Chapter 20

Ion Channels Relevant to Pain

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ION CHANNELS AND PAIN

A wide variety of voltage- and ligand-gated ion channels are needed for the generation and propagation of neuronal action potentials. Many of these channels are generically expressed by a wide variety of neurons. Certain ion channels, however, appear to be localized to neurons conveying nociceptive information. Such channels are potential therapeutic targets; selective blockade could produce analgesia. An understanding of the ion channels expressed on first order Aδ- and C-fibers, as well as second and third order neurons in the dorsal horn and thalamus is obviously of considerable interest to pain research. This chapter is limited to a discussion of voltage-dependent calcium and sodium channels, as well as certain members of the families of potassium and acid-sensing channels, the TRP family, and purinoceptors implicated in pain transmission. Throughout, an emphasis will be placed on studies examining intracranial pain.

VOLTAGE-DEPENDENT CALCIUM CHANNELS

Voltage-dependent calcium channels (VDCC) are composed of α_1, $\alpha_2\delta$, β, and γ subunits; the α_1 subunit contains the selective calcium ion pore, a voltage sensor, and the binding site for selective high-threshold VDCC blockers. Ten isoforms of the α_1 subunit have been identified and this forms the basis for the most recent classification system (52). The 10 isoforms are grouped into three families according to their sequence homology (Table 20-1). VDCCs have also been grouped into six classes: L, N, P, Q, R, and T according to their electrophysiologic properties and pharmacologic susceptibility to specific blocking agents. At the most basic level, voltage-gated calcium channels may be divided electrophysiologically into two groups depending on their threshold of activation. Low-threshold VDCCs (T-type VDCCs) are activated at more hyperpolarized membrane voltages relative to the high-threshold (L-, N-, P-, and Q-type) channels (see references in Catterall ([22]).

VDCC are differentially distributed at the neuronal level. P/Q-type VDCCs are located predominantly at presynaptic sites (174,175), N-type are found both pre- and post-synaptically (173), and T- and L-type channels are mainly located on the proximal dendrites and soma of neurons (33,69,172). Synaptic release of neurotransmitters depends on the influx of calcium ions through voltage-gated calcium channels. P/Q-type VDCCs appear to be the most prevalent exocytotic calcium channel within the central nervous system (CNS) (49) controlling the release of excitatory amino acids, monoamines and peptide neurotransmitters (95,127,159,163). Exocytotic release of a variety of neurotransmitters is also inhibited by the N-type blocker ω-conotoxin GVIa (45,95,147,159,163). Excitatory glutaminergic neurotransmission is not completely prevented by blockade of N-type channels, indicating that several VDCCs are involved in neurotransmitter release (159,163). Antagonism of one type of calcium channel may therefore not significantly affect sensory signaling, especially if the presynaptic neuron is strongly depolarized. L-type channels do not appear to have a very significant role in neurotransmitter release in the CNS (although the nature of the stimulus used to evoke neurotransmitter release may be crucially important; nifedipine may inhibit release of substance P from dorsal root ganglion cells when they are depolarized with KCl but not electrical stimulation [73]). L- and N-type channels may have a more important role regulating neuronal membrane properties and synaptic integration. In the dorsal horn of the rat, just as in other neuronal systems such as cortical and hippocampal pyramidal cells, high-threshold calcium currents can generate both regenerative and plateau potentials (77,142,166). Plateau potentials sustained by calcium currents may result in a shift of the resting membrane potential toward threshold levels and contribute to the nonlinear response properties observed in dorsal horn neurons (109). The response of a dorsal horn neuron to nociceptive stimulation may therefore be dramatically enhanced

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TABLE 20-1 VDCC α_1 Subunit Classification, Distribution, and Functional Characteristics

Threshold of Activation	*Ca^{2+} Channel*	*Ca^{2+} Current Type*	*Previous Name of α_1 Subunits*	*Specific Blockers*[a]	*Tissue Distribution*
High threshold	$Ca_V1.1$	L-type	α_{1S}	Dihydropyridines	Skeletal muscle
	$Ca_V1.2$	L-type	α_{1C}	Dihydropyridines	Cardiac muscle Endocrine cells Neurons
	$Ca_V1.3$	L-type	α_{1D}	Dihydropyridines	Endocrine cells Neurons
	$Ca_V1.4$	L-type	α_{1F}	Calciseptine (?)	Retina
	$Ca_V2.1$	P/Q-type	α_{1A}	ω-Agatoxin IVA	Nerve terminals Dendrites
	$Ca_V2.2$	N-type	α_{1B}	ω-Conotoxin GVIA	Nerve terminals Dendrites
	$Ca_V2.3$	R-type	α_{1E}	SNX-482 (?)	Cell bodies Dendrites Nerve terminals
Low threshold	$Ca_V3.1$	T-type	α_{1G}	Ethosuximide	Cardiac muscle Skeletal muscle Neurons
	$Ca_V3.2$	T-type	α_{1H}	Ethosuximide	Cardiac muscle Neurons
	$Ca_V3.3$	T-type	α_{1I}	Ethosuximide	Neurons

[a] Specificity of VDCC blockers depends on concentration.
Adapted from Catterall WA. Structure and regulation of voltage-gated Ca^{2+} channels. *Annu Rev Cell Dev Biol.* 2000;16:521–555.

following activation of calcium (possibly L-type VDCCs) mediated plateau potentials (108). Calcium-channel conductance is also subject to the modulatory effects of various neurotransmitters and peptides, which can further affect a neuron's response characteristics at any given voltage (48,84,112).

It is unsurprising that VDCCs may be targets for compounds with potential analgesic properties. Gabapentin, a drug commonly used for the treatment of neuropathic pain, is also used as a prophylactic treatment for migraine. This calcium-channel modulator inhibits high-threshold VDCCs in dorsal root ganglion neurons (157) and may influence excitatory and inhibitory neurotransmitter release in the spinal dorsal horn (8). The N-type channel blocker ziconotide has also shown promise in clinical trials as a treatment for postoperative and cancer pain (155). Difficulties arise, however, when trying to determine pharmacologically the role of VDCCs in pain pathways. As we shall see, the effects of blocking individual channels may differ depending on the type of drug used in each study, as well as the dosage and their route of administration. The nature of the nociceptive stimulus may also crucially influence the outcome (for comprehensive reviews see Vanegas and Schaible [164] and Prado [129]). It is not clear, for example, whether results from models of chronic inflammatory and neuropathic pain are equally applicable to intracranial nociceptive neurotransmission, although it is reasonable to assume a degree of commonality.

L-Type VDCCs

Although blockers of L-type VDCCs have demonstrated analgesic properties against nociceptive stimuli (39,70,97,110,116,162), negative results have been reported as well (97,151,152). Behavioral studies on mice in which the gene encoding the $Ca_V1.3$ (α_{1D}) subunit has been ablated do not appear to support a role for L-type channels in acute thermal nociceptive signaling (28). Visceral pain transmission may rely in part on L-type channels; several studies show an analgesic effect for selective antagonists (such as verapamil and diltiazem) (39,70,105); however, this has not been universally demonstrated (74). L-type channels have also being implicated in inflammatory pain signaling, at least in models that use chemical irritation of peripheral nerves and joints as a nociceptive stimulus. In these models, two behavioral phases are noted, an early and a late phase, the later of which correlates with the onset of central sensitization (44). Although L-type VDCCs have a doubtful role in the early phase, they do have a modest effect reducing behavioral responses associated with the late inflammatory phase (29,97,116).

N-Type VDCCs

Blockers of N-type channels have demonstrated analgesic properties against acute mechanical and thermal nociceptive stimulation in several animal studies (97,115,116),

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although again these results have not been replicated in all experiments (151). Preliminary clinical data further suggest that N-type channels may be important in human pain pathways. The selective blocking agent ziconotide has analgesic properties when administered intrathecally (5,155); however, side effects in the initial titration period remain a serious problem. N-type channels also have an important function in chronic inflammatory and neuropathic pain. Upregulation of the $Ca_v2.2$ subunit is correlated with pain behavior following neural or chemical injury (25,180). The delayed response to inflammatory agents is attenuated by treatment with N-type blockers (41,103,115,116,151,152) and they are still effective at reducing nociceptive behaviour, even if central sensitization has become established. The advent of genetically modified mice lacking functional N-type channels confirms their importance in the development of chronic pain states (144). Studies on the behavioral responses elicited by acute noxious thermal and mechanical stimulation, however, have yielded conflicting results (71,86). In general these mice do not manifest significantly altered behavioral responses, which questions their role in the transmission of acute pain (111,145).

P/Q-Type VDCCs

Because P/Q-type channels are involved in both excitatory and inhibitory synaptic neurotransmission (49,95,159,163), it is not surprising that P/Q-type channel blockers are reported to have inhibitory, facilitatory, or even no effects on the responses of spinal neurons to nociceptive stimulation (110,114,151,152). It is probably simplistic to think that P/Q-type channels are only involved in excitatory mechanisms of pain transmission. Behavioral studies using natural mutant *"leaner"* mice confirm that mutations of P/Q-type channels may modulate noxious sensory information in complex ways. Although analgesic behavior is demonstrated following mechanical testing, hyperalgesic responses are observed after noxious thermal stimulation (121). It is also becoming apparent that P/Q-type channels have important actions in GABAergic inhibitory circuits. When mutant Familial Hemiplegic Migraine type 1 (FHM1) P/Q-type channels are expressed in inhibitory interneurons, it has been reported that they are less able to sustain GABAergic synaptic currents (17). Application of ω-agatoxin GIVa to the brainstem leads to an increase in spontaneous firing of medullary dorsal horn neurons (while also inhibiting the responses to noxious stimulation of the dura mater), possibly because of an action on GABAergic interneurons (50). A similar disinhibitory action may also be observed following micro-injection of P/Q-type blockers into the periaqueductal grey (PAG) (88). P/Q-type channels may therefore have a role in both inhibitory as well as excitatory neurotransmission, influencing the gating of sensory information at multiple levels in the nervous system. P/Q-type channels also have a role in the perception of inflammatory pain. Primary and secondary hyperalgesia resulting from chronic inflammation is prevented by pretreatment with P/Q- blockers (41,97,114,151), which suggests that P/Q-type channels may have an important role in the development of central sensitisation.

R-Type VDCCs

Until recently, studying the role of R-type VDCCs was limited by a lack of specific blocking agents. Attempts have been made to circumvent this limitation by generating $Ca_V2.3$ (α_{1E}) knockout mice. Whereas both homozygous $Ca_V2.3$-*null* and heterozygous mice exhibit normal responses to acute noxious thermal, mechanical, and chemical stimuli, homozygous mutant mice demonstrate reduced behavioral responses to somatic inflammatory pain. Heterozygotes but not $Ca_V2.3$-null mice also appear to have impaired responses following nociceptive stimulation of the viscera (143,145). Recently it has been suggested that the peptide SNX-482 may act as a relatively selective R-type blocker (14). Intrathecal administration of SNX-482 appears to have complex actions on nociceptive behavior. In the formalin test the late-phase response is attenuated in a dose-dependent manner but the early phase is either unaffected or even potentiated (110). At present it appears that R-type channels are involved in the development of somatic inflammatory pain and possibly also visceral pain, but it is not clear what—if any—role they play in the transmission of acute nociceptive information.

T-Type VDCCs

Blockade of T-type channels with ethosuximide reduced spinal dorsal horn neuronal firing in response to electrical, mechanical, and thermal stimulation in a dose-dependent manner in a model of neuropathic pain (102). Such hyperalgesic behavior may be mediated in part by a synergistic interaction between T-type VDCCs and neurokinin 1 receptor activation in lamina I neurons (80). In addition to mechanically induced neuropathic pain (47), T-type blockers also appear to be effective in combating nociceptive behavior resulting from chemotherapy-induced neuropathy (55). Further evidence supporting a role for these channels in nociceptive transmission is provided by the antisense targeting of $Ca_V3.2$ mRNAs. This results in a significant reduction of T-type channel currents with a concomitant antinociceptive effect in models of both acute and chronic somatic pain (15). T-type currents also have a central function modulating thalamic neuronal firing. The transition from tonic to burst mode may have important sensory gating properties, regulating the flow of visceral nociceptive information (87).

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VOLTAGE-GATED CALCIUM CHANNELS AND INTRACRANIAL NOCICEPTION

Although it cannot be assumed that the same array of high-threshold VDCCs are involved in spinal and trigeminovascular nociceptive signaling, initial studies do indicate that N- and P/Q-type channels have a role in transmitting sensory information from the dura (50). Blockade of N-type channels effectively inhibits the responses of neurons in the spinal trigeminal nucleus to cold and inflammatory stimulation of the dura mater. Blockade of P/Q-type channels have a less profound effect, whereas L-type block does not have a significant action. Interestingly in both cases application of L- and P/Q-type VDCC blockers produce an increase in spontaneous firing rates, suggesting a disinhibitory action on dorsal horn neurons. Because VDCC blockers were applied to the exposed brainstem and upper cervical cord, the possibility that these effects were the result of actions at other sites, such as in the PAG, cannot be excluded (88). This problem of anatomic localization has been overcome by microiontophoretic ejection of VDCC blockers directly onto trigeminal neurons (149). In this study L-, N- and P/Q-type channels were all shown to contribute to action potential generation by second order neurons in the trigeminocervical complex (Figs. 20-1 and 20-2). Furthermore, neuronal firing triggered by electrical stimulation of the superior sagittal sinus (which is known to cause pain in humans) could also be inhibited by L- and N-type blockers (Fig. 20-3). Although evidence for the effects of L-type channel blockers in the trigeminal nucleus is conflicting, their action on neurogenically induced dural vasodilation suggests that these channels may be involved in trigeminovascular nociceptive pathways in the periphery (1). The potent L-type channel blocker calciseptine can inhibit the presynaptic release of CGRP from trigeminovascular neurons, as did P/Q- and N-type VDCC blockers.

Given the central contribution that VDCCc make toward action potential generation and synaptic neurotransmission, it is hardly surprising that VDCCs have an

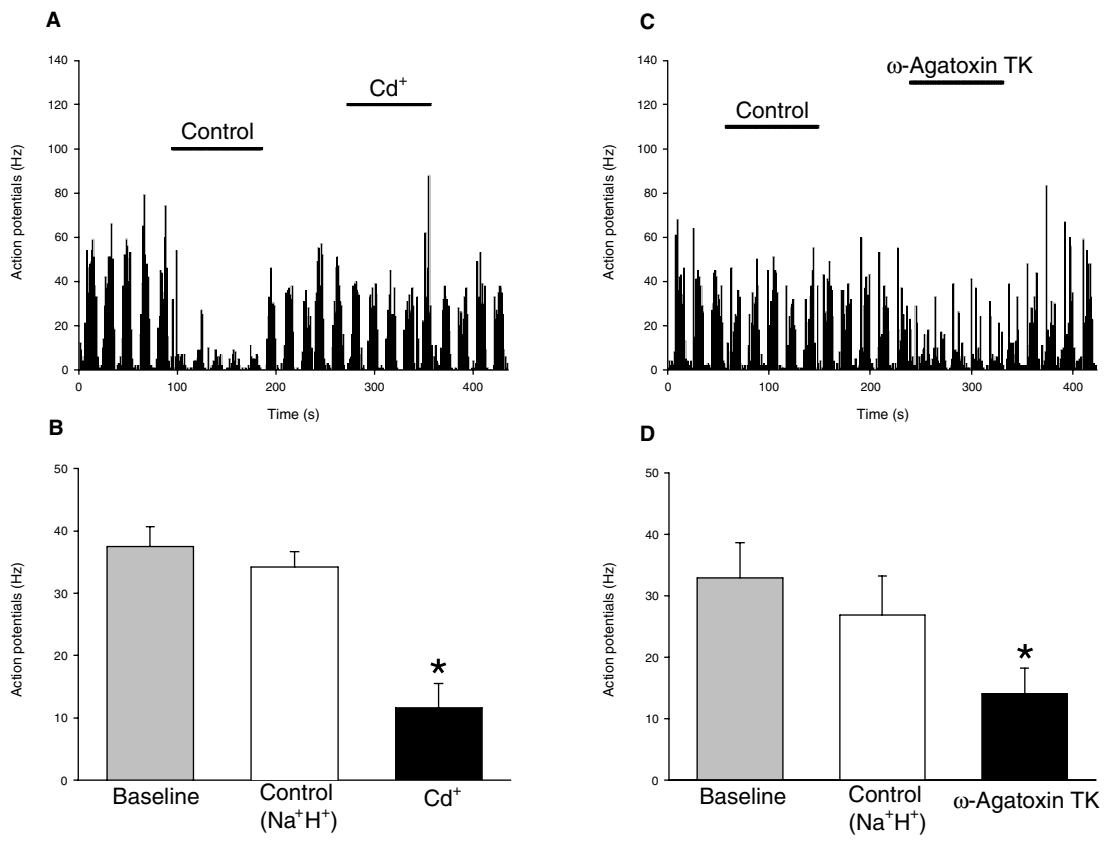

FIGURE 20-1. Effects of VDCC blockers on L-glutamate induced firing by neurons in the trigeminocervical complex. All neurons were activated by electrical stimulation of the superior sagittal sinus. Recordings from individual neurons are shown in the upper panels (**A** and **C**) and mean firing rates are illustrated in the histograms (**B** and **D**). Both cadmium ions (Cd^{2+})—nonselective VDCC blocker—and the selective antagonist of P/Q-type channels, ω-agatoxin TK, both inhibited neuronal firing. ($^*P < .05$.) Adapted from Shields KG, et al. Post-synaptic high threshold voltage dependent calcium channels modulate trigeminovascular nociceptive neurotransmission in the trigeminovascular complex of the cat. *Neuroscience*. (In press.)

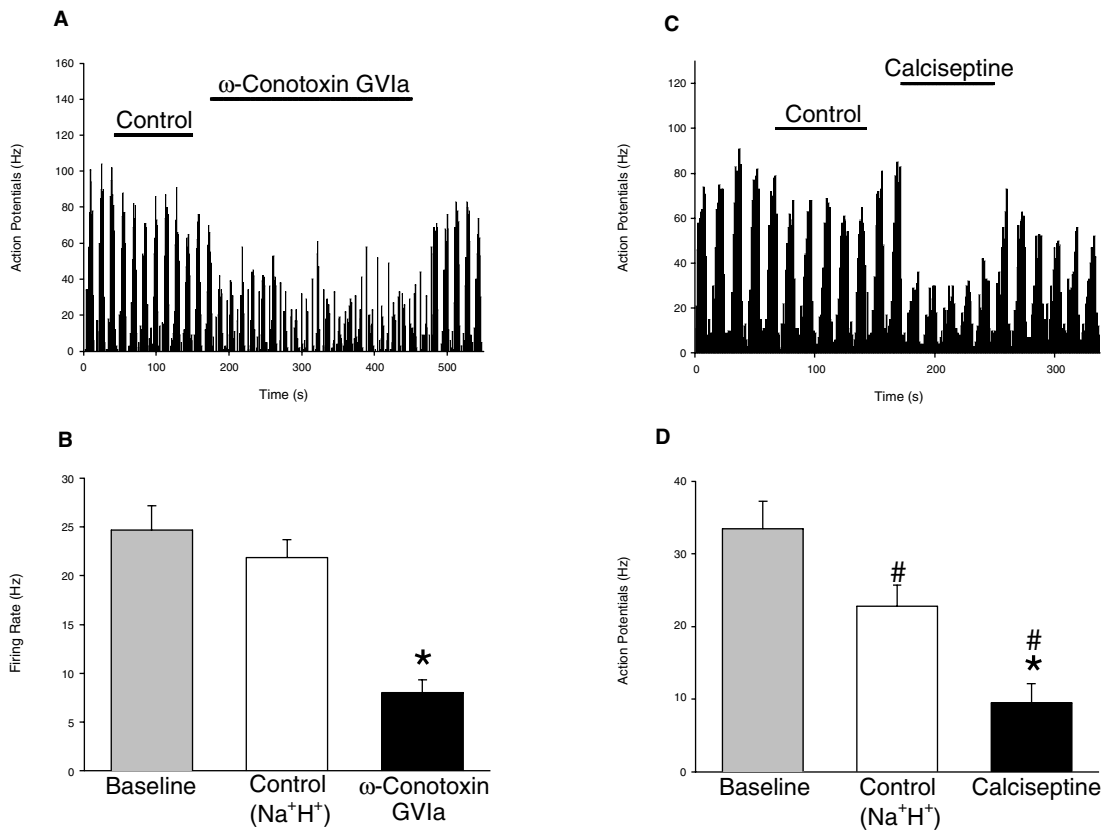

FIGURE 20-2. Blockade of N-type channels with ω-conotoxin GVIa (**A** and **B**) and L-type channels with calciseptine (**C** and **D**) both inhibit neuron firing by neurons in the trigeminocervical complex. (*# $P < .05$.) Adapted from Shields KG, et al. Post-synaptic high threshold voltage dependent calcium channels modulate trigeminovascular nociceptive neurotransmission in the trigeminovascular complex of the cat. *Neuroscience*. (In press.)

important role in pain signaling. Blockade of individual channels may not necessarily prevent transmission of nociceptive information, but certain channels appear to serve a more integral part in pain transmission than others. All VDCCs appear to have complex actions, and in some cases this may involve modulating activity in GABAergic interneurons as well as descending inhibitory circuits. Whether this can be translated into further successful therapeutic interventions remains to be seen.

VOLTAGE-GATED SODIUM CHANNELS

Voltage-gated sodium channels (VGSCs) play a central role in the excitability of all neurons, mediating the influx of sodium ions following depolarization of the cell membrane (92). Ten pore-forming α-subunits have been identified in vertebrates (21,62). These differ in their activation threshold, rate of inactivation, and sensitivity to tetrodotoxin (TTX) (Table 20-2). The potential importance of VGSCs in the pathophysiology of headache is demonstrated by the efficacy of lidocaine, a nonselective blocker of VGSCs, in the treatment of migraine, cluster headache (12,135), and short-lasting unilateral neuralgiform headache with conjunctival injection and tearing (SUNCT) (101). Antiepileptic drugs such as lamotrigine may also exert some of their therapeutic effects on headache through an action on VGSCs.

TTX-Sensitive Channels

In sensory neurons the TTX-sensitive current is inactivated by TTX at nanomolar concentrations. The VGSCs responsible for generating this current are activated at relatively depolarized membrane potentials (−55 to −40 mV). Most have rapid activation and inactivation kinetics, but recover slowly from their inactivated state (92). TTX-sensitive channels such as $Na_v1.6$ and $Na_v1.7$ are present in almost all sensory neurons (10) and TTX-sensitive channels are essential for action potential conduction in myelinated and unmyelinated axons (61,137). TTX-sensitive channels are therefore reasonable therapeutic targets for pain control and two channels, $Na_v1.3$ and $Na_v1.7$, may have a particular function in the genesis of neuropathic pain.

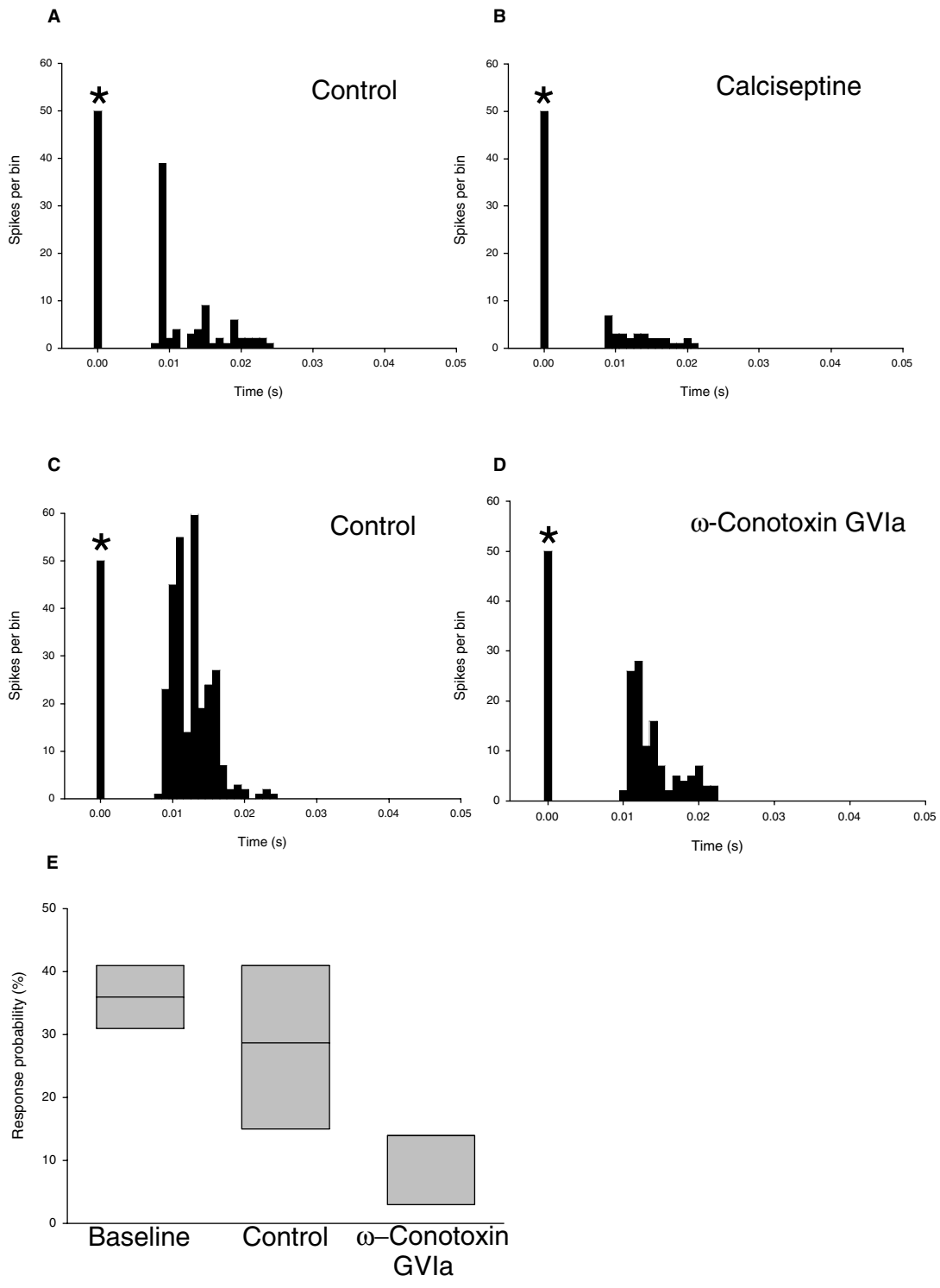

FIGURE 20-3. VDCC blockers inhibit nociceptive neurotransmission in the trigeminocervical complex. Post-stimulus histograms demonstrating the inhibitory effect of calciseptine, a blocker of L-type channels, (**A** and **B**) and ω-conotoxin GVIa, a selective antagonist of N-type channels, (**C, D,** and **E**) on the responses of neurons in the trigeminocervical complex following electrical stimulation of the superior sagittal sinus. The median response probabilities with quartile ranges under the three test conditions of the cells treated with ω-conotoxin GVIa are shown in panel E. (*Stimulus artifact). Adapted from Shields KG, et al. Post-synaptic high threshold voltage dependent calcium channels modulate trigeminovascular nociceptive neurotransmission in the trigeminovascular complex of the cat. *Neuroscience*. (In press.)

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TABLE 20-2 Classification, Distribution, and Characteristics of Voltage-Gated Sodium Channels

Threshold of Activation	*Na^+ Channel*	*Sensitivity to TTX*	*Rate of Inactivation*	*Tissue Distribution*
Low	$Na_V1.1$	Sensitive	Fast	CNS, DRG neurons, motor neurons
Low	$Na_V1.2$	Sensitive	Fast	CNS
Low	$Na_V1.3$	Sensitive	Fast	Embryonic neurons, mature CNS
Low	$Na_V1.4$	Sensitive	Fast	Skeletal muscle
Low	$Na_V1.5$	Resistant	Medium	Heart, embryonic DRG neurons
Low	$Na_V1.6$	Sensitive	Fast	Motor and DRG neurons and CNS
Low	$Na_V1.7$	Sensitive	Fast	DRG neurons and CNS
	$Na_V1.8$	Resistant	Slow	DRG neurons (80% on small- and 20% on large-diameter neurons)
Low	$Na_V1.9$	Resistant	Medium	Small-diameter DRG neurons, CNS

Abbreviations: DRG, dorsal root ganglia; CNS, central nervous system.
Adapted from Lai J, et al. Voltage-gated sodium channels and hyperalgesia. *Annu Rev Pharmacol Toxicol.* 2004;44:371–397.

The expression of $Na_V1.3$ is developmentally regulated. $Na_V1.3$ is usually only expressed in significant quantities by embryonic sensory neurons (168). Unlike many other TTX-sensitive channels, $Na_V1.3$ recovers rapidly from inactivation (36). Abnormal expression of $Na_V1.3$ (in addition to other sodium channels) by adult sensory neurons may be induced in models of neuropathic (34,168) and chronic inflammatory pain (9). Interestingly similar changes are observed in spinal horn neurons following spinal and peripheral nerve injury (65,66). Re-expression of $Na_V1.3$ therefore contributes to the neuronal excitability, which occurs following peripheral nerve injury.

$Na_V1.7$, which is found predominantly on sympathetic and peripheral sensory neurons, is also implicated in nociception (113). $Na_V1.7$ mRNA is upregulated in a model of inflammatory pain (9) and mutations in the *SCN9A* gene, which encodes the channel cause primary erythermalgia (179). This rare, autosomal-dominant peripheral neuropathy, which may be treated with intravenous lidocaine (94), results in episodic burning pain, edema, and erythema (124). The painful nature of this condition may arise from a hyperpolarizing shift in the activation kinetics of the mutated channels (35).

TTX-Resistant Channels

TTX-resistant channels are insensitive to micromolar (>10 μmol) concentrations of TTX (92,122) and two, $Na_V1.8$ and $Na_V1.9$, appear to have an important role in the transmission of nociceptive sensory information from the periphery. $Na_V1.8$ and $Na_V1.9$ are confined to nociceptive primary afferent neurons (3,16,46,53). $Na_V1.8$ channels are preferentially found on small-diameter neurons and to a lesser extent on medium- and large-diameter cells. They are localized to the soma and also the terminal arbors of sensory neurons (54). $Na_V1.9$ is generally confined to small-diameter neurons (43,54).

These currents have an important role in action potential generation by mechanoreceptive C-fibers in the dura mater (Fig. 20-4) (156). Propagation of action potentials by unmyelinated axons and C-fiber activation of dorsal horn neurons (81) are also reliant on TTX-resistant channels (16,61,134). TTX-resistant channels are modulated by acute inflammatory mediators such as PGE_2 (51,85,141), adenosine (60), serotonin (18,123), and bradykinin. As a result, the TTX-resistant current is activated at more hyperpolarized membrane potentials and its magnitude is also increased. This effect, mediated by G proteins, appears to increase neuronal excitability and contributes to the induction of sensitization (177).

The current generated by $Na_V1.8$, also known as sensory-neuron–specific channel, has a high threshold for activation ($\sim$−36 mV) and steady-state inactivation. Unlike TTX-sensitive channels, it activates and inactivates slowly, but recovers rapidly from its inactivated state (89,122,138,148). This channel has a specialized function in nociceptive sensory neurotransmission. $Na_V1.8$-*null* mutants exhibit analgesic behavior following noxious mechanical stimulation (2). $Na_V1.8$ channels also have a role in neuropathic (61,91), inflammatory, and visceral pain (93).

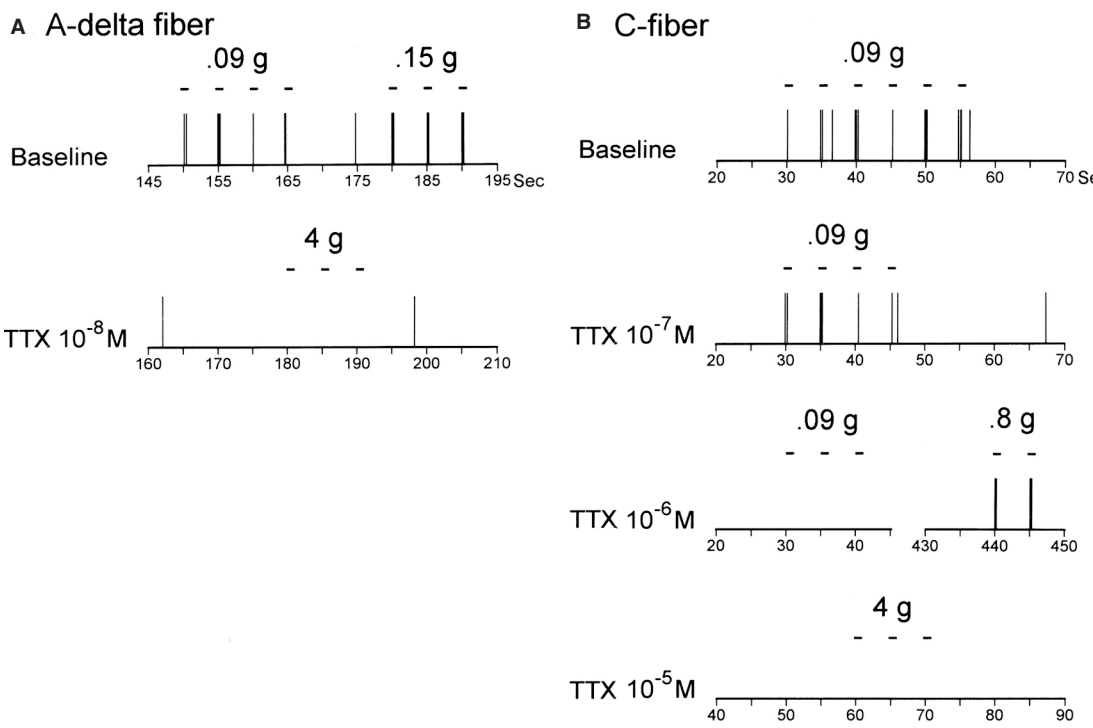

FIGURE 20-4. Demonstration of the role that VGSCs play in action potential generation in Aδ **(A)** and C-fibers **(B)** arising from the dura mater. TTX-resistant sodium channels play an important role in action potential generation by C-fibers—in this case triggered by mechanical stimulation of the dura mater. Taken from Strassman AM, Raymond SA. Electrophysiological evidence for TTX-resistant sodium channels in slowly conducting dural sensory fibers. *J Neurophysiol.* 1999;8152:413–424.

$Na_v1.9$ does not contribute directly to the generation of action potentials; rather, it appears to have an important role in modulating neuronal excitability (6,32,140). This channel has a very low threshold for activation and produces a "persistent" current (42), which may have a significant depolarizing effect on the resting membrane potential (shifting it from −70 to −49 mV). It may also enhance the responsiveness of neurons to inputs normally subthreshold for action potential generation (72). Although the balance of evidence does support an important nociceptive function for $Na_v1.9$ channels, not all studies support this (30,128)

POTASSIUM ION CHANNELS

Members of this large family of structurally related channels are characterized by their high conductance and almost total selectivity for K^+ ions (96). Potassium-channel diversity is related to their different mechanisms of gating and pharmacologic sensitivity. In the current classification system, four families are recognized: voltage-gated (K_V), calcium-activated (K_{Ca}), tandem pore domain (K_{2P}), and the inward rectifier channels (K_{ir}) (64). Although each family has multiple members, evidence exists demonstrating that specific types of K^+ channel are implicated in the antinociceptive actions of several drugs. These include the ATP-sensitive (K_{ATP}) members of the K_{ir} family, G-protein–regulated inwardly rectifying K^+ channels ($K_{ir}3$)—otherwise known as GIRK channels, $K_V1.1$ and the small- (SK) and large- (BK) conductance calcium-activated channels (for a comprehensive review see Ocana et al. [120]).

$K_V1.1$ channels are found on small-diameter (<30 μm) trigeminal neurons projecting to the superficial layers of the cervical dorsal horn (161) (in addition to larger diameter spinal dorsal horn neurons [136]) and also Aδ and C visceral sensory neurons (58), where they have important effects on neuronal excitability. Hyperalgesia is observed in mice lacking the gene encoding the $K_V1.1$ α subunit, further indicating its role in nociceptive signaling pathways (27). Other potassium channels are also found in high concentrations in the superficial laminae of the spinal and medullary dorsal horn including G-protein–regulated (99), calcium-activated (which colocalize with immunoreactivity for CGRP) (107) and ATP-sensitive K^+channels (181). This provides further supporting evidence for the multiplicity of potassium channels involved in nociceptive neurotransmission.

Potassium ion channels appear to have complex modulatory effects on trigeminal neurons, potentially regulating electrical behavior and neuronal excitability

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(132,133,154). K^+ channel opening is implicated in the antinociception produced by several G-protein–coupled receptors in the trigeminal system such as $GABA_B$ (160) and opioid (161) receptors. Potassium channels may therefore act as a common pathway for metabotropic receptors influencing the functional state and firing properties of dorsal horn neurons (40). They may also have more widespread actions, being a common mechanism in the modulation of nociceptive signaling by brainstem monoaminergic nuclei (68). Many drugs, including several used for acute (4,125,126) and prophylactic treatment of migraine (56,106), may also rely on potassium-channel opening for their analgesic effects.

ACID-SENSING CHANNELS

Noxious stimuli in the form of trauma, inflammation, and ischemia result in tissue injury and acidification of the extracellular milieu. Acid-sensing ionic channels (ASICs) belong to ligand-gated non–voltage-gated Na^+ channels and are members of the degenerin/epithelial sodium channel (Deg/ENaC) superfamily (for a comprehensive review see Krishtal [90]). ASICs are transiently activated by a rapid decrease of extracellular pH in the microcirculation, although there is doubt whether such changes do occur in vivo. ASICs can be blocked by amiloride and modulated by protein kinase C (7) and snail neuropeptide FMRFamide. The ASIC family consists of several subunits: ASIC1, −2, −3, and −4. Both ASIC1 and ASIC2 give rise to two isoforms: ASIC1a, ASIC1b, ASIC2a, and ASIC2b. Channels are assembled from four subunits in homomeric and specific heteromeric configurations. Expression of ASICs is predominantly, but not exclusively, in the nervous system, both in the brain and the periphery (in sensory ganglia). ASIC1a, ASIC2a, and ASIC2b are expressed both in the periphery and the CNS. High levels of all three channels are found in the spinal dorsal horn (178). Proton-induced current in dorsal horn neurons appears to be largely generated by homomeric ASIC1a and heteromeric ASIC1a + 1b channels. ASIC1b and ASIC3 channels are primarily expressed in sensory neurons (98)—in the rat trigeminal ganglion both small- and medium-sized neurons demonstrate immunoreactivity for ASIC3 (as well as CGRP) (78). ASIC3 channel expression and current density may be upregulated on dorsal root ganglion neurons by inflammatory mediators—with potentially significant effects on neuronal excitability (98). Such changes may, however, be prevented by treatment with nonsteroidal anti-inflammatory drugs (165).

Much of what is known about the exact involvement of ASICS in these processes comes from knockout mice. ASIC1-*null* and ASIC1a overexpressor mice demonstrated either impaired hippocampal synaptic plasticity accompanied by behavioral defects in spatial learning and fear conditioning (169,171) or enhanced fear conditioning (170). ASIC1 does not, however, appear to contribute to the behavioral responses observed in a model of chronic inflammatory pain (150). In contrast, ASIC2a and ASIC3 knockout mice seem to point to a role for these proteins in mechanosensation and nociception. ASIC2a seems essential for "light touch" (131), whereas ASIC3 may have complex actions in pain perception. ASIC3 may modulate the perception of moderate-to-high intensity pain sensation (23,130), while also contributing to the hyperalgesic behavioral responses observed in a model of chronic pain (150).

TRP FAMILY

Mammalian nonselective cation Transient Receptor Potential (TRP) channels are composed of six related protein families (for a review see Clapham [26]). TPR channels are the vanguard of our sensory systems, responding to a variety of stimuli including temperature, touch, pain, and osmolarity. Within the TRPV subfamily several channels play a role in nociception. The vanilloid receptor (TRPV1, previously VR1) (20) is activated by several activators including capsaicin, the pungent ingredient in hot chili peppers, protons, and heat (see below). TRPV1 receptors are located on small- and medium-sized neurons that are unmyelinated C-fibers or thinly myelinated Aδ-fibers (82). TPV1 receptors are expressed in neurons in the trigeminal and dorsal root ganglia (63,79). Intravenous capsaicin promotes release of the proinflammatory neuropeptides, substance P and neurokinin A from trigeminal neurons and causes dural extravasation in rat (100). Also because of co-immunoreactivity of TRPV1 and CGRP in certain trigeminal ganglion cells (59), TRPV1 has been considered a target for development of antimigraine drug. In addition, desensitization to capsaicin (or its analog resiniferatoxin) has therapeutic potential and has been tried in chronic intractable pain through vasomotor rhinitis of the overactive bladder (158).

Several neuronal members of the TRPV subfamily are activated by thermal stimuli that are painfully hot (e.g., $>42^\circ$C). TRPV1 and TRPV2 (VRL-1) are activated at temperatures above 43°C and 52°C, respectively. TRPV4 channels and TRPM8 (CMR1), a member of the TRPM family, respond to moderate non-noxious heat stimuli, whereas TRPN1 (ANKTM1) is activated at 17°C and evokes a feeling of pain (for a review see Huang [76]). In accordance with their role in sensory temperature sensation, most of them are expressed in subsets of DRG neurons. Coexpressed TRPN1 with TRPV1 in a subpopulation of DRG neurons might allow simultaneous response to cold and other agonists, and promote hyperalgesia. The involvement of TRP channels in vivo has been demonstrated by administration of antibodies against TRPV1 to diabetic mice resulting in amelioration of thermal allodynia and hyperalgesia (83). Studies in TRPV1-*null* mice showed that sensory neurons

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were deficient in responses to the capsaicin, protons, and moderate noxious heat (19,38). In these mice behavioral responses to capsaicin were absent and responses to acute thermal stimuli were diminished. Transgenic models for the other TRP channels involved in nociception will become instrumental to investigate the exact in vivo consequences of individual TRP channels and to what extent they act together.

ATP RECEPTORS

Ion channels opened by ATP are called ionotrophic *P2X receptors,* whereas G-protein coupled receptors activated by ATP belong to the P2Y family. The P2X receptor family consists of seven members, P2X1 through -7, most of which are expressed in small nociceptive sensory neurons including the trigeminal ganglia (166,167). On sensory neurons, homomeric P2X3 or heteromeric P2X3/P2X2 receptors are the main transducers of fast ATP signaling. The origin of extracellular ATP is probably from neurons as well as non-neuronal cells (104). The participation of P2X receptors in pain mechanisms is well established (24,119) and certain ATP receptors, especially P2X3 type, might be important for head pain.

P2X3 receptors are readily desensitized (119) even by low concentrations of extracellular ATP (153). Such a desensitization state is modulated by extracellular Ca^{2+} (31) and Mg^{2+} (57), possibly via an action on the extracellular loop of the P2X3 receptor. Desensitization may help to avoid indiscriminate, long-lasting activation of such receptors with consequent persistent pain. Experiments on knockout mice together with pharmacologic data provide evidence that P2X3 receptors participate in pain mechanisms like hyperalgesia following sensitization. The pronociceptive effect of P2X3 receptor activation is also strongly potentiated by inflammatory mediators (67,119); for example, CGRP (which is of particular interest to headache research) appears to interact with the purinogenic system in a complex fashion. CGRP potentiates the response of P2X receptors to ATP (176), whereas activation of P2Y receptors indirectly triggers the release of CGRP from small-diameter DRG and trigeminal neurons (146,182). Trigeminal nerve terminals possess a high density of P2X3 receptors (167) and these may modulate the sensitization of trigeminal sensory neurons as demonstrated in a model of chronic inflammatory dental pain (75). It is also worth noting that glial cells bear ionotropic and metabotropic ATP receptors that may also be involved in pain mechanisms (104). Although P2X receptors are reported to transiently enhance high-threshold Ca^{2+} currents (37), N-type channels are negatively modulated by P2Y receptors (11), a phenomenon supposed to counteract the ATP algogenic action mediated by P2X receptors (13). We can thus envisage a novel dynamic interaction between P2X and P2Y receptors and Ca^{2+} channels in the mechanism of head pain generation and a potential target for new analgesic treatment.

CONCLUSION

An astonishing amount of data has been generated over the last few years in identifying and characterizing ion channels that are relevant for pain, and more specifically, head pain. Only by a detailed investigation of the complex actions of these channels will we fully appreciate their importance for headache disorders. Not only will our knowledge increase over the coming years, but hopefully we will be able to use these insights as a starting point for the development of promising novel drug therapies.

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P1: KWW/KKL P2: KWW/HCN QC: KWW/FLX T1: KWW
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