⁸ The introduction of procaine, an ester local anesthetic that could be easily sterilized, the recognition that anesthesia could be prolonged significantly if local vasoconstriction was established with coadministration of epinephrine, and the introduction of agents with medium and long durations of action and reduced toxicity (lidocaine and bupivacaine, respectively), greatly expanded the usefulness of sodium-channel blockade. Today, the administration of sodium-channel blockers with topical, regional, epidural, or intrathecal technique is used not only for the control of surgical pain but also for the management of chronic pain conditions.

The use of local anesthetics is so familiar that it is easy to lose sight of the fact that the ability of these drugs to prevent nerve impulse propagation is achieved only with very high local concentrations (.1-1.0 mmol/L)—concentrations that would far exceed the lethal threshold if given systemically (plasma levels of 40-60 $\mu\text{mol/L}$).

The possibility that relatively low, systemically tolerable doses of sodium-channel blockers might be useful was first made clear by the discovery that procainamide and lidocaine suppress cardiac arrhythmias and that lidocaine and other local anesthetics have antiseizure activity.⁵⁶ For lidocaine, these therapeutic actions are obtained with plasma levels of 5-10 $\mu\text{mol/L}$. Several lines of evidence showing that there are useful low-dose actions of sodium-channel blockers arose from attempts to treat neuropathic pain. Early evidence suggested analgesic activity with orally available lidocaine congeners like tocainide and mexiletine (for review, see Kalso et al¹²¹). It has long been known that patients with painful peripheral neuropathy sometimes receive weeks of relief following a single local anesthetic block of the painful region.^{12,26,149} Such a long period of pain relief from drugs that were known to have short half-lives invited the suspicion that the mechanism of action was a placebo response or even evidence of psychoneurosis. However, there is now abundant evidence^{10,41,150,196} that rats with an experimental painful peripheral neuropathy also obtain many days of pain relief following single systemic exposures to local anesthetic, and it is therefore apparent that the effect is physiologic, although the mechanism is still not understood. The intravenous lidocaine plasma concentrations that relieve clinical and experimental neuropathic pain are in the 5-10 $\mu\text{mol/L}$ range, far below the concentrations that are needed to block nerve impulse propagation. There is firm evidence that tricyclic antidepressants (TCAs) relieve neuropathic pain via a mechanism that is separate from that by which they modulate mood,¹⁵³ and there is now reason to believe that their analgesic action may be at least partly mediated via sodium-channel blockade (see following). More recently, evidence has begun to appear that indicates that changes in the expression of sodium-channel isoforms are involved in chronic inflammatory pain condi-

tions and that sodium-channel blockers may also have a role in the treatment of these conditions.

Here we review the evidence for a role of neuronal sodium channels in the pathogenesis of chronic neuropathic and inflammatory pain conditions. In addition, we highlight a variety of mechanisms that might contribute to the pain relief that is obtained with sodium-channel blockers, including mechanisms engaged by very low doses that may have nothing to do with sodium-channel binding.

The Voltage-Gated Sodium Channel and its Isoforms

Voltage-gated sodium channels are transmembrane molecular pores that open and close in response to changes in the local electric field across the membrane. The structural features that support channel function are remarkably similar among all of the known isoforms of voltage-gated sodium channels.

Structure and Function

The sodium channel is composed of 1 very large continuous protein, the α -subunit, and 1 or 2 smaller ancillary β -subunits.³⁸ The α -subunit contains all of the features necessary for a functional ion channel, including voltage sensor, activation gate, ion pore, and inactivation gate. The β -subunits are important for chaperoning the α -subunits to the plasma membrane and for stabilizing them there, and they can modulate channel gating and pharmacology.

The α -subunit (Fig 1) consists of 4 homologous domains (D1-D4), each of which contains 6 transmembrane helices (S1-S6) and a short nonhelical region between S5 and S6, the P-segment, which is thought to line the ion permeation pathway which is formed by close apposition of the P-segments from each of the 4 domains. The ion-conducting pore enables ions of the appropriate charge and diameter (sodium being the most permeant) to pass through the otherwise relatively impermeable lipid bilayer. The narrowest part of the pore appears to be closer to the outer surface of the channel, and this is the locus where selectivity among ions occurs, the "selectivity filter."^{105,200} The inner opening of the pore that faces the cytoplasm appears to have a considerably wider vestibule, accommodating large organic cations that cannot pass through the narrower selectivity filter but can reach the cytoplasm by permeation through the lipid bilayer of plasma membranes. The cytoplasmic opening also contains regions where drugs bind to parts of the channel involved in inactivation, the pore-closing process that is driven by depolarization.

Opening of sodium channels from their resting closed state involves several steps of conformational change, most in response to forces put on the channels by the changed electric field that accompanies membrane depolarization. Charged segments of the channel, identified as multiple positive amino acid residues on at least 3 of the 4 S4 segments,²³¹ "sense" these electrical field changes and move within the membrane (in ways that

are still debated¹⁴⁷), effectively displacing their fixed charges “outward” from a location closer to the intracellular to one closer to the extracellular surface. Such voltage-dependent “activation gating” is associated with a proportional displacement of charge across the membrane, the so-called “gating current.”^{11b} Movement of S4 segments is apparently coupled to movement of S6 segments (again in ways unknown), subtly changing the dimensions of the pore and allowing ions to pass through this open state of the channel.

Open channels preferentially transform to a closed inactivated state, a conformation from which reopening is highly unlikely. However, at negative membrane potentials, eg, around the resting potential, inactivated channels will transition to the closed state, a conformation from which they are relatively easy to open upon subsequent depolarization. The rapid inactivation phase appears to be initiated by the binding of a cytoplasmic loop, which connects D3 to D4 in the inner vestibule of the channel, effectively placing an obstruction over the inner opening.

Many different amphipathic amine molecules, including local anesthetics and class I antiarrhythmics, inhibit the function of sodium channels. Screens of all 4 domains by site-directed mutagenesis reveal that major contributors to local anesthetic binding are located on S6 segments that line the channel^{214,215} and that blocking potency is also sensitive to mutations of the inner ring.²⁰⁰ Some of these residues are also involved in binding the inactivation moiety of the D3-D4 linker loop.

Although the binding site for local anesthetics is in the pore and in contact with S4 segments, the gating charge movement that signals the major voltage-dependent conformational change during channel activation is strongly suppressed by local anesthetics,^{34b,125b} further evidence for the conformational coupling between “gating” and pore domains. Although the binding pharmacophore for local anesthetics is very similar among all channel isoforms, differences in their activation regions might indirectly influence the effective potency of local anesthetic-like blockers.

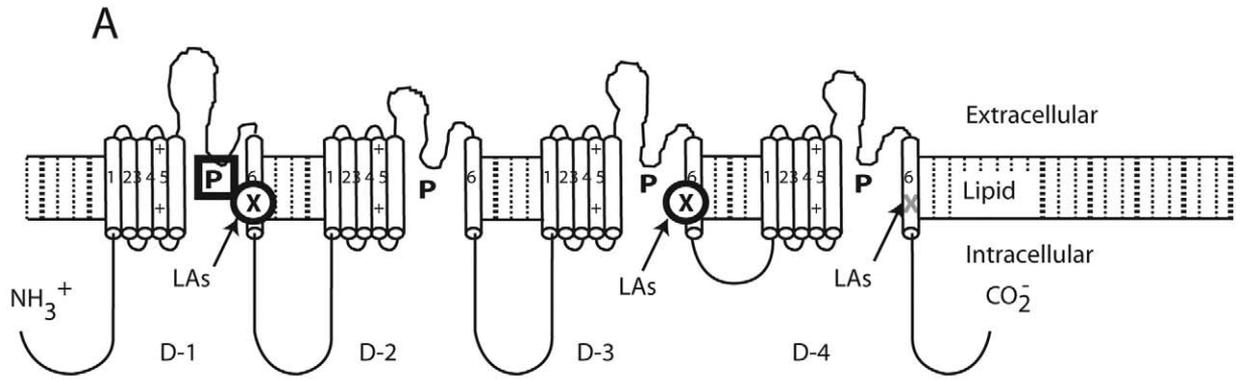
Channel Isoforms

Nine genes have been identified which encode voltage-gated sodium channel α -subunits in mammals.⁹³ They differ in terms of their tissue distribution in the adult and developing organism, their electrophysiologic properties, their pharmacology, and their response to nerve injury and inflammation. Somatosensory primary afferent neurons (ie, dorsal root ganglion (DRG) neurons) in the adult rat and mouse express 6 of the 9 isoforms; another 2 isoforms are present in the normal embryonic DRG (Table 1). Coexpression of multiple isoforms is the rule²³; no DRG neuron is known to express only one isoform. Different functional classes of primary afferent neurons (eg, low-threshold mechanoreceptors and nociceptors) express different mixtures of isoforms. However, there is no evidence that any particular mixture is unique to any particular type of neuron.

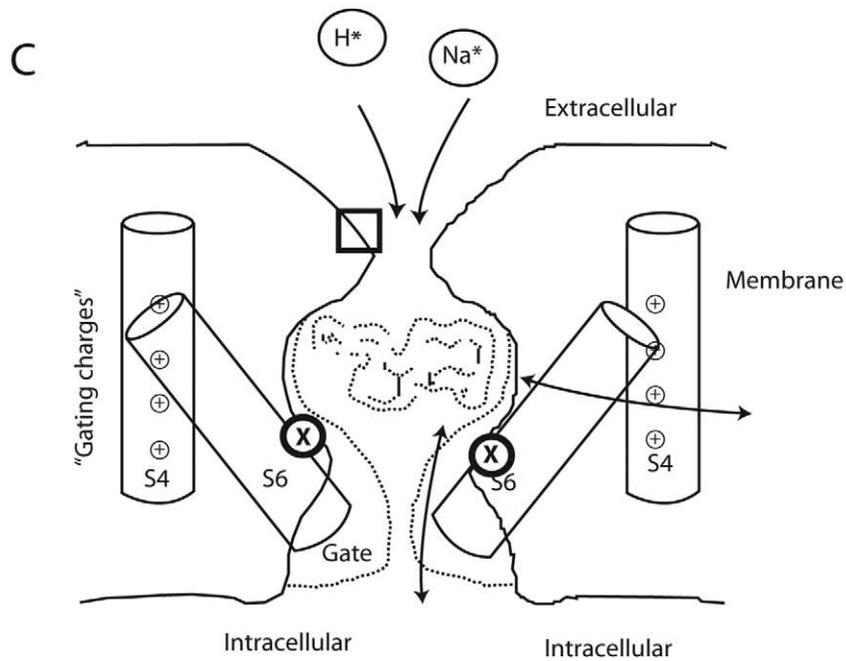
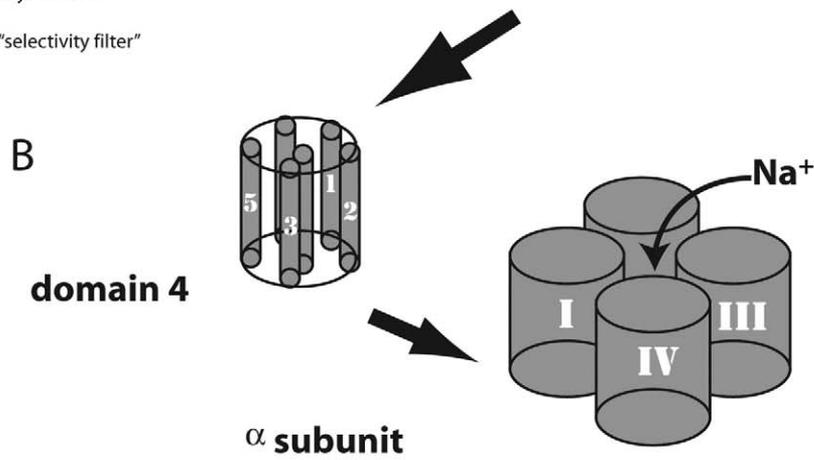
We have long known that sodium channels are es-

sential for the propagation of nerve impulses, and one might imagine that a single type of sodium channel would be sufficient for such a purpose. Why then are there 9 α -subunit isoforms? Impulse propagation is not the only phenomenon mediated via voltage-gated sodium channels. In the periphery, the sensory neuron's receptor terminals express macromolecular complexes that transduce various forms of energy (eg, mechanical, thermal, or chemical stimuli) into a graded membrane depolarization, the generator (or receptor) potential. When the generator potential crosses a certain threshold, the graded depolarization is converted into a stream of nerve impulses. Generally, increasing the magnitude and duration of the generator potential gives rise to nerve impulse streams of increasing frequency and duration. Roughly the opposite occurs when a sensory nerve impulse enters the spinal cord dorsal horn: The impulse stream is decomposed into a graded depolarization that invades the terminal branches of the sensory axon and depolarizes its synaptic boutons (the presynaptic potential), causing neurotransmitter release. All of these processes—the generator potential, its conversion into an impulse stream, the conduction of the impulse stream along the sensory axon into the spinal cord, the spread of depolarization to the synaptic boutons, and the release of neurotransmitter—vary depending on which sodium-channel α -subunit isoforms are present. It thus seems possible that the diversity among isoforms reflects evolutionary pressures to fine-tune these phenomena in the context of the different functional classes of sensory neurons. Moreover, it has been hypothesized that different isoforms may associate with a variety of receptor molecules to form specialized macromolecular complexes that regulate nociceptor excitability.²¹⁸

The multiplicity of isoforms may be just the tip of the iceberg. It is known that the genes that encode the α -subunits have many alternative splice variants, that some of these variants appear to be unique to DRG neurons, and that peripheral nerve injury changes the patterns of alternative splice variants observed.¹⁷⁷ Moreover, it is essential to remember that the functions of the α -subunits are modulated by their associated β -subunits. Thus, voltage-gated sodium channel activity in neuropathic and inflammatory conditions may arise from injury-evoked changes in the β -subunits. Five β -subunits have been identified to date, and there is evidence that at least 3 of them are present in DRG neurons. Avulsion of the dorsal roots in man has been shown to decrease the expression of β 1 and β 2 but not β 3 in DRG neurons.³⁹ In animals with experimental nerve injury,^{169b} the levels of the β 2-subunit are increased in the DRG somata of those cells whose axons have been injured, and to a lesser extent also in their uninjured neighbors. Moreover, the null mutant mouse that lacks β 2-subunits develops less mechanical allodynia than the wild type following the spared-nerve injury.^{169b}



- X** LA potency general
- Stereoselectivity selected
- Inner ring of "selectivity filter"



Electrophysiologic Properties of Sodium-Channel Isoforms

The channel isoforms have different electrophysiologic properties. They differ in their thresholds for opening and in the length of time that the channel remains open. They also vary in the amount of time that the channel spends in the inactivated state and in the effect of membrane potential on this kinetic parameter. At negative membrane potentials, the different channel isoforms transition from the closed inactivated state to the resting closed state at different rates.

Prior to the molecular identification of the channel isoforms, sodium channels were classified functionally with respect to gating of the current that flowed through them (eg, low- or high-threshold, fast- or slow-inactivating, etc) in conjunction with their sensitivity to blockade by tetrodotoxin (TTX), with some being very sensitive to TTX and others being relatively resistant. The molecular and electrophysiologic classifications are not easily reconciled. Equating particular currents with their corresponding channel isoforms *in vivo* is difficult, because channel kinetics can be modified by the β -subunits and by a variety of endogenous regulators. However, this difficulty is mostly overcome by experiments that use recombinant α -subunit isoforms expressed in *Xenopus* oocytes and other cells, a less complicated situation that has provided a wealth of unambiguous detail on the functional characteristics of the different isoforms.^{92,93} We can now assign the various kinds of currents (and TTX sensitivity) to their respective channel isoforms with a high degree of confidence in most cases (Fig 2).

In the normal animal, DRG neurons express a complex array of sodium currents, and they are unusual in that they express both TTX-sensitive and TTX-resistant sodium currents.¹³³ The population of large DRG neurons, which contain mostly low-threshold mechanoreceptive neurons ("touch receptors"), appears to mostly express isoforms that are TTX sensitive and have fast kinetics. The population of small DRG neurons, which contain mostly nociceptive neurons, expresses a mixture of channel isoforms with both TTX-sensitive fast kinetics and TTX-resistant slow kinetics. Much recent research has focused on the 2 TTX-resistant channels that are expressed in nociceptors, Na_v1.8 and Na_v1.9.

Na_v1.8 produces a slowly activating and inactivating current which has relatively depolarized voltage dependences of activation and steady-state inactivation. TTX-resistant sodium current in DRG neurons is believed to be carried primarily via the Na_v1.8 isoform. Na_v1.8 is preferentially expressed in nociceptive afferents,⁶⁷ where it appears to be localized in the cell body,¹⁸¹ peripheral receptor terminals,^{30,31,37,127,232} and central terminals within the spinal cord dorsal horn.^{99,116} In peripheral terminals, the channel appears to underlie spike initiation.³⁰ It is yet to be demonstrated that functional Na_v1.8 channels are present in central terminals, but a TTX-resistant current appears to contribute to the release of transmitter from the central terminals of primary afferents.⁹⁹

Another type of TTX-resistant current, called the persistent current, is also predominantly expressed in small, mostly nociceptive, DRG neurons.^{52,178} This current is likely produced by the Na_v1.9 isoform.^{62,65,73} The channel has a relatively low threshold for activation, such that the channel may be active at resting membrane potentials,⁵² and it has an exceedingly slow activation rate, suggesting that it contributes little to action potential propagation. The channel also has an exceedingly slow inactivation rate, resulting in a "persistent" current that may contribute to the generation of burst discharge.¹⁰⁴ When the channel was first identified, it was believed to be restricted to the peripheral nervous system, particularly to small-diameter sensory neurons.^{65,204} Subsequent anatomic⁷⁹ and electrophysiologic⁷³ evidence confirms that expression of this channel in sensory neurons is largely restricted to nociceptive A δ - and C-neurons. Recent data suggest that the channel is also present in the enteric nervous system.¹⁸⁶

Is There a Cocktail Effect?

Most of the currently available data focuses on the role of individual isoforms; however, the complex mixture of sodium-channel isoforms in DRG neurons raises the possibility that in any particular neuron the mixture may have emergent properties. There are few *in vivo* or *in vitro* data on this question, but preliminary analyses have been performed *in silico*.^{104,196}

Figure 3 shows results obtained with a computer

Figure 1. Structural features of the Na⁺ channel that determine local anesthetic interactions. (A) The consensus arrangement for the single peptide of the Na⁺ channel α -subunit in a plasma membrane. Four domains with homologous sequences (D-1 through D-4) each contain 6 alpha helical segments that span the membrane (S1-S6). Each domain folds within itself to form 1 cylindrical bundle of segments, and these bundles converge to form the functional channel's quaternary structure (B). Activation gating that leads to channel opening results from the primary movement of the positively charged S4 segments in response to membrane depolarization (C). Fast inactivation of the channel follows the binding to the cytoplasmic end of the channel of part of the small loop that connects D-3 to D-4. Ions travel through an open channel along a pore defined at its narrowest dimension by the P region formed by the partial membrane penetration of the 4 extracellular loops of protein connecting S5 and S6 in each domain. Intentional directed mutations of different amino acids on the channel indicate those residues that are involved with local anesthetic (LA) binding in the inner vestibule of the channel (*bold X*, on S6 segments) and at the interior regions of the ion-discriminating "selectivity filter" (*bold square*, on the P region) and are also known to influence stereoselectivity for phasic inhibition (*bold circle*, also on S6 segments). (C) A schematic cross-section of the channel speculates on the manner in which S6 segments, forming a "gate," may realign during activation to open the channel, allowing the entry and departure of a bupivacaine molecule by the "hydrophilic" pathway. The closed (inactivated) channel has a more intimate association with the LA molecule whose favored pathway for dissociation is no longer between S6 segments (the former pore). Reprinted with permission from Strichartz GR, Berde CB: Local anesthetics, in Miller RD (ed): Miller's Anesthesia. Philadelphia, PA, Elsevier Churchill Livingstone, 2005, pp 573-603.¹⁹⁵

Table 1. Properties of Voltage-Gated Sodium Channel α -Subunit Isoforms

CHANNEL α -SUBUNIT ISOFORM	INACTIVATION RATE (TTX SENSITIVE/RESISTANT)*	DISTRIBUTION IN NORMAL ADULT RAT DRG†	CHANGE POST-NERVE INJURY	CHANGE POST-INFLAMMATION
Na _v 1.1	Fast (S)	+ 75% of all cells; esp. high levels in large cells	↓ SNL injured DRG ¹³⁰ No Δ DN ⁵¹	No Δ ²⁴
Na _v 1.2	Fast (S)	+ Low levels; 40% of all cells	↓ SNL injured DRG ¹³⁰	No Δ ²⁴
Na _v 1.3	Fast (S)	– Very low or absent in adult, but high levels in embryo	↑ Axot esp small cells; but no Δ Rhizot ^{22,217} ↑ SNL injured DRG; esp. medium and large cells ¹³⁰ ↑ CCI small cells ⁶³ ↑ DN small and large cells ⁵¹	↑ small cells ²⁴
Na _v 1.4	Fast (S)	–	n/a	n/a
Na _v 1.5	Slow (R)	– (embryo only)	n/a	n/a
Na _v 1.6	Fast (S)	+ 87% of all cells, esp. high levels in medium and large cells	↑ DN small and large cells ⁵¹	No Δ ²⁴
Na _v 1.7	Fast (S)	+ All cells	DN ¹¹⁵	↑ esp. small cells ^{24,95}
Na _v 1.8	Slow (R)	+ 50% all cells	↓ Axot small cells; but no Δ Rhizot ¹⁹¹ ↓ CCI small cells ⁶³ ; but no Δ CCI found by Novakovic et al ¹⁶⁰ ; decrease only in axot cells ⁵⁷ no Δ SNL uninjured DRG ⁹⁰ Translocated to axons in CCI, Axot., SNL uninjured DRG ^{90,136,160} ↓ DN small and large cells ^{51,115}	↑ esp. small cells ^{24,95,203}
Na _v 1.9	Very slow (persistent) (R)	+ 80% of small cells, rare in large cells; ca. 50% of all A δ and C nociceptors; 90% of "silent" C-nociceptors	↓ Axot small cells; but no Δ Rhizot ¹⁹¹ ↓ CCI small cells ⁶³ ; decrease only in axot cells ⁵⁷ ↑ DN small and large cells ⁵¹	No Δ ²⁴

Abbreviations: \uparrow , \downarrow , no Δ , increase, decrease, or no change in expression of mRNA and/or protein. Axot, axotomy (nerve transection); DN, diabetic painful peripheral neuropathy; SNL, spinal nerve ligation model of Kim and Chung¹³¹; CCI, chronic constriction injury model of Bennett and Xie¹⁷; Rhizot, dorsal rhizotomy.

*Data from International Union of Pharmacology: <http://www.iuphar-db.org/iuphar-ic/sodium.html>.

†Data from Black et al²³; Dib-Hajj et al⁶²; Fang et al⁷³; Kim et al¹³⁰; Renganathan et al¹⁷⁹; and Waxman et al.²¹⁷

model of neuronal firing that includes channel isoforms with only fast- or a mixture of fast- and slow-inactivating kinetic parameters matching those measured in DRG neurons. In response to a single brief (.1 msec) supra-threshold depolarizing stimulus, the model neuron with 100% fast-inactivating channels fires a single impulse. Remarkably, changing only 5% of the channels to the slowly-inactivating type yields repetitive discharge of relatively long duration. It is far from clear whether our current methods could detect such a small change.

Pharmacology of Sodium-Channel Isoforms

State-Dependent Binding of Sodium-Channel Blockers

A critical aspect of local anesthetic binding that underlies many of their therapeutic actions is state-selective affinity. Local anesthetics bind relatively weakly to resting closed channels, more tightly and rapidly to open channels, and as tightly but more slowly to inactivated

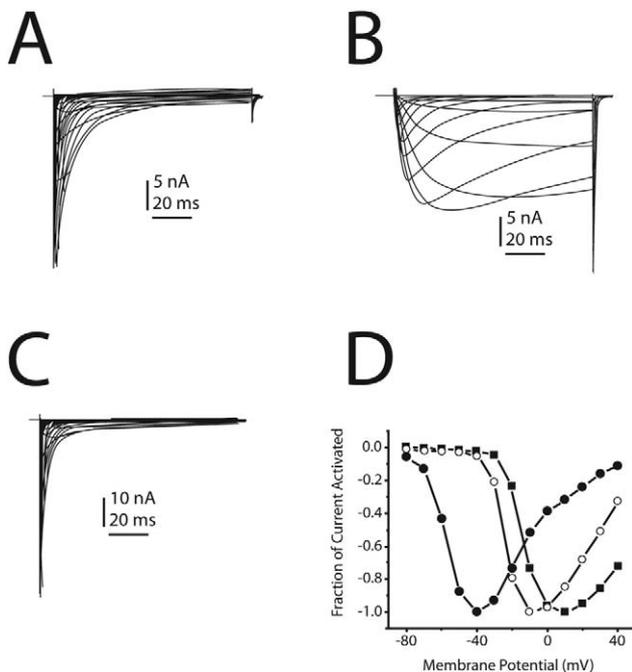


Figure 2. Primary afferent somatosensory neurons express multiple voltage-gated sodium channels with distinct voltage dependence and kinetic properties. (A) Slow TTX-resistant currents. (B) Persistent TTX-resistant currents. (C) Fast TTX-sensitive currents. (D) Comparison of voltage dependence of activation for the currents shown in A (solid squares), B (solid circles), and C (open circles). All currents were recorded from small-diameter rat DRG neurons. The currents in A and B were recorded in the presence of 500 nmol/L TTX.

channels.^{28,45} As a consequence of these differences, patterns of membrane potential that present more of the high-affinity states result in a larger effective blockade.^{28,188} Two such patterns are germane to the discussion of pain: the trains of ectopic impulses that occur after peripheral nerve injury and the spontaneous discharge of sensitized nociceptors innervating inflamed tissue. A train of rapidly firing impulses presents a sequence of open channels that increasingly bind local anesthetic, to the point that the cumulative binding leaves too few unbound channels available to support the action potential.¹⁶⁹ The scenario for such use-dependent block depends on the concentration and binding rate constants for the drug, the frequency of the impulses and, often overlooked, the margin of safety for impulse generation and propagation in the zone of firing.¹⁹⁶ Moreover, the prolonged depolarizations produced by some channel isoforms yield a relatively large number of inactivated channels that support a slow binding of local anesthetics. When the long depolarization is due to a persistently open population of channels, the blockade by the local anesthetic will shorten the depolarization.¹²⁹ This situation might have particular significance in the case of sodium channels expressed on primary afferent terminals in the spinal cord. A high density of slowly inactivating sodium channels would support a prolonged depolarization, allowing voltage-gated calcium channels on the terminal's membrane to remain

open longer, which would allow a proportionately larger influx of calcium into the terminal and thereby result in a relative increase in neurotransmitter release. Blockade of only a fraction of the persistently open sodium channels, and of more of those channels that are in the inactivated state, could dramatically shorten the duration of depolarization, and this would profoundly reduce the release of neurotransmitter.

Pharmacologic Diversity Among Isoforms

The structural diversity among the various isoforms, particularly in those regions of the molecule not involved with voltage sensing and pore formation, suggests that they may have different pharmacologic profiles. Relatively little data is available on this question. We have already noted that there is differential binding for TTX among the various isoforms. Natural toxins from the venoms of poisonous toads, spiders, marine mollusks of the genus *Conus* (cone snails), dinoflagellates, and other animals also discriminate among isoforms.^{25,163} The observation that different isoforms have different kinetics for open, inactivated, and closed states implies differential sensitivity to drugs exhibiting state-dependent binding, and there is indeed evidence for diversity of local anesthetic effects on the different isoforms. For example, the mammalian cardiac channel, $Na_v1.5$, appears to be much more sensitive than the neuronal channels, eg, $Na_v1.2$, to lidocaine block. This difference in potency results from a difference in the levels of resting activation between the 2 channels and therefore reflects gating differences rather than drug affinity differences between the channels.^{161,213,221} In vitro experiments using the $Na_v1.7$ and $Na_v1.8$ isoforms coexpressed with the β_1 subunit show that $Na_v1.8$ is 4 times more sensitive to lidocaine.⁴⁶

Moreover, the isoforms present different potential substrates for several protein kinases, and some may also be directly modified by G-proteins.³⁵ Exposure of neurons to inflammatory mediators (eg, prostaglandin (PG) E_2), algogenic substances (eg, endothelin-1), and neurotransmitters (eg, serotonin) can modify TTX-R channel gating through enzymatic phosphorylation of intracellular portions of the α - and β -subunits. However, there is little evidence that TTX-S channels are subject to such

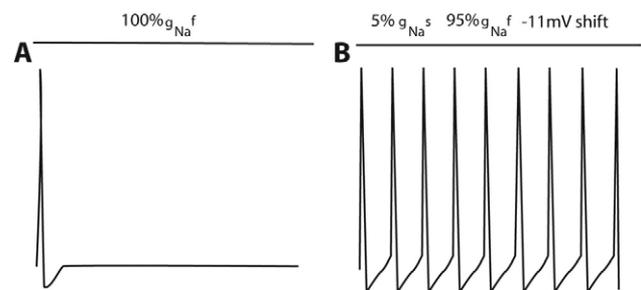


Figure 3. Results of a computer simulation of the responses of a neuron that expresses only sodium channels with fast inactivation rates (A) and the change produced by switching 5% of the channels to the slowly inactivating type (B). Responses are to a single .1-msec depolarizing stimulus. Data from Gent et al, unpublished observations.

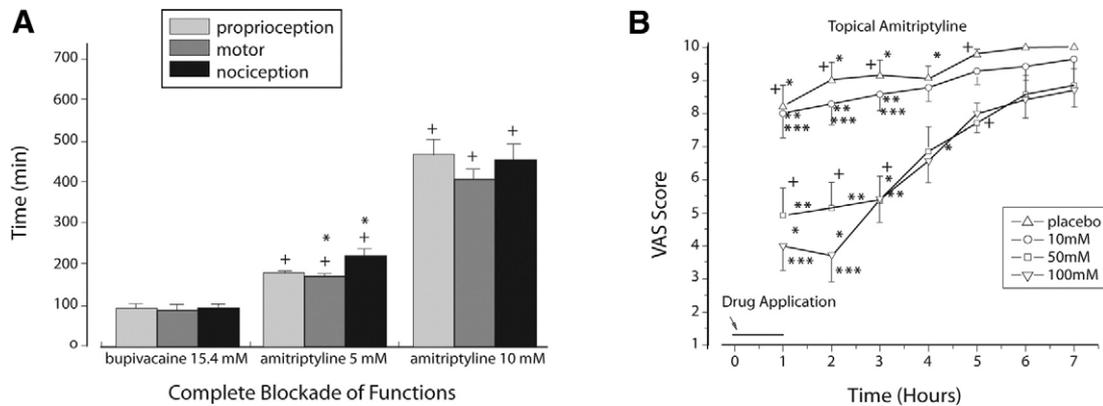


Figure 4. (A) Time of complete proprioceptive, motor, and nociceptive blockade produced by bupivacaine vs amitriptyline when applied to rat sciatic nerve. +: Block durations of all 3 functions in the amitriptyline groups are significantly longer than in the bupivacaine group. *: Nociceptive blockade is significantly longer than motor blockade in the 5 mmol/L amitriptyline group ($P < .05$). Data from Gerner et al.⁸⁵ (B) Long-lasting analgesia in human volunteers with a topical preparation of amitriptyline. $P < .05$ for *(placebo vs 100 mmol/L amitriptyline), ** (10 vs 50 mmol/L amitriptyline), *** (10 vs 100 mmol/L amitriptyline), and + (placebo vs 50 mmol/L amitriptyline). Reprinted with permission from Gerner P, Kao G, Srinivasa V, Narang S, Wang GK: Topical amitriptyline in healthy volunteers. *Reg Anesth Pain Med* 28:289-293, 2003.⁸⁴

receptor-driven phosphorylation in primary nociceptors, although such channels and their related currents are sensitive to direct activation of protein kinase (PK) A and its intracellular pathways in cultured cells.³⁵ The extent to which these mechanisms contribute to neuropathic pain in vivo is unknown.

The differential distribution of channel isoforms between different tissues raises the hope that isoform-selective drugs that modify function in primary afferent neurons may be relatively free of the toxic central nervous system (CNS) and cardiac side effects that constrain the use of currently available sodium-channel blockers. We focus here on the role of neuronal sodium-channel isoforms, but we note that sodium channels are also expressed by glia cells in both the peripheral and central nervous systems; glia cells may also be a target of sodium-channel blocking drugs.¹⁹²

Tricyclic Antidepressants as Sodium-Channel Blockers

Any discussion of the role of sodium channels in pain states involves evidence derived from the use of local anesthetic drugs. However, we must also consider the evidence from tricyclic antidepressants (TCAs). This group of drugs is commonly used in the treatment of neuropathic and other chronic pain states.^{32,153} All of the TCAs are known to have a complex mixture of actions which might contribute to their analgesic action. Early work concentrated on their ability to inhibit the reuptake of norepinephrine and serotonin.¹⁵³ TCAs have also been shown to block sodium, potassium, and calcium voltage-gated channels.¹⁶⁷ We focus here on whether activity at sodium channels contributes to their analgesic efficacy.

Potent Blockers of Sodium Channels

Compared to bupivacaine, many TCAs show a longer duration of blockade of nerve impulse propagation

when tested in animal preparations (Fig 4). Amitriptyline was more potent than bupivacaine in a subcutaneous infiltration model¹²⁶ and when applied intrathecally in rat and sheep.⁸³ However, when amitriptyline was evaluated for ulnar nerve blockade in healthy human volunteers, it was found to be less effective than bupivacaine—contrary to the results from a large number of animal studies. This might be due to the thicker nerve sheaths present in humans as compared to rats, presenting a larger barrier to penetration for amitriptyline into the nerve.⁸⁰ When applied topically, the pain-blocking activity of amitriptyline was found to be significantly more effective than placebo in healthy human volunteers,⁸⁴ and some subjects had a complete analgesia lasting several hours (Fig 4).

Local anesthetics and TCAs both bind more tightly to the inactivated state of the sodium channel.³⁴ Thus, as with local anesthetics the nerve block obtained with TCAs is use dependent, and this dependency may be even more pronounced for TCAs.¹⁶⁷

There are data suggesting that various TCAs differ in their ability to block sodium channels. Whereas amitriptyline, doxepin, and imipramine were superior to bupivacaine in blocking nerve impulse propagation in a rat sciatic nerve preparation, trimipramine and desipramine were somewhat less effective than bupivacaine, and nortriptyline, protriptyline, and maprotiline were clearly inferior.¹⁹⁸ However, it is unclear whether the differences among TCAs are due to differences in their activity at sodium channels or to differences in their ability to pass through various membrane barriers within peripheral nerve trunks.

It remains to be seen whether various TCAs show similar binding affinities for the various sodium-channel isoforms. There is evidence for 2 distinct binding affinities for bupivacaine and amitriptyline at physiologically relevant membrane potentials.⁸⁵

Contribution of Sodium Channels to Neuropathic Pain Conditions

Clinical Evidence

Several clinical studies have demonstrated that therapeutic agents that exhibit use-dependent block of sodium channels show efficacy against painful peripheral neuropathy. Systemic administration of lidocaine and other sodium-channel blockers relieves the symptoms of neuropathic pain in patients with postherpetic neuralgia, painful diabetic neuropathy, idiopathic trigeminal neuralgia, and other conditions.^{12,74,123,149,184,189} A topical preparation of lidocaine relieves postherpetic neuralgia,^{182,183} and preliminary studies suggest that it may also have efficacy in painful diabetic peripheral neuropathy.¹¹ Intravenous lidocaine has shown efficacy in a double-blind placebo-controlled study in patients with neuropathic pain following spinal cord injury.⁷⁵ Sodium channel blockade is a likely mechanism through which at least some antiepileptics might suppress neuropathic pain. As noted above, the well established efficacy of TCAs may be due, at least in part, to their ability to block sodium channels.

Experimental Evidence

The expression of sodium-channel isoforms in primary afferent neurons is dramatically altered after peripheral nerve transection and in animal models of post-traumatic and diabetic painful peripheral neuropathy (Table 1). $Na_v1.1$ and $Na_v1.2$ are down-regulated following axotomy.¹³⁰ $Na_v1.3$ is not normally expressed in adult DRG, but its expression is greatly up-regulated after complete and partial nerve injuries and after the induction of diabetes.^{22,51,63,130,167,191,217} In contrast, dorsal rhizotomy, which severs the central branch of the DRG neuron's axon, has no effect on $Na_v1.3$ expression.^{22,217} The de novo expression seen after nerve transection can be normalized by treatment with glia-derived neurotrophic factor (GDNF) and nerve growth factor (NGF).¹³⁹ The expression of $Na_v1.6$ is up-regulated in small and large DRG cells in experimental diabetic painful neuropathy.⁵¹ The expression of $Na_v1.8$ is down-regulated in primary afferent neurons following transection of their peripheral axons, but no change is seen when their central axons are cut.¹⁹¹ In partial nerve injuries, the intact afferent neurons (perhaps only the C-nociceptors) show little or no change in the expression of $Na_v1.8$ ⁹⁰ but redistribute these channels from their cell bodies in the DRG to their axons.^{90,160} Contrary results have appeared concerning the fate of $Na_v1.8$ expression in the chronic constriction injury (CCI) model.¹⁷ A decrease in small cells has been reported by Dib-Hajj et al,⁶³ but Novakovic et al¹⁶⁰ found no change. In the CCI model, the DRG contain a mixture of axotomized and intact neurons; it is likely that there is a decrease of $Na_v1.8$, but only in the axotomized population.⁵⁷ $Na_v1.8$ is down-regulated in small and large DRG neurons in diabetic rats.⁵¹ Peripheral nerve transection, but not dorsal rhizotomy, decreases the expression of $Na_v1.9$.¹⁹¹ A decrease is also seen in small cells in the

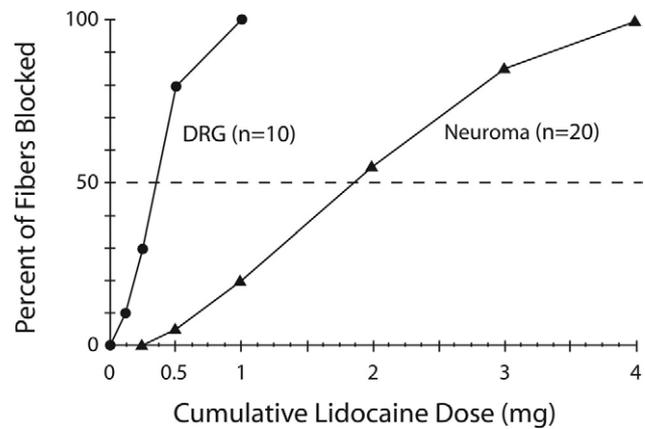


Figure 5. Cumulative dose of lidocaine (repeated IV bolus dosing) producing a complete block of spontaneous ectopic discharge in primary afferent neurons recorded after sciatic nerve transection in the rat. Ectopic discharge arising from the axotomized neuron's cell body in the DRG is 4-5× more sensitive to lidocaine than discharge arising from the site of nerve injury (neuroma). Note that the graph shows the percentage of neurons whose spontaneous discharge was completely blocked by the indicated dose. A significant reduction in discharge frequency would be obtained by plasma levels less than those achieved with the indicated dose. Reprinted with permission from Devor M, Wall PD, Catalan N: Systemic lidocaine silences ectopic neuroma and DRG discharge without blocking nerve conduction. *Pain* 48:261-268, 1992.⁶¹

CCI model⁶³; but in this case the change is also probably found only in the axotomized cells.⁵⁷ Interestingly, $Na_v1.9$ is increased in both small and large cells in diabetic rats.⁵¹

Ectopic Discharge

A large body of evidence suggests that the development of discharge from ectopic foci after nerve injury may represent an underlying mechanism that drives neuropathic pain.^{1,60,98} These abnormal discharges may arise at the site of nerve injury or in the injured primary afferent neuron's cell body in the DRG.^{58,119,120,132,151,210,211} Moreover, it is now clear that in partial nerve injuries, intact axons also have ectopic discharge when they are neighbors to degenerating axons.²²² Electrophysiologic studies show that both myelinated and unmyelinated primary afferent axons show spontaneous activity after nerve injury, and it is thus probable that such discharge is present in low-threshold mechanoreceptors and in nociceptors.⁶⁰

Seminal discoveries in this field were reported by Devor et al.^{59,61} First, they showed that sodium channels accumulate at the stump of severed axons. Second, they demonstrated that ectopic discharge in axotomized primary afferent neurons is very sensitive to lidocaine, being blocked by doses that have no effect on the initiation of nerve impulses by natural stimuli or on the propagation of nerve impulses. Moreover, they showed that ectopic discharge originating in the DRG is about 5 times more sensitive to lidocaine than the ectopic discharge originating at the site of nerve injury (Fig 5). There is evidence that mexiletine is even more potent than lido-

caine in blocking ectopic discharge.⁴⁰ These effects were found at doses that are far below those that yield cardio- or neurotoxicity.

The presence of spontaneous ectopic discharges may provide a mechanism to account for spontaneous pain and dysesthesiae after nerve injury. However, there are several caveats associated with this hypothesis. First, the presence of spontaneous discharge in injured fibers, or in their uninjured neighbors, may play a role in spontaneous pain. But work with experimental animal models of neuropathic pain rarely measures spontaneous pain. Experimentally, we usually measure the response of a nerve-injured animal with light touch, noxious heat, or cold stimuli, and changes in the threshold for the nocifensive reflex response to these stimuli are indices of allodynia or hyperalgesia. Many, but perhaps not all, peripheral neuropathy patients have spontaneous pain in addition to various kinds of allodynia and hyperalgesia,¹⁶ but we do not know if there is a causal link between spontaneous and stimulus-evoked pains.

A second argument against the interpretation of spontaneous discharge of injured afferents being “the” mechanism of spontaneous neuropathic pain is the issue of time dependency. It is known that spontaneous discharges occur soon after injury but, in the animals at least, they diminish rapidly and appear to revert to normal within 20 to 50 days, whereas behavioral signs of evoked neuropathic pain are present for many weeks afterwards.^{19,103,145,148} Even during the period immediately following nerve injury, the correlation between the degree of spontaneous activity and evoked neuropathic pain behaviors may be weak.^{72,143-145} These findings emphasize the distinction between the mechanisms which initiate neuropathic pain and those that maintain it. It is possible that the maintenance phase relies on the evolution of CNS mechanisms.^{33,70,98,118}

Channel Isoforms and Neuropathic Pain

Na_v1.3

At least some primary afferent ectopic discharge and at least some of the symptoms of painful peripheral neuropathy are TTX sensitive.¹⁶⁴ For example, low concentrations of TTX, which have no effect on nerve impulse propagation, block spontaneous ectopic discharge and mechanoallodynia in the spinal nerve ligation model.^{47,146} Na_v1.3 is TTX sensitive, and its expression in small, presumably nociceptive, DRG neurons (as well as in medium and large, presumably nonnociceptive, neurons) is up-regulated following axotomy in the SNL, CCI, and diabetic neuropathy models.^{22,47,51,63,115,130,141,217} The post-injury increase in Na_v1.3 expression parallels the appearance of a rapidly repriming sodium current in small DRG neurons.²² These observations suggest that Na_v1.3 may make a key contribution to neuronal hyperexcitability.

Post-nerve injury treatment with GDNF normalizes Na_v1.3 expression, reduces ectopic discharge in A-fibers, and reduces pain.^{27,212} However, a 50% decrease in the expression of Na_v1.3 in rat DRG cells, obtained via the

antisense method, has no effect on mechanoallodynia or cold-allodynia in the rat spared-nerve injury model.¹⁴¹

Na_v1.7

Nassar et al¹⁵⁷ have produced mice in which the Na_v1.7 gene is knocked out selectively in nociceptive DRG neurons. These animals do not differ from their wild-type controls in the development of mechanoallodynia in the Chung model¹³¹ of post-traumatic painful peripheral neuropathy. Moreover, mechanoallodynia develops normally in mice in which both Na_v1.7 and Na_v1.8 have been deleted.¹⁵⁶ However, expression of Na_v1.7 is increased in the DRG of rats with streptozotocin-induced painful diabetic neuropathy, and there is also an increase in the level of phosphorylation of the channel.¹¹⁵

Erythromelalgia (also “erythromalgia”) is a rare autosomal dominant condition in which the patient experiences episodes of burning pain and red hot skin, usually in the extremities and usually exacerbated by warmth and relieved by cooling. This condition has been variously ascribed to neuropathic and vasomotor mechanisms and to small-fiber neuropathy.^{55,166} There is now definitive evidence that at least some erythromelalgia patients have an inherited mutation in the gene that encodes the Na_v1.7 channel.^{64,69,155,231} The mutant channel has alterations in its activation and deactivation kinetics, a lowered threshold for activation, and an increase in current amplitude, resulting in hyperexcitability and high-frequency discharges in nociceptive DRG neurons.^{53,64} It remains to be seen whether similar changes in Na_v1.7 function are present in acquired painful peripheral neuropathy.

Na_v1.8

Several lines of evidence suggest that Na_v1.8 is important in painful peripheral neuropathy. Na_v1.8 makes a major contribution to the TTX-resistant current found in small, presumably nociceptive, DRG neurons. Kral et al¹³⁵ showed that the TTX-resistant current in small DRG cells isolated from CCI rats had activation thresholds that were more negative than normal and that there was a reduction in the average density of this current. DRG levels of Na_v1.8 are down-regulated in rats with streptozotocin-induced diabetes at times when the animals display mechanoallodynia.^{51,115} In the rat spinal nerve ligation (SNL) model of Kim and Chung,¹³¹ where the axons of L5 and L6 DRG neurons are transected, Na_v1.8 levels are decreased in L5 DRG, but increased in the intact neurons of L4.²³⁴

Treatment with antisense, but not mismatch, oligodeoxynucleotide (ODN) to Na_v1.8 significantly reduced channel expression and produced a temporary reversal of allodynia and hyperalgesia in SNL rats (Fig 6).^{90,136}

Because the expression of Na_v1.8 is down-regulated in the L5/L6 small cell bodies whose axons have been injured in this model,¹⁹¹ the site of action of Na_v1.8 inhibition may lie elsewhere.^{90,136} Following axotomy and in the CCI model, stores of Na_v1.8 within the neuron’s cell body in the DRG are translocated to the neuron’s axon, and this correlates with an increase in TTX resistance in

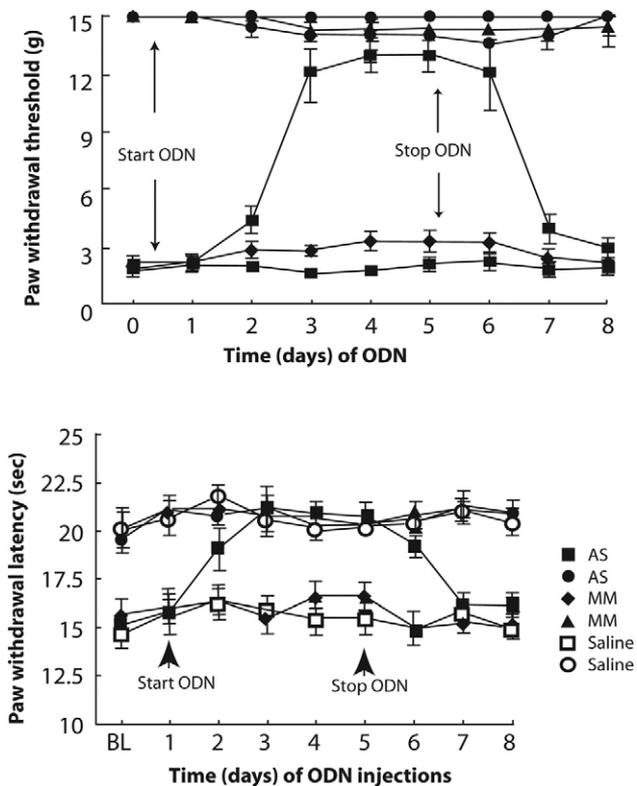


Figure 6. Antisense targeting of $Na_v1.8$. Rats with the spinal nerve ligation (SNL) model of post-traumatic painful peripheral neuropathy developed the expected ipsilateral mechanoallodynia (Top) and heat-hyperalgesia (Bottom), as shown at baseline (BL) testing. One day later, the animals were injected intrathecally with antisense (AS) oligodeoxynucleotide (ODN) to block the expression of the $Na_v1.8$ sodium channel, with mismatch (MM) ODN as a control or saline as an additional control. MM and saline had no effect, but AS reversed the allodynia and hyperalgesia on the nerve-injured side for as long as it was administered. Reprinted with permission from Lai J, Gold MS, Kim CS, Bian D, Ossipov MH, Hunter JC, Porreca F: Inhibitions of neuropathic pain by decreased expression of the tetrodotoxin-resistant sodium channel, $Na_v1.8$. *Pain* 95:143-152, 2002.¹³⁶

slowly conducting axons (almost certainly nociceptors).^{90,160} This translocation has also been seen in the uninjured L4 DRG neurons in the SNL model, and there is evidence of ectopic discharge in the axons of L4 neurons in that model. Thus, the pain-relieving effect of antisense-mediated inhibition of $Na_v1.8$ in models of partial nerve injury is likely due to changes in the uninjured afferent axons.

In contrast to these results, other evidence indicates that $Na_v1.8$ is not essential for the development of neuropathic pain. Kerr et al¹²⁵ found no difference between normal mice and $Na_v1.8$ -null mutants, and Nassar et al¹⁵⁶ reported no effect on mechanoallodynia in the Chung model in $Na_v1.8$ null mice or in mice with a double knockout for $Na_v1.8$ and $Na_v1.7$. The reason for the discrepancy between the antisense and knockout experiments is unclear. It may be of importance that the antisense experiments were done in rats, whereas the knockout experiments were done in mice.

The possibility that the $Na_v1.8$ is critical for ectopic dis-

charge in injured primary afferent fibers is supported by the results of experiments in genetically modified mice that lack the $Na_v1.8$ isoform. In the wild-type mouse strain from which the mutants were prepared, section of the saphenous nerve and the subsequent formation of a nerve-stump neuroma is associated with the appearance of a high incidence of ectopic discharge in A- and C-fibers. By 3 weeks after nerve injury, 20% of the fibers in the wild-type mice had ectopic discharge, but less than 1% of the fibers had ectopic discharge in mice lacking $Na_v1.8$.¹⁸⁵

It is important to note that there is evidence suggesting that the function of $Na_v1.8$ may change with time following nerve injury. In the first 2-3 weeks following nerve injury, the channel appears to contribute to neuropathic pain by enabling activity in uninjured afferents. However, with time, expression of the channel appears to be up-regulated again in injured axons and may even accumulate at the site of nerve injury.⁵⁰

$Na_v1.9$

The persistent current carried by the $Nav1.9$ channel is abundantly expressed in small DRG neurons, particularly the nonpeptidergic IB4-binding neurons that terminate in the spinal cord substantia gelatinosa. Mice lacking functional $Na_v1.9$ channels have no apparent change in normal pain sensation. Moreover, tests in mutant mice with nonfunctional $Na_v1.9$ channels¹⁷⁴ and in mice where the channel's expression has been inhibited by the oligodeoxynucleotide antisense method¹⁷² have failed to show any effect on the expression of neuropathic pain.

Membrane Potential Oscillations and Ectopic Discharge

One might hypothesize that the ectopic discharge in primary afferent neurons results from the classic (Hodgkin-Huxley) repetitive firing process in which a sustained depolarization repeatedly draws the membrane potential above threshold. However, accumulating evidence acquired both in vitro and in vivo suggests that at least in the cell bodies of primary afferent sensory neurons in the DRG, the ectopic discharge results from a quite different process—subthreshold membrane potential oscillations.^{6-8,122,143,168,223,226,227}

The Oscillatory Mechanism Is Voltage Sensitive

When first penetrated by a microelectrode, most DRG neurons have a stable resting potential. However, a minority (about 5%-10%) of the A-neurons (those that have myelinated axons) exhibit periodic sinusoidal membrane potential oscillations of small amplitude (≤ 2 mV peak-to-peak) with a mean frequency of about 100 Hz (Fig 7).^{7,143,227} The oscillations are voltage sensitive (Fig 7): Both their prevalence and amplitude are enhanced upon depolarization.^{7,143,168} Accordingly, the percentage of oscillating A-neurons is increased from about 6%

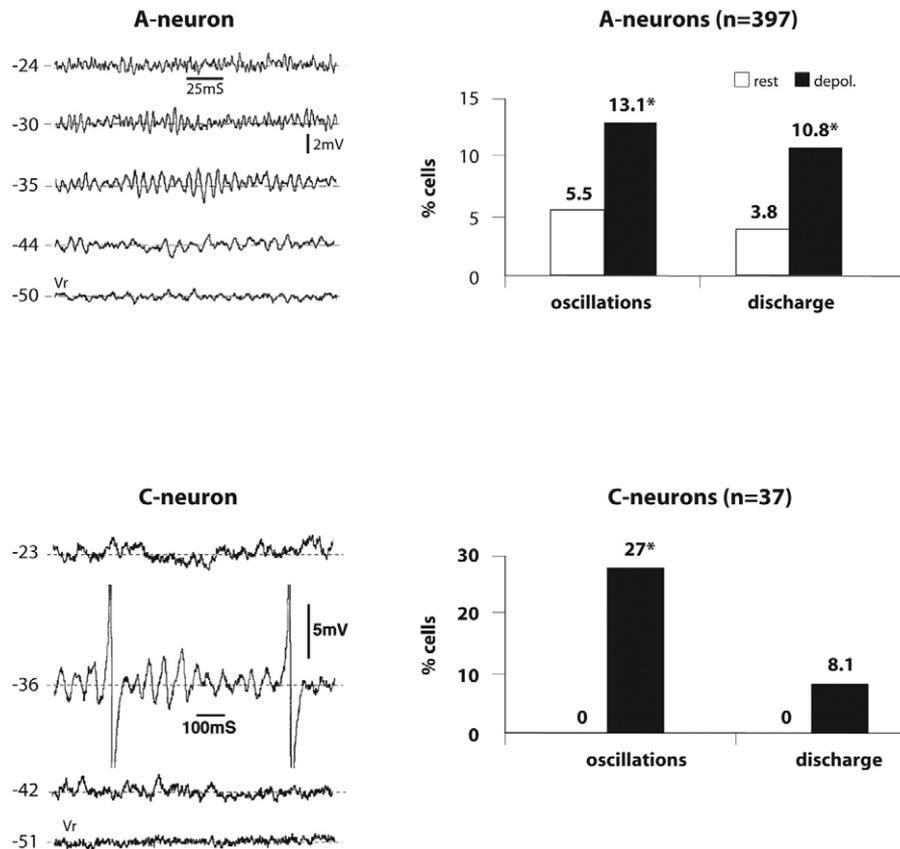


Figure 7. Subthreshold sinusoidal membrane potential oscillations from noninjured rat DRG neurons. (Left) Oscillations in A- and C-neurons at various membrane potentials. Vr, resting potential. Note ectopic impulses arising from 2 of the oscillations in the C-neuron. (Right) Percentage of DRG A- and C-neurons with membrane potential oscillations and ectopic discharge at the neuron's resting potential (*open bars*) and when depolarized (*solid bars*). * $P < .001$ vs control. Reprinted with permission (left side of figure) from Amir R, Michaelis M, Devor M: Membrane potential oscillations in dorsal root ganglion neurons: Role in normal electrogenesis and neuropathic pain. *J Neurosci* 19:8589-8596, 1999.⁷ Copyright ©1999 by the Society for Neuroscience.

at the resting potential to about 13% upon depolarization (pooled data from experiments with both immature and adult rats).^{7,143,144} In addition, oscillations are revealed in about 27% of the depolarized C-neurons (those with unmyelinated axons) (immature and adult rats).⁷

Nerve Injury Enhances the Oscillatory Mechanism

Nerve injury induces an increase in the prevalence of DRG neurons with subthreshold oscillations. For example, the proportion of depolarized A-neurons with oscillations is increased from about 10% to 24% (adult rats, pooled data from both sciatic and spinal nerve transection),^{7,143} and in C-neurons the proportion increased from 28% to 44% (adult rats, sciatic transection).⁷

The Oscillatory Mechanism Is Essential for the Generation of the Ectopic Discharge

The oscillatory mechanism is essential to the generation of repetitive ectopic discharge.^{7,143,144,227} Only oscillating neurons show repetitive ectopic firing; conversely, neurons without subthreshold oscillations do

not, even upon depolarization.^{7,143} Some neurons fire ectopic singlet spikes at low frequency with an irregular pattern. Each such spike is triggered by the upstroke of a sinusoidal oscillation that crosses threshold, indicating a causal relation between the oscillations and the discharge.^{7,143} Other neurons fire sustained trains of regularly spaced spikes, or sequences of spike bursts. Here, the first impulse in the train (or burst) is consistently triggered by an oscillation sinusoid, and each subsequent spike is triggered by a depolarizing after-potential.⁸ Consistent with the hypothesis, because the prevalence of oscillating neurons is relatively low at resting potential, spike discharge is also rare, with only about 4% of the A-neurons firing spontaneously. However, on depolarization, as the underlying oscillatory mechanism is enhanced, this percentage is increased to 11% (pooled data from both immature and adult rats).^{7,143} In addition, about 8% of the C-cells begin to fire repetitively (immature and adult rats).⁷ Because nerve injury increases the prevalence of oscillating neurons, it consequently enhances the prevalence of ectopically active neurons. Accordingly, the proportion of depolarized A-neurons that fire repetitively is increased from about 10% to 23% (adult rats, pooled data from both sciatic and spinal

nerve transection),^{7,143} and in C-neurons the proportion increases from 12% to 27% (adult rats, sciatic transection).⁷

Thus, the question of what causes repetitive ectopic discharge becomes 2 questions: 1) What causes membrane potential oscillations? and 2) what causes the depolarizations that raise them to the firing threshold? There are several processes capable of depolarizing DRG neurons. These include mechanical stress (eg, during movement or straight-leg raising),¹⁵⁹ cell-to-cell cross-depolarization (where activity in a subpopulation of DRG neurons affects adjacent neurons),^{4,5,8} and sympathetic efferent activity.^{8,171,226} Indeed, all of these mechanisms increase ectopic firing in vivo.⁶⁰ Oscillatory behavior in DRG neurons can thus be thought of as a “motor” ready to be engaged when the “clutch” of a slow-onset physiologic depolarization is released.

Sodium Channels Are Important for the Generation of Subthreshold Membrane Potential Oscillations

The ionic basis of the oscillatory mechanism in DRG neurons has been studied.^{6,7} A voltage-sensitive and TTX-sensitive sodium conductance appears to be important for the rising phase of the oscillations in both A- and C-neurons. The oscillations are eliminated by partial substitution of sodium ions with choline in the perfusion solution, and by bath application of TTX or lidocaine.^{7,168,227} Once eliminated, oscillations could not be restored by depolarization, but they generally reappeared following washout of the blocker. Calcium-channel blockers were not effective at blocking the oscillations.^{7,168} Suppression of oscillations with sodium substitution, or via TTX or lidocaine block, also suppressed ectopic firing, consistent with the idea of a causative relation between the oscillations, the ectopic discharge, and sodium channels. Oscillations and resulting ectopic discharge were consistently blocked at times when propagation of impulses evoked by axonal stimulation persisted, indicating that the effect was specific to the ectopic discharge mechanism, rather than a local anesthetic effect.^{7,227} Ectopic firing in vivo is also suppressed using lidocaine concentrations insufficient to block axon conduction.⁶¹

Although there are considerable data supporting the hypothesis that a TTX-sensitive channel underlies the membrane potential oscillations, there are 2 pieces of data to suggest that, at least under certain conditions, TTX-resistant current may also contribute to oscillations. First, depolarization-potentiated oscillations have been described in trigeminal ganglion neurons, and these oscillations are resistant to TTX. In contrast, the oscillations from DRG neurons are persistent and TTX sensitive.¹⁷⁶ This may correlate with a difference between trigeminal and DRG neurons; in the rat, trigeminal nerve transection evokes relatively little ectopic discharge.²⁰² Second, membrane potential oscillations induced in dissociated A-neurons appear to have a voltage dependence that is inconsistent with the biophysical properties of TTX-sensitive

channels present in sensory neurons.¹⁴² The voltage dependence of oscillation amplitude in these neurons peaks at about -20 mV, a potential at which 100% of TTX-sensitive channels would be inactivated and therefore unable to contribute to the depolarization phase of the oscillation.

Sodium Channel Isoforms That Are Likely to Contribute to the Oscillatory Mechanism

Among the sodium channel subtypes, $Na_v1.3$ is the most likely to contribute to the oscillatory mechanism. It has fast enough gating kinetics, it is up-regulated at precisely the same time after axotomy that oscillations, ectopic firing, and behavioral allodynia commence, and TTX suppresses all 4.^{22,144,217} Of course, this does not mean that other sodium-channel isoforms might not play important roles. Selective block of any one of the isoforms might be enough to attenuate the oscillations and bring at least some cells below the threshold for ectopic firing. The TTX-resistant isoforms $Na_v1.8$ and $Na_v1.9$ are particularly interesting in this regard. Moreover, the relatively persistent depolarizations produced when $Na_v1.8$ and $Na_v1.9$ open may be important both in generating the oscillations and in bringing them to the firing threshold.

Hyperpolarization-Activated, Cation-Nonselective, Cyclic Nucleotide-Modulated Channels

It is known that sodium currents in DRG neurons are carried not only by the channels that respond to membrane depolarization but also by a family of channels designated as HCN—for hyperpolarization-activated, cation-nonselective, cyclic nucleotide-modulated—that respond to membrane hyperpolarization. These channels pass both sodium and potassium. They are involved in cardiac pacemaker activity, and in DRG neurons they are known to be involved in the production of rhythmic discharge and to modulate the membrane's resting potential.^{68,207} Four isoforms have been identified, of which HCN1-3 are clearly expressed in rat DRG cells; the presence of HCN4 is uncertain.^{42,207} Large- and medium-diameter DRG neurons that probably correspond to fibers with A β and A δ conduction velocities express these channels abundantly. However, about 15% of DRG cells expressing HCN also express TRPV1, suggesting that at least some C-nociceptors may also express this channel.⁶⁸ HCN1 and HCN2 are significantly, but partially, down-regulated in the L5/L6 DRG of rats in the SNL model. Nevertheless, in a rat nerve-ligation model, ZD7288, an inhibitor that is specific for HCN channels, significantly decreases mechanoallodynia and the ectopic firing frequency in both A β and A δ fibers (by 90% and 40%, respectively).⁴² There is also evidence that HCN channels in the hippocampus have a role in epilepsy.¹⁴ The pharmacologic characteristics of HCN channels are not known in detail. Local anesthetics block HCN channels in rat small DRG neurons at clinically relevant concentrations.²¹ The

data from SNL rats and the clear role that HCN channels play in rhythmic repetitive firing and in regulating the membrane resting potential suggest that they may have a role in neuropathic pain.

However, it does not seem likely that HCN channels contribute to the subthreshold membrane potential oscillations described above. First, the current conducted via HCN channels in DRG neurons is minimal or inactivated at membrane potentials required for oscillation activity. Second, the current carried by HCN channels is consistently inhibited upon depolarization, whereas the oscillatory mechanism is enhanced. Third, the HCN current has very slow kinetics, which is inconsistent with the relatively high frequency of the subthreshold membrane potential oscillations. Fourth, HCN1 and HCN2 are down-regulated following SNL at times when the oscillatory mechanism is enhanced.^{42,143}

Sodium Channels on CNS Neurons

There is evidence that sodium channels expressed by CNS neurons also may be involved in neuropathic pain. $Na_v1.3$ is up-regulated in spinal cord dorsal horn neurons in CCI rats. Inhibiting this increase with antisense ODN decreased dorsal horn neuron hyper-responsiveness and reduced the animal's abnormal pain responses.¹⁰¹ The expression of $Na_v1.3$ is increased in dorsal horn neurons also following a traumatic spinal cord injury that produced the central pain syndrome. Here too, the antisense ODN procedure decreased neuronal and behavioral signs of abnormal pain.¹⁰⁰ Very recent work shows that spinal cord injury causes an increase in the excitability of nociceptive neurons in the ventrobasal thalamus and an up-regulation of $Na_v1.3$ in thalamic neurons. Inhibition of $Na_v1.3$ expression by antisense ODN given via lumbar intrathecal administration reversed the excitability changes and decreased $Na_v1.3$ expression in the thalamus.¹⁰² It is not clear how treatment at the spinal level affected thalamic expression levels.

Microinjection of lidocaine into the rostroventral medial medulla (RVM) blocks both tactile and thermal hyperesthesia^{134,170} when tested 4 days and later but not within the first 2 days after SNL. The time course of lidocaine activity emphasizes the possibility of a time-dependent plasticity in the CNS that may maintain neuropathic pain. The systemic delivery of quaternary derivatives of lidocaine, QX222, and QX314, which do not cross the blood-brain barrier, has been shown to reverse thermal hyperalgesia,¹⁶⁴ but not tactile hyperesthesia, in rats with SNL.⁴⁴ In contrast, QX314 reversed tactile hyperesthesia when microinjected into the RVM.⁴⁴

The mechanisms by which the QX compounds achieve their effects are not fully understood. Voltage-clamp studies in both myelinated and nonmyelinated axons show that the QX compounds block TTX-S sodium channels only from the intracellular compartment,^{79b,194b} yet they reduce the C-fiber elevation of the compound action potential of rat nerves¹⁶⁴ with a strong "frequency-dependent" inhibition; frequency-dependent block is a hallmark of local anesthetic drug actions on sodium

channels.^{194b} Systemically administered QX compounds relieve symptoms of neuropathic pain in rats and reduce injury-related ectopic impulse activity, with impulses arising from the injury site and the DRG being considerably more sensitive than those recorded in the dorsal horn. A possible interpretation of these results is that systemic QX compounds have easier access to peripheral nerve sodium channels but are much slower to reach the CNS. One might speculate that the sites of action for these "therapeutic" effects are primarily TTX-R sodium channels, whose structure at the outer pore differs from that at TTX-S channels, similar to the differences wrought by site-directed mutagenesis of sodium channels that allows extracellular local anesthetics to reach their blocking site from the outer opening of the channel.^{176b} However, whether the actions of the QX compounds truly involve only sodium channels and, if so, the degree of isoform selectivity of this effect are important questions that remain unanswered.

The observations described suggest that sodium channels may have different roles in the different kinds of stimulus-evoked pain abnormalities (ie, mechanoallodynia evoked by input from A β low-threshold mechanoreceptors vs heat-hyperalgesia evoked by input from C-nociceptors). This is consistent with the idea that the different kinds of neuropathic stimulus-evoked pains have at least partly different pathophysiologic mechanisms. There is considerable evidence that this is true in the animal models²²⁴ and some evidence that it is true in man. For example, using selective nerve block techniques in human volunteers who received topical application of mustard oil, it has been shown that mechanoallodynia is evoked by input from the large-diameter myelinated A β fibers, whereas heat-hyperalgesia is evoked by input from unmyelinated C-nociceptors.¹³² The possibility that tactile allodynia is mediated by large fibers raises the unanswered question of how "knockdown" of $Na_v1.8$ by antisense ODN can produce antiallodynic actions, because $Na_v1.8$ is almost exclusively found on small DRG neurons in normal animals.

Peripheral Changes in Sodium Channels Drive CNS Changes

Several lines of evidence suggest that after nerve injury, enhanced peripheral neuronal activity, including the ongoing discharge of C-nociceptors, results in central changes at spinal levels. Central sensitization²¹⁹ is the most thoroughly studied example. Recent studies suggest that post-injury changes at supraspinal levels also contribute. For example, there is evidence that nerve injury is followed by activation of descending pain-facilitatory mechanisms in the brain stem that serve to maintain pain hypersensitivity.¹⁷³ As noted, $Na_v1.8$ is found primarily in small-diameter nociceptors, yet knockdown with antisense ODN, or normalization of its distribution with growth factors (see below), reverses both mechanoallodynia and heat-hyperalgesia after SNL. Although acute systemic administration of QX314 was not active against mechanoallodynia in nerve-injured rats, mech-

anoallodynia was reversed by long-term (several days) administration of QX314.¹⁶⁴ This suggests that long-term suppression of peripheral activity with QX314 may be necessary to enable reversal of CNS changes, although the result could also reflect a slow accumulation of QX314 in the CNS during systemic delivery.

The first hypothesis is also supported by recent observations made with artemin, a member of the GDNF neurotrophic factor family.⁸² Prolonged systemic administration of artemin reversed both mechanoallodynia and heat-hyperalgesia in rats with SNL.⁸² The injury-induced redistribution of Na_v1.8 along the sciatic nerve was returned to the normal state by artemin, as was up-regulation of spinal dynorphin and other neuropeptides.⁸² Artemin acts through the GFR α 3 receptor, which is found exclusively on small diameter nociceptors.¹⁶⁵ These findings support the concept that chronic treatments that can interfere with peripheral changes after nerve injury can result in a cascade of events that serve to normalize the chronic pain state.

It is important to note that accumulating evidence indicates that neuropathic pain is a chronic abnormal state whose mechanisms evolve over time. After peripheral nerve injury, a number of central and peripheral neural changes occur that are essential for the initiation and maintenance of the neuropathic pain state. Either the prevention or the reversal of these central or peripheral adaptations may return the nervous system to a normalized state, resulting in prevention of pain expression. Sodium-channel blockade, over an extended time period, might accomplish this goal and might therefore be useful as a mechanism of disease modification. Critically, although the primary site of action of sodium-channel blockers may be in the primary afferent fiber, or perhaps within the CNS, the ultimate action may result from normalization of function at both central and peripheral sites.

Long-Lasting Pain Relief With Short-Duration Exposure to Sodium-Channel Blockers

Neuropathic pain patients often report days or weeks of pain relief after brief treatments with lidocaine and other sodium-channel blockers. It is now firmly established that an analogous phenomenon occurs in rats with experimental peripheral neuropathies who receive infusions of lidocaine. For example, Chaplan et al⁴¹ showed that an intravenous infusion of lidocaine, which was maintained at constant plasma levels for 30 minutes to several hours, eliminated the animal's mechanoallodynia not only during the infusion but also for at least 3 weeks afterwards. In addition, Mao et al¹⁵⁰ have shown that a single bupivacaine block of the sciatic nerve in CCI rats relieves heat-hyperalgesia for at least 2 days.

Strichartz et al^{10,190,196} have shown that there is a threshold lidocaine plasma concentration of 2.5–5 μ mol/L required for long-lasting (48 h post-infusion) pain relief. They also found that there was a limit to the degree of long-lasting relief attainable in each individual rat; neither repeated infusions to the same effective

plasma concentration nor graduated infusions to increasingly higher levels (which were still well below toxic levels), delivered every 48 h, could elevate the amount of pain relief above an individual's "ceiling level." Some rats achieved complete and persistent relief, but most had a more limited recovery. A minority, about a quarter of those tested, responded insubstantially to lidocaine and had no relief during or after the infusion. The reason for these individual differences has yet to be identified. There was a strong correlation between the acute reversal of allodynia during the infusion and the degree to which it was sustained 2 days later, implying that these 2 phenomena are mechanistically coupled. In identical experiments with mexiletine, the same degree of acute relief during the infusion was never followed by a persistent recovery, suggesting a special activity for lidocaine. Because the half-life of lidocaine in the rat's circulation is at best a few hours, and its less potent (with respect to impulse blockade) metabolites are present for only a few hours longer,¹²⁴ the enduring recovery is unlikely to be due to any residual drug.

Detailed examination of the time course of this phenomenon revealed 3 distinct phases of activity (Fig 8). In animals infused 2 days after nerve injury, substantial relief was seen during the 30-min infusion (with mechanoallodynia reduced by about 70%). Thirty minutes after the end of the infusion, mechanoallodynia returned completely. Remarkably, beginning about 6 h later and continuing over the next 24 h, mechanoallodynia slowly disappeared again and remained at a greatly reduced level for 1 to 2 weeks. Therefore, the sustained recovery from allodynia was not a continuation of the relief during drug infusion, but was instead a delayed and slowly evolving effect.

Identical infusions of lidocaine at 7, rather than 2, days after injury gave essentially identical acute effects and the same delayed rise in threshold over the next 24 h, but in this case the recovery was not sustained and the therapeutic effect faded over the following week.

In summary, these observations suggest that the late persistent phase of pain relief obtained with lidocaine is mechanistically distinct from that seen acutely, that the long-lasting pain relief may or may not be coupled to sodium-channel blockade, and that the long-lasting relief may depend critically on the pathophysiologic mechanisms underlying mechanoallodynia, with these mechanisms varying over time and from animal to animal.

Contribution of Sodium Channels to Inflammatory Pain Conditions

Recent evidence suggests that sodium channels play a key role in the pain and hypersensitivity associated with tissue inflammation, and there is a possibility that this role is especially important in the context of chronic inflammation. A δ - and C-nociceptors become sensitized when the tissue that they innervate becomes inflamed. Primary afferent nociceptor sensitization is characterized by the appearance of 3 abnormal properties: 1) spontaneous discharge, 2) a lowered threshold to the

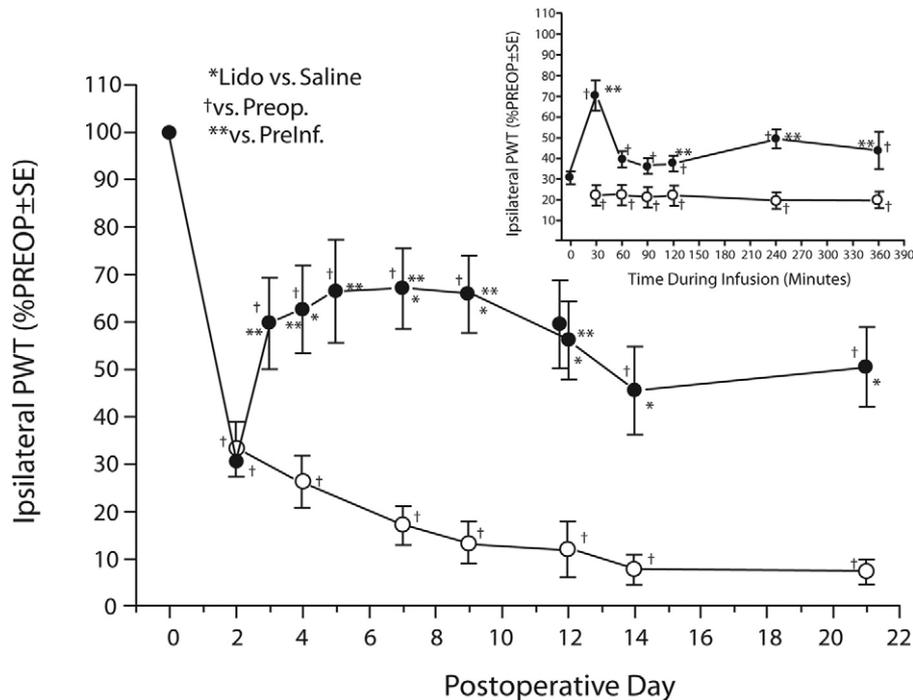


Figure 8. Brief infusions of lidocaine ($4 \mu\text{g/mL}$ plasma = $14.7 \mu\text{mol/L}$) at 2 days after sciatic nerve ligation (SNL) in rat reverse mechanical allodynia ipsilateral to the ligation in 3 phases: 1, peri-infusion; 2, transient postinfusion; and 3, slowly developing sustained. Solid circles = lidocaine infusion; open circles = saline infusion. Reprinted with permission from Araujo MC, Sinnott CJ, Strichartz GR: Multiple phases of relief from experimental mechanical allodynia by systemic lidocaine: Responses to early and late infusions. *Pain* 103:21-29, 2003.¹⁰

adequate stimulus, and 3) a stimulus-response (S-R) function that is shifted leftwards. These changes contribute to the generation of ongoing (“spontaneous”) pain, hyperalgesia, and allodynia. Of course, ongoing pain, hyperalgesia, and allodynia are also symptoms of neuropathic pain. The question is therefore to what extent the phenomena found in both inflammatory and neuropathic pain states share common physiologic mechanisms, including contributions from sodium channels. Tissue inflammation also activates a class of nociceptors that are not normally excitable. These “silent” nociceptors become excitable when exposed to an inflammatory milieu; when activated, they behave like sensitized normal nociceptors.¹⁵⁴ It is not known whether “silent” nociceptors are engaged in neuropathic pain states, and there are no data on their sensitivity to sodium-channel blockade. However, it is of interest to note that 90% of silent C-nociceptors express $\text{Na}_v1.9$.⁷³ It is important to note that most of our knowledge about nociceptor sensitization comes from experiments using acute inflammatory states. As we note in the following, there is evidence that acute and chronic inflammatory pain have at least partly different mechanisms.

Clinical Evidence

Sodium-channel blockers are not usually considered in the therapy for chronic inflammatory pain, but recent open-label trials^{11,54,81} suggest that topical lidocaine may relieve low back pain, the pain of osteoarthritis, and myofascial pain. The mechanism that produces such ef-

fects is unclear; it seems very unlikely that anesthetic block of cutaneous nociceptors could relieve pain that is coming from deep tissues. Topical lidocaine in these studies results in low plasma levels ($<.2 \mu\text{g/mL}$), so an effect can not be ascribed to blockade of nerve impulse propagation.

Experimental Evidence

The inflammatory pain state created by a subcutaneous injection of complete Freund’s adjuvant (CFA) has been studied extensively. Studies using an antiserum that recognizes an epitope common to all Na_v -channel isoforms have shown that CFA injection evokes a dramatic up-regulation of sodium channel expression. The effect is of rapid onset, with peak levels of up-regulation occurring within about a day, and the effect persists for at least 2 months.^{94,97}

There have been 2 studies of lidocaine’s effects on spontaneous discharge in nociceptors innervating inflamed tissue where the plasma levels have been measured. Puig and Sorkin¹⁷⁵ (Fig 9) used a continuous intravenous infusion to examine lidocaine’s effects on 14 cutaneous C-fiber nociceptors with spontaneous discharge following formalin injection in the hind paw (approximately 30 min before lidocaine). Decreases in firing frequency and complete block of spontaneous discharge were seen with lidocaine plasma levels of $15\text{--}34 \mu\text{mol/L}$ (mean $21 \mu\text{mol/L}$). The mean threshold lidocaine concentration was not reported, but effects were clearly seen at about $6.5 \mu\text{mol/L}$. Mechanical stimulation of the C-fiber’s

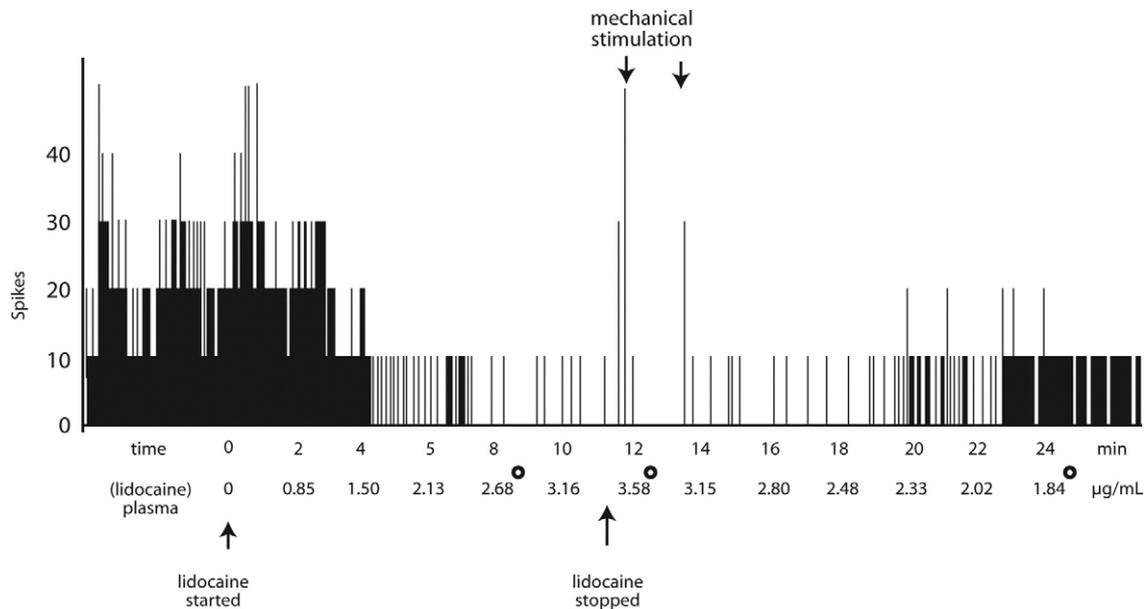


Figure 9. Effect of lidocaine infusion on the ongoing inflammation-evoked discharge of a C-fiber cutaneous nociceptor in the rat. Infusion began 27 min after injecting formalin into the hind paw and continued for the period marked by the *lower arrows*. Plasma levels were estimated from the Stanpump program and confirmed by 3 plasma samples drawn at the times marked with *open circles*. Inhibition is first seen clearly at a concentration of about 1.5 µg/mL. Note that a mechanical stimulus still initiated discharge even when the spontaneous discharge was blocked. Reprinted with permission from Puig S, Sorkin LS: Formalin-evoked activity in identified primary afferent fibers: Systemic lidocaine suppresses phase-2 activity. *Pain* 64:345-355, 1995.¹⁷⁵

cutaneous receptive field evoked discharge even when the ongoing discharge was completely inhibited.

Xiao et al²²⁵ used a continuous intravenous infusion to examine lidocaine's effects on 9 Aδ- and C-fiber cutaneous nociceptors with spontaneous discharge following administration of an antibody against GD2 ganglioside. This antibody is used to treat pediatric neuroblastoma patients; its infusion frequently causes the rapid onset of pain and mechanoallodynia. Intravenous infusion in rats also causes mechanoallodynia of rapid onset. The mechanism by which the antibody produces pain and allodynia is not known, but it is likely to involve a neuroimmune response with similarity to the neuroimmune phenomena that occur during inflammation. Xiao et al²²⁵ found that plasma lidocaine levels of 1.4-8.6 µmol/L produced a >50% decrease in spontaneous discharge without affecting the fiber's mechanical threshold. The block of spontaneous discharge often persisted for many minutes after the infusion (45-60 min in 2 extreme cases). Based on these 2 studies, the threshold plasma lidocaine level needed to block the ongoing discharge of cutaneous Aδ- and C-nociceptors in the context of an acute inflammation is likely to be in the range of 1.3-6.5 µmol/L. Note that these were studies of cutaneous nociceptors; there are no data on the lidocaine sensitivity of nociceptors innervating inflamed deep tissues.

Behavioral indices of spontaneous pain and hyperalgesia in rats with an experimental arthritis are reduced by repeated systemic administration of the sodium-channel blockers mexiletine and clobenxetine.¹³⁷ The analgesic effects became larger with repeated doses, suggesting that the mechanisms underlying the drug effect and/or

the mechanisms of the pain and hyperalgesia evolved with time. Systemically tolerable doses of mexiletine have also been shown to block behavioral and electrophysiologic indices of pain in the formalin model.^{43,117}

Channel Isoforms and Inflammatory Pain

Na_v1.7 Channel

An increase in the expression of Na_v1.7 and its redistribution may contribute to inflammation-induced nociceptor sensitization. Early experiments showed that a brief exposure of PC12 cells to NGF, which is known to play a major role in inflammatory hyperalgesia,²²⁰ results in an increase in Na_v1.7 expression and an increase in channel density at neurite terminals.^{205,206} Inflammation induced by CFA is accompanied by a rapid increase of Na_v1.7 expression, and this increase is blocked by pretreatment with cyclooxygenase inhibitors.⁹⁵ An increase of Na_v1.7 expression is seen after the administration of NGF.⁹⁶ Nerve growth factor not only increases expression of Na_v1.7, but also increases the density of the channel in sensory nerve terminals *in vitro*.²⁰⁶ Inflammation of the tooth pulp is associated with a redistribution of Na_v1.7 to the nodes of Ranvier in thinly myelinated axons.¹⁹³ Recent studies have shown that ablating Na_v1.7 in nociceptive neurons greatly reduces inflammatory pain responses.^{156,157}

Na_v1.8 Channel

There is a large body of evidence suggesting that modulation of Na_v1.8 contributes to the initiation of inflammation-induced nociceptor sensitization and hyperalgesia. Inflammation evoked by CFA is associated with a

rapid increase in Na_v1.8 expression in DRG that is inhibited by ibuprofen.⁹⁵ The persistent hyperalgesia evoked by repeated injections of PGE₂ is also accompanied by an up-regulation of Na_v1.8 in DRG.²⁰⁹ Inflammatory mediators induce a rapid increase in maximal sodium conductance, a small hyperpolarizing shift in the voltage dependence of activation, and an acceleration of TTX-resistant current activation, all of which enhance excitability.^{87,88} A large number of inflammatory mediators have been demonstrated to induce these same changes in Na_v1.8 channels: PGE₂,^{71,88} serotonin,^{36,88} adenosine,⁸⁸ epinephrine,¹²⁸ endothelin-1,²³⁶ and NGF.²³⁴ In addition, the increased excitability of DGR neurons evoked by PGE₂ and forskolin in vitro is TTX resistant.⁷¹ The Na_v1.8 channel might therefore act as a final common target for a diverse array of inflammatory mediators acting through a number of distinct second-messenger pathways, including PKA,^{71,87} PKC,^{87,128} a nitric oxide,³ extracellular signal-regulated kinase,⁶⁶ and a ceramide-dependent kinase.^{233,234} At least some of these effects appear to reflect a direct phosphorylation of the Na_v1.8 channel.⁷⁷ A role for Na_v1.8 in the initiation of inflammatory hyperalgesia is further supported by the observation that antisense knockdown of the channel blocks the development of hyperalgesia induced by a single injection of PGE₂^{127,209} as well as the long-lasting hyperalgesia produced by repeated injections of PGE₂.²⁰⁹ Moreover, agonists of the μ-subtype of opioid receptor⁸⁶ and group II metabotropic glutamate receptor²³⁰ block both the modulation of the channel and the development of inflammatory hyperalgesia¹⁴⁰ and allodynia.²²⁹

Further evidence for a role for Na_v1.8 in the early phase of inflammatory pain comes from studies in mice in which its gene has been knocked out.^{2,138} For example, these animals have normal acute pain responses to visceral irritants (intracolonic instillation of saline and intraperitoneal acetylcholine). However, intracolonic administration of the inflammatory and nociceptor-sensitizing agents capsaicin and mustard oil produced significantly less pain-related behavior in the mutants that lacked Na_v1.8 channels. Colonic inflammation in the wild-type mice was associated with referred hyperalgesia—a reduction of pain thresholds to noxious stimuli applied to the hind paw or abdominal skin. Mice lacking the Na_v1.8 gene had little or no referred hyperalgesia.

Given that persistent inflammation is associated with elevated levels of inflammatory mediators, the associated modulation of Na_v1.8 is also likely to contribute to the maintenance of inflammatory hyperalgesia. An increase in the expression of the channel may also have a role. Support for this suggestion comes from the observation that there is an increase in Na_v1.8 mRNA in DRG following peripheral injection of a high dose of carrageenan and repeated injections of PGE₂.^{24,203,209} There is also an increase in the relative density of the TTX-resistant current in visceral sensory neurons following inflammation of the gastric mucosa²⁰ and colon.¹⁸ That these changes in expression may be associated with an increase in the transport of the channel to the peripheral terminals is supported by observations indicating that the

CFA-induced inflammation results in an increase in Na_v1.8 in peripheral axons⁴⁸ (but see Okuse et al¹⁶²). Moreover, a 4.5-fold increase in Na_v1.8 levels has been found in the pulp extracted from painful teeth compared to pulp from nonpainful teeth¹⁸⁰; the innervation of the dental pulp is almost entirely nociceptive. Further evidence implicating Na_v1.8 comes from experiments showing that antisense knockdown of Na_v1.8 attenuates CFA-induced hyperalgesia,¹⁷² the neuronal hyperexcitability observed in an animal model of cystitis,²³² and the long-lasting hyperalgesia seen after repeated injections of PGE₂ in rat.²⁰⁹

It should be noted that some of the data obtained in the Na_v1.8 null mutant mouse² do not support results obtained with other approaches. For example, whereas NGF-induced hyperalgesia is significantly attenuated, PGE₂-induced hyperalgesia is unaffected in mice lacking Na_v1.8.¹²⁵ Moreover, the onset of CFA-induced hyperalgesia is delayed, but not inhibited,² and the hyperalgesia seen with cyclophosphamide-induced cystitis is unaffected.¹³⁸ The basis for the different results obtained with mice lacking Na_v1.8 and those obtained with other experimental manipulations is not clear. It is possible that the genetic mutation that eliminates Na_v1.8 induces compensatory changes in the expression of other ion channels or other mechanisms and that these compensatory mechanisms mask the effects of the gene deletion.² It is also possible that the involvement of Na_v1.8 in mediating inflammatory pain varies with different inflammatory mediators.

In fact, there is evidence to suggest that the role of the Na_v1.8 channel differs during inflammation of different tissues. For example, both the proportion of afferents sensitized and the magnitude of the inflammation-induced sensitization of colonic sensory neurons¹⁹⁷ appear to be greater than what is observed following inflammation of cutaneous afferents.⁹ Both the magnitude of the modulation and the proportion of neurons in which Na_v1.8 is modulated are greater in colonic afferents than in cutaneous afferents.⁹¹ That similar differences are observed in isolated neurons in vitro when exposed to the same inflammatory mediators suggests that these differences do not merely reflect differences in the underlying tissue or the mode of inflammation.⁸⁹

Na_v1.9 Channel

Many inflammatory mediators act via G-protein-coupled receptors on voltage-gated sodium channels. Indirect evidence in support of a role for Na_v1.9 in inflammatory hyperalgesia comes from the observation that guanosine triphosphate (GTP) and its nonhydrolyzable analog GTPγS results in a significant increase in Na_v1.9-mediated current.¹³ This GTPγS-mediated increase in current is blocked with protein kinase antagonists, suggesting that the increase is the result of a phosphorylation event. Phosphorylation of Na_v1.9 is likely to be initiated via inflammatory mediators such as PGE₂.¹⁸⁷ Studies of Na_v1.9 currents in mice lacking the Na_v1.8 channel suggest that Na_v1.9

channels may be dynamically modulated by a variety of factors.¹⁵² Data on the role of Na_v1.9 in the maintenance of inflammatory hyperalgesia have been mixed. Support for such a role comes from the observation that Na_v1.9 expression can be modulated by neurotrophins, in particular, GDNF.⁷⁸ However, application of GDNF to cultured neurons did not appear to increase channel expression but rather restored expression levels to normal values. No change in Na_v1.9 expression is seen after carrageenan injection,²⁴ but there is evidence for a decrease in the amount of Na_v1.9-like immunoreactivity that appears to be transported to the periphery.⁴⁸

Mice with nonfunctional Nav1.9 channels have abnormal responses in inflammatory pain models.¹⁷⁴ Their phase I response in the formalin model is normal, but they fail to display the phase II response. Following CFA injection, they develop heat-hyperalgesia to a normal degree for the first few hours, but over the ensuing days the hyperalgesia resolves much more quickly than normal. Moreover, they do not develop the heat-hyperalgesia that is normally seen after subcutaneous injection of PGE₂.

Actions of Sodium-Channel Blockers That Do Not Involve Sodium Channels

Although sodium channel actions are undoubtedly the primary site of action for the classical effects of local anesthetics, they are certainly not the sole target of these drugs. Interactions with other signaling systems have been reported for many years, but have not received much attention, because the clinical importance of such effects has never been firmly established.

The majority of studies looking at actions not mediated via sodium channels have used lidocaine as the test agent. For example, lidocaine's interactions with calcium channels could in theory have an analgesic effect. However, concentrations required for half-maximal block of calcium channels are between .5 and 2 mmol/L.¹⁰⁶ Delayed rectifier-type potassium channels in *Xenopus* sciatic nerve are blocked by lidocaine, but only at approximately 1 mmol/L.²⁹ In anterior pituitary cells, lidocaine inhibits such potassium currents at 1.9 mmol/L, and sodium channel inhibition occurs at 170 μmol/L.²²⁸ Whether disregarding these interactions because of these high concentration requirements is appropriate remains an open question.

Calcium-signaling G protein-coupled receptors (GPCRs), the activation of which results in the release of calcium from intracellular stores, have been identified as a target for lidocaine.^{107,113} Several findings make this discovery of particular interest. First, lidocaine inhibition of several GPCRs can occur at concentrations that are clinically relevant, eg, the plasma levels seen after an epidural block. Remarkably, some of these receptors are inhibited at nanomolar concentrations. Second, it appears that a number of lidocaine's well described clinical actions that are difficult to explain by sodium-channel block can be accounted for by

inhibition of GPCR signaling. In particular, the inflammatory-modulating actions of lidocaine may result from interactions with GPCR-mediated inflammatory signaling molecules. Importantly, prolonged (hours) exposure of cells to lidocaine greatly enhances its effects on GPCR signaling, whereas the actions on ion channels take minutes in their onset and to reach steady-state.

Molecular Mechanisms of Lidocaine-GPCR Interactions

The mechanisms of action of lidocaine on GPCR signaling have been elucidated largely by studies in recombinant systems, usually the *Xenopus* oocyte. Initial findings demonstrated a wide range of sensitivities of various GPCR to lidocaine. Thus, when expressed in *Xenopus* oocytes, the M₁ muscarinic acetylcholine receptor is remarkably sensitive to lidocaine, with a 50% inhibition concentration (IC₅₀) of 18 nmol/L.¹⁰⁸ On the other end of the spectrum, the AT_{1A} angiotensin receptor is essentially completely insensitive to lidocaine.¹⁵⁸ Other receptor systems, such as those for the inflammatory mediator thromboxane A₂ (TXA) and the putative inflammatory signaling molecule lysophosphatidate (LPA), show intermediate sensitivities to local anesthetics.^{114,158}

Investigations of lidocaine's effects on GPCR function have used selective extracellular vs intracellular exposure to QX314 (obtained with bath application and intracellular injection, respectively), a nonpermeant permanently charged lidocaine analog. Whereas sensitivity to extracellular QX314 varies greatly (discussed in the following), the sensitivity to intracellular QX314 is similar among receptors. For example, IC₅₀ values for QX314 of TXA, LPA, M₁, and M₃ muscarinic receptors are .53, .72, .96, and .45 mmol/L, respectively. Because the molecular structures of these receptors are quite different, this finding suggests that intracellular QX314 may be interacting instead with similar targets that are downstream along the signaling pathways engaged by these receptors.

The GPCRs activate 1 of several G proteins (G_q, G₁₁, G₁₄, or G_o), which in turn activate phospholipase C (PLC). Activated PLC cleaves membrane phosphatidylinositol-bisphosphate into intracellular inositoltrisphosphate (IP₃) and diacylglycerol (DAG). Inositoltrisphosphate induces intracellular calcium release by activating a receptor channel on intracellular stores. Diacylglycerol is a direct PKC activator which works synergistically with Ca⁺² to elevate PKC's activity. In addition to this enzyme-mediated action, G proteins may directly activate membrane channels; an example is the activation of the cardiac inwardly rectifying K⁺ channel by the βγ-subunit released after muscarinic receptor activation by the vagus nerve.

The sensitivity of the various G proteins to local anesthetic has been investigated. Calcium release induced by direct injection of IP₃ into oocytes was found to be insensitive to local anesthetic,¹⁹⁹ indicating that the IP₃ channel and its downstream signaling pathway

are not targets. The similar potencies for intracellular QX314 on various receptors, and the lack of effect on the downstream signaling pathway, suggest an effect on the G proteins that are coupled to receptors. These findings have been confirmed by direct knockdown of various G protein α -subunits using antisense oligonucleotides. First, the G protein subtypes coupling to various GPCRs were determined. In *Xenopus* oocytes, the LPA receptor, for example, couples to G_q and G_o , the trypsin receptor to G_q and G_{14} , and the M_1 muscarinic receptor to G_q and G_{11} . Effects of intracellular local anesthetic on receptor function were then studied after selective knockdown of each of these G protein subunits. In each case, the effect of local anesthetic was eliminated if, and only if, the G_q protein was removed; knockdown of any of the other G protein α -subunits was without effect on local anesthetic sensitivity.¹¹³ Interestingly, the lack of local anesthetic effect on angiotensin signaling could now be explained also, because this receptor was found not to be coupled to G_q in this model, but instead to G_o and G_{14} . Further evidence for an action on G_q signaling was provided by studies where mammalian G_q was introduced into the cell after knockdown of endogenous G_q protein; in this setting, local anesthetic sensitivity was regained.¹¹¹ Therefore, the intracellular effects of local anesthetics on GPCR signaling are likely explained by an inhibitory action on the G_q α -subunit or its downstream substrate(s). These findings are compatible with other investigations of local anesthetic actions on GPCR modulation of ion channels.²²⁸

It remains possible that the apparent action of local anesthetic on the G_q protein is indirect. For example, it is conceivable that intracellular QX314 activates a protein kinase, which in turn phosphorylates G_q and thereby inhibits its function. Evidence against this hypothesis has been obtained for PKC by testing for local anesthetic sensitivity in the presence of a PKC inhibitor,¹⁹⁹ but an indirect effect through another kinase cannot be formally excluded.

Intracellular inhibition of most GPCRs by QX314, mimicking the actions of intracellular lidocaine, occurs at an IC_{50} of approximately .5-1 mmol/L, which is much higher than the nmol/L concentrations required for inhibition of muscarinic receptor signaling by extracellular lidocaine. Which of these sites, intracellular or extracellular, and which corresponding potency, is germane to the clinical actions of systemic local anesthetics? Approximately 2-20 μ mol/L concentrations are attained systemically in the clinical setting, when lidocaine is given either intentionally intravenously or as byproduct of the epidural administration of high concentrations and volumes of the local anesthetic. Two important observations suggest that these low concentrations exert effects that might be relevant in the clinical setting: first, synergistic interactions with extracellular receptor domains; and second, a profound time dependence of the effect.

Interactions at Extracellular Receptor Domains

It appears that the high sensitivity of some GPCRs to local anesthetics can be explained by additive or even superadditive (synergistic) interactions between 1 (or more) intracellular binding site (of which G_q appears by far to be the most prominent) and 1 (or more) extracellular site. This has been investigated by comparing the effects of QX314, applied either intracellularly or extracellularly, with the effects of the compounds applied both intracellularly and extracellularly. When this experiment was performed with the M_1 muscarinic receptor, the IC_{50} values were approximately 50-fold less when the compound was applied to both sides of the membrane compared with selective intracellular or extracellular application. By isobolographic analysis, there was a significant superadditivity between the intra- and extracellular interactions.¹⁰⁸ The M_3 muscarinic receptor, which is largely similar in structure to the M_1 receptor, shows different sensitivity: Whereas lidocaine blocks M_1 receptors with an IC_{50} of 18 nmol/L, inhibition of the M_3 receptor requires approximately 370 nmol/L.¹¹² This is congruent with the presence of a major extracellular binding site for lidocaine that is present on the M_1 receptor but not on the M_3 receptor. Studies using chimeric M_1/M_3 receptors demonstrated that the N-terminus and third extracellular loop were required to obtain a significant increase in extracellular inhibition by QX314.

Time Dependence of Lidocaine's Effect on GPCR

The actions of local anesthetics on GPCR have been found to be profoundly time dependent. When oocytes expressing TXA receptors were exposed to bupivacaine for 4 h at 1.2 μ mol/L (1/10 of the IC_{50} obtained with acute exposure) receptor signaling was reduced to 25% of control.¹¹⁰ Similar results were obtained with lidocaine and with other receptors. In general, inhibitory potency is increased at least 4-fold by 48-h exposure. The site of action of this time-dependent effect was shown to be intracellular and dependent on the presence of G_q protein: After selective G_q knockdown, time-dependent effects were eliminated. The molecular mechanism behind this time-dependent modulation of G protein function has not been determined. However, in experiments using GTP γ S (a G protein activator) the inhibition of induced currents by bupivacaine is also time-dependent,¹¹⁰ indicating that the site of action within the G protein pathway is downstream of GDP-GTP exchange.

Other G Proteins May Show Different Responses to Local Anesthetics

Whereas the selective actions on calcium-signaling G proteins have been investigated in some detail, the effects of local anesthetics on other G proteins are not well known. Any such effect is likely to be different from the effect shown on the G_q pathway. Local anesthetic effects on G_i have been examined in a reconstituted adenosine

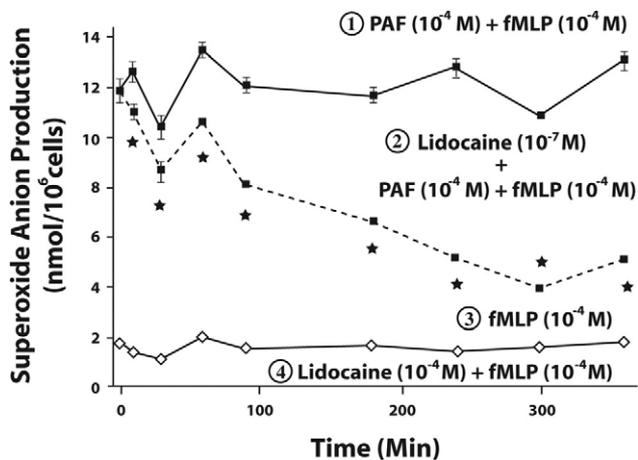


Figure 10. Effects of lidocaine (10^{-7} mol/L) on priming of superoxide release from human neutrophils in vitro. (1) Priming neutrophils with an exposure to PAF and subsequently activating them with formyl-methionine-leucine-phenylalanine (fMLP) evokes a large release of superoxide anion. (2) Including lidocaine with PAF in the priming step significantly decreases ($*P < .01$) the neutrophil response to subsequent activation (3 and 4; the two curves superimpose) Activating neutrophils with fMLP or fMLP + lidocaine evokes a much smaller response in the absence of priming (ie, prior exposure to PAF). Data from Hollmann et al.¹⁰⁹

signaling system.¹⁵ Lidocaine was found to potentiate G_i -coupled signaling through the A1 receptor by reducing cyclic adenosine monophosphate production. G_i was shown to be the target, indicating that in this class of G proteins lidocaine may have an enhancing, rather than an inhibiting, receptor-signaling action.

Local Anesthetic Effects on the Immune System Response to Tissue Damage

The pain associated with tissue inflammation and nerve injury occurs in the context of a complex immune response. The immune response is likely to make significant contributions to nociceptor activation and sensitization, and to the generation of ectopic discharge, for example, via the release of proinflammatory cytokines (for review, see Watkins and Maier²¹⁶). Therefore, a local anesthetic effect on the immune response is likely to modify both inflammatory and neuropathic pain states. There is now evidence that local anesthetics do affect the immune response and that this occurs at low plasma levels.

Effects on Human Neutrophils

The immune response to tissue injury is initiated by the rapid infiltration of neutrophils. Human neutrophils do not express sodium channels, and any actions of local anesthetic on these cells are therefore by definition sodium-channel independent. Neutrophil functioning is known to be regulated by GPCR signaling; therefore, the GPCR actions discussed above may also be relevant to the immune system. Neutrophils have a complex array of responses to activation by mediators, including superox-

ide release, chemotaxis, and release of further mediators; local anesthetics have been shown to inhibit all of these.^{76,201}

Local Anesthetics Inhibit Neutrophil Priming but Not Activation

An area that has been investigated in some detail is neutrophil priming. Priming refers to a process where an initial exposure to an agonist, such as platelet-activating factor (PAF), induces little or no response from neutrophils but results in a major increase in their response to a subsequent activating agonist.¹⁰⁹ The enhanced release of superoxide from primed neutrophils has been shown to be an important pathogenic factor in a number of inflammatory diseases.⁴⁹

The effects of various local anesthetics on the activation and priming pathways have been investigated in human neutrophils in vitro (Fig 10). At clinically relevant concentrations, local anesthetics are essentially without effect on the activating pathway; but priming is inhibited substantially by even very low levels.^{76,109} Importantly, the effect on priming in human neutrophils is strongly time dependent: Lidocaine at $.1 \mu\text{mol/L}$ inhibited PAF-induced priming to 39% of control after a 5-hour exposure.¹¹⁰ The mechanism behind the selective action on priming can be explained by the findings already discussed. Neutrophil activation is mediated primarily by a G_i -coupled pathway, whereas priming is mediated primarily by a G_q -coupled pathway. A selective effect of local anesthetic on G_q signaling explains why activation is unaffected while priming is inhibited.

Conclusions

It is clear that changes in the expression and function of voltage-gated sodium channel isoforms play an important role in neuropathic pain. These changes are likely to be key factors in the pathogenesis of both spontaneous and evoked ectopic discharge in damaged primary afferent neurons and probably also in the ectopic discharge that develops in their undamaged neighbors. Changes in channel isoforms may also produce pathologic changes in the process that regulates neurotransmitter release. Changes in sodium channel expression in CNS neurons, leading to altered states of neuronal excitability, are also likely to be involved. Moreover, accumulating evidence points to changes in sodium channel expression as key factors in inflammatory pain states. Such changes include many of the phenomena implicated in neuropathic pain, with the additional factor of a role for changes in sodium channel expression in the process of nociceptor sensitization. There are data to suggest that alterations in sodium channel expression may be particularly important in chronic inflammatory pain, and this is an area that deserves far more investigation. Whether the key change is in a single channel isoform or whether a particular mix of channels is the change in neuropathic or inflammatory pain states remains to be determined,

but a change in the “cocktail” of channels is likely to be significant.

At least some neuropathic pain phenomena, and perhaps chronic inflammatory pain phenomena as well, are unusually sensitive to local anesthetics. This sensitivity is likely to involve the drugs’ action on sodium channels in some cases (eg, ectopic discharge), whereas other cases (eg, the long-duration pain relief seen after a brief infusion) may involve other pharmacologic pathways.

The clinical efficacy of lidocaine and other sodium-channel blockers for the control of inflammatory and neuropathic pain is very likely to include mechanisms of action that involve binding to the sodium channel.

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