

Review

Calcium-permeable acid-sensing ion channel in nociceptive plasticity: A new target for pain control

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ABSTRACT

The development of chronic pain involves increased sensitivity of peripheral nociceptors and elevated neuronal activity in many regions of the central nervous system. Much of these changes are caused by the amplification of nociceptive signals resulting from the modulation and altered expression of specific ion channels and receptors in the central and peripheral nervous system. Understanding the processes by which these ion channels and receptors are regulated and how these mechanisms malfunction may lead to new treatments for chronic pain. Here we review the contribution of the Ca²⁺-permeable acid-sensing ion channel (ASIC_{Ca}) in the development and persistence of chronic pain, and the potential underlying mechanisms. Accumulating evidence suggests that ASIC_{Ca} represents an attractive new target for developing effective therapies for chronic pain.

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Abbreviations: ASICs, acid-sensing ion channels; AMPA, α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; BDNF, brain-derived neurotrophic factor; CGRP, calcitonin gene-related peptide; CaMKII, calcium/calmodulin-dependent protein kinase II; CNS, central nervous system; CFA, complete Freund's adjuvant; cAMP, 3',5'-cyclic AMP; CRE, cAMP response element; CREB, cAMP response element binding protein; DRG, dorsal root ganglion; ERKs, extracellular signal-regulated protein kinases; GFAP, glial fibrillary acidic protein; IP₃, inositol (1,4,5)-trisphosphate; IB4, isolectin B4; LTP, long-term potentiation; NGF, nerve growth factor; NMDA, N-methyl-D-aspartate; NK1, neurokinin 1; NPFF, neuropeptide FF; NPAF, neuropeptide AF; PNS, peripheral nervous system; PLC, phospholipase C; PKA, protein kinase A; PKC, protein kinase C; PKG, protein kinase G; RIP, receptor interaction protein; SDH, spinal dorsal horn.

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1. Introduction

Pain is the sensation resulting from noxious stimuli that evokes unpleasant experience. A peripheral nociceptive stimulus normally evokes a myriad of physiological events that trigger the acute pain. Alterations in these events can further result in the development of chronic pathological pain (for review, see Woolf and Salter, 2000). In general, chronic pain falls into two categories—*inflammatory pain* caused by inflammation associated with tissue damage, and *neuropathic pain* resulting from lesions of the nervous system. After inflammation or neural injury, dramatic alterations occur in the sensory system, amplifying nociceptive responses and increasing the neuronal sensitivity to peripheral stimuli, so that normally innocuous or low-intensity stimuli cause pain. Such exaggerated nociceptive sensation (hypersensitivity) can originate from either an increased responsiveness of peripheral nociceptors (peripheral sensitization) or plasticity in the central processing of nociceptive inputs (central sensitization). Because Ca²⁺ plays an essential role in neuronal and synaptic plasticity in both the peripheral (PNS) and central nervous system (CNS), Ca²⁺-permeable ion channels are likely to be the mediators of peripheral and central sensitization underlying chronic pain (for review, see Scholz and Woolf, 2002). Indeed, a large body of evidence indicates that various types of Ca²⁺-permeable channels can regulate neuronal excitability and synaptic transmission in the nociceptive sensory pathway and are important for the induction and expression of pain hypersensitivity. These include several TRP (Transient Receptor Potential) channels such as TRPV1 (Caterina et al., 1997, 2000), TRPM8 (Proudfoot et al., 2006; Xing et al., 2007) and TRPA1 (Kwan et al., 2006; Obata et al., 2005), voltage-gated Ca²⁺ channels (for review, see Altier and Zamponi, 2004), NMDA receptors (Haley et al., 1990; Woolf and Thompson, 1991), P2X receptors (for review, see Chizh and Illes, 2001), and Ca²⁺-permeable AMPA (Gu et al., 1996; Hartmann et al., 2004) and kainite receptors (Kerchner et al., 2001a, 2001b). Moreover, some of these channels are redistributed and/or up-regulated under conditions of chronic pain. Here we review recent evidence for Ca²⁺-permeable acid-sensing ion channel (ASIC) as a major player in pain hypersensitivity, with special emphasis on the underlying mechanisms for central sensitization. The role of ASICs in peripheral nociceptors as noxious stimulus detectors is discussed in detail elsewhere (for review, see Lingueglia, 2007; Voilley, 2004).

2. Property and distribution of Ca²⁺-permeable ASIC (ASIC_{Ca})

Subunits of ASICs are members of the degenerin/epithelial Na⁺ channel protein superfamily. To date, seven ASIC subunits have been identified: 1a, 1b, 1b2, 2a, 2b, 3, and 4 (for review, see Lingueglia, 2007; Wemmie et al., 2006). They form a variety of homomeric and heteromeric ion channels that are activated by the reduction of extracellular pH, triggering membrane depolarization via Na⁺ influx into target neurons.

2.1. Distribution profiles

The expression patterns and properties of these channels make them attractive candidates for major sensors of tissue acidosis associated with inflammation, ischemia, and cancer metastases (for review, see Wemmie et al., 2006; Xiong et al., 2008). Heterologous expression studies using a combination of various ASIC subunits showed that, unlike other homomeric or heteromeric ASICs, homomeric ASIC1a channel is also permeable to Ca²⁺ in addition to Na⁺ (Yermolaieva et al., 2004). It can be activated by moderate reduction of extracellular pH, with the half maximal activation pH_{0.5} of ~6.2 (Waldmann et al., 1997). This rather unique Ca²⁺ permeability of the homomeric ASIC1a channel, referred to hereafter as ASIC_{Ca}, suggests its potential involvement in nociceptive plasticity.

In most neurons, the variety of ASICs and their exact subunit compositions remain unclear. However, ASIC1a subunit is distributed throughout CNS and PNS (for review, see Krishtal, 2003). Studies using Ca²⁺ imaging and electrophysiological recording provided several lines of evidence showing that the ASIC_{Ca} is expressed in neurons of the spinal dorsal horn (SDH) (Duan et al., 2007; Wu et al., 2004), inferior colliculus (Zhang et al., 2008), midbrain (Pidoplichko and Dani, 2006), amygdala (Wemmie et al., 2003), hippocampus (Gao et al., 2005; Yermolaieva et al., 2004; Zha et al., 2006) and neocortex (Xiong et al., 2004). In addition, the use of a peptide tarantula (*Psalmopoeus cambridgei*) toxin Psalmotoxin 1 (PcTX1), which specifically blocks ASIC_{Ca} (Escoubas et al., 2000), further confirmed the presence of this channel in a variety of neuronal types (Duan et al., 2007; Gao et al., 2005; Pidoplichko and Dani, 2006; Xiong et al., 2004; Zhang et al., 2008).

2.2. Functional significance

The precise functions of ASICs are still under active investigation. In peripheral sensory neurons, their primary functions include nociception (Mamet et al., 2002, 2003; Sluka et al., 2003; Ugawa et al., 2002; Voilley et al., 2001), mechanosensation (Page et al., 2005; Price et al., 2000, 2001) and taste transduction (Lin et al., 2002; Ugawa, 2003). In the CNS, ASIC_{Ca} is involved in spine development (Zha et al., 2006), synaptic plasticity, spatial learning (Wemmie et al., 2002), and fear memory (Wemmie et al., 2003, 2004). Under pathological conditions of tissue acidosis, activation of ASIC_{Ca} contributes to axon degeneration (Friese et al., 2007) and neuronal death (Xiong et al., 2004) associated with multiple sclerosis and severe focal cerebral ischemia, respectively. Furthermore, the activation of NMDA receptors during global ischemia also enhances the ASIC_{Ca} function via Ca²⁺-calmodulin kinase II (CaMKII)-mediated phosphorylation, exacerbating ischemia-induced neurotoxicity (Gao et al., 2005). Interestingly, ischemia also increases ASIC2a expression in the CA1 and CA3 regions of the hippocampus. However, ASIC2a induction does not occur in dying neurons (Johnson et al., 2001), suggesting that ASIC2a may have neuroprotective functions by forming Ca²⁺-impermeable heteromeric ASICs. As will be discussed below, accumulating evidence indicates a critical role of ASIC_{Ca} in spinal nociception.

3. Identification of ASIC_{Ca} in spinal dorsal horn neurons

3.1. Morphological evidence

As the first relay station of sensory processing, the spinal dorsal horn (SDH) plays essential roles in the integration, transmission and amplification of nociceptive information (Fig. 1). The transcripts of ASIC1a, ASIC2a and ASIC2b are detected in SDH neurons (Wu et al., 2004), and expression of ASIC1a and ASIC2a subunits are shown by Western blotting and immunohistochemistry (Duan et al., 2007; Wu et al., 2004). Moreover, in contrast to the high-level ASIC1a expression in both the dorsal and ventral rat spinal cord, ASIC2a is highly expressed only in the ventral spinal cord (T.-L.X. and B.D., unpublished results), suggesting that ASIC1a plays the primary role in the rat SDH. Furthermore, ASIC1a was not co-localized with the astrocyte marker, glial fibrillary acidic protein (GFAP), the oligodendrocyte marker, receptor interacting protein (RIP), or the microglia marker OX-42. Interestingly, although ASIC1a is expressed in most dorsal root ganglion (DRG) neurons, it was not found in either peptidergic or non-peptidergic nociceptive afferent terminals containing calcitonin gene-related peptide (CGRP) and isolectin B4 (IB4), respectively (Duan et al., 2007), suggesting that ASIC1a-containing channels in DRG may function in peripheral terminals or cell bodies.

3.2. Functional evidence

Electrophysiological and pharmacological properties of ASICs have been examined in acutely dissociated and cultured SDH

neurons and ASIC_{Ca} is suggested to be responsible for the acid-induced current in these neurons (Duan et al., 2007; Wu et al., 2004). First, the electrophysiological properties, such as the pH values for threshold and half-maximum activation, desensitization rate, ion selectivity, Ca²⁺ and Zn²⁺ sensitivity (Wu et al., 2004), are all similar to those of homomeric ASIC1a and/or heteromeric ASIC1a/2b channels expressed in non-neuronal cell lines (Hesseler et al., 2004; Waldmann et al., 1997). Second, acid-induced currents in SDH neurons are largely blocked by PctX1, the specific inhibitor for ASIC_{Ca}. Third, using ASIC1a-specific siRNA, ASIC_{Ca} is shown to be the predominant ASIC in rat SDH neurons (Duan et al., 2007). Finally, the presence of the ASIC_{Ca} is confirmed by Ca²⁺ imaging studies—the acid-induced Ca²⁺ influx in rat SDH neurons is abolished by specific siRNA suppression of ASIC1a expression (Duan et al., 2007).

Nociceptive afferents make synapses onto both excitatory projection neurons inhibitory interneurons in the SDH (Perl, 1984; Willis and Coggeshall, 1991). It remains unexplored whether ASIC_{Ca} would play a differential role in both types of SDH neurons. The recent demonstration of ASIC_{Ca} activation of hippocampal inhibitory interneurons (Ziemann et al., 2008) provides an important beginning for further investigation of how ASIC_{Ca} influences nociceptive neuronal circuits in the SDH.

4. ASIC_{Ca} in inflammatory pain

Inflammation-induced sensitization of nociceptive sensory processing has been extensively studied over the past two decades. Recent evidence indicates that ASIC_{Ca} in SDH neurons plays

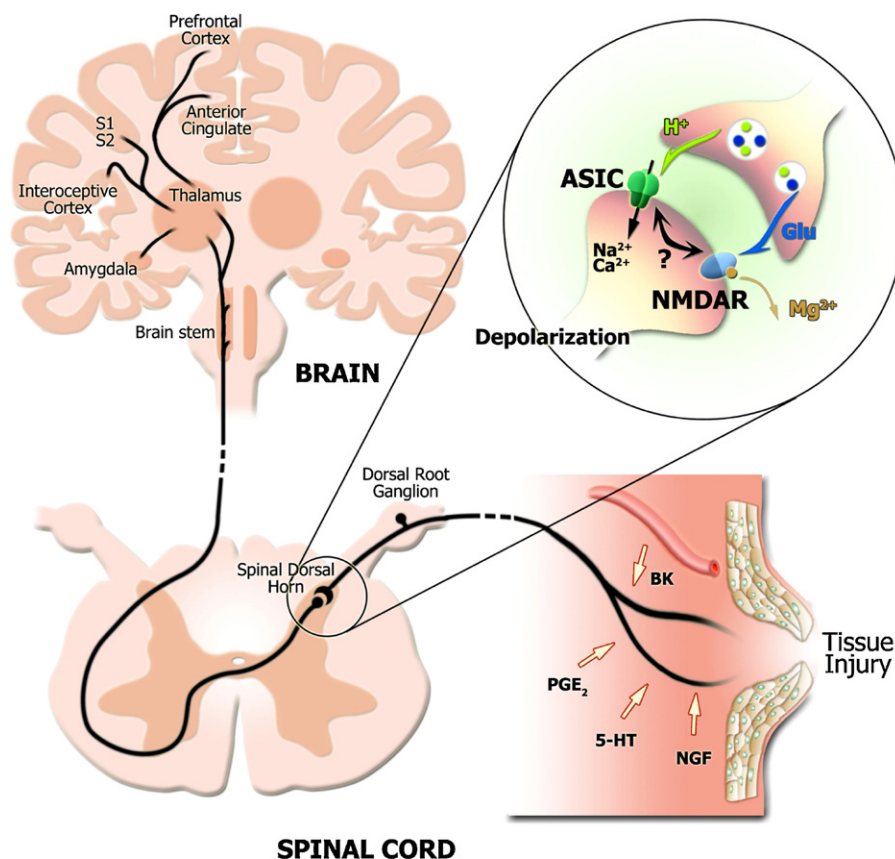


Fig. 1. Ca²⁺-permeable ASIC1a channel in SDH neurons. During tissue injury, inflammatory factors, including peptides (bradykinin), lipids (prostaglandins), neurotransmitters (5-HT) and neurotrophins (NGF), sensitize or excite the terminals of the nociceptor by interacting with cell-surface receptors expressed by these neurons. Activation of the nociceptor triggers a release of excitatory transmitter glutamate in the spinal dorsal horn (SDH). And then the noxious stimuli are transmitted to the supraspinal structures [brainstem, thalamus, amygdala, insular cortex, somatosensory cortex (S1), secondary somatosensory cortex (S2), anterior cingulate cortex and prefrontal cortex]. In the SDH, peripheral inflammation induces up-regulation of ASIC1a in postsynaptic SDH neurons. Synaptically released protons activate the Ca²⁺-permeable ASIC1a channel in the postsynaptic membrane, inducing depolarization, which in turn releases the well-defined Mg²⁺ block on the NMDA receptor (NMDAR).

important roles in the development of central sensitization underlying chronic pain induced by peripheral inflammation.

4.1. Role of glutamatergic transmission

Spinal nociceptive inputs from primary afferents are mainly mediated at glutamatergic synapses onto SDH neurons through the activation of AMPA, NMDA and kainate subtypes of ionotropic glutamate receptors as well as metabotropic glutamate receptors (mGluRs), leading to the excitation of SDH nociceptive neurons (for review, see Fundytus, 2001; Furue et al., 2004). In the meantime, activation of these glutamate receptors may elevate intracellular Ca^{2+} and trigger the activation of many protein kinases, including protein kinase A (PKA), protein kinase C (PKC), protein kinase G (PKG), CaMKII, as well as extracellular signal-regulated protein kinases (ERKs). Furthermore, persistent hyperactivity of peripheral nociceptors following inflammation can dramatically alter glutamatergic signaling cascades and modify neuronal excitability and synaptic function in the spinal cord and supraspinal areas, leading to central sensitization (for review, see Woolf and Salter, 2000; Zhuo, 2008). During peripheral inflammation, primary afferent terminals also release substance P (SP) (for review, see Levine et al., 1993) and brain-derived neurotrophic factor (BDNF) (for review, see Pezet and McMahon, 2006) that activate postsynaptic G-protein coupled neurokinin 1 (NK1) receptors and the tyrosine kinase receptor TrkB, respectively, resulting in elevated PKA, PKC, and ERK activities. Activation of these receptors, ion channels, and kinases all appear to be essential for central sensitization and chronic pain (Fig. 2; for review, see Ji et al., 2003).

4.2. New role of ASIC_{Ca}

In addition to the above well-known signaling processes, behavioral evidence showed that ASIC_{Ca} in the SDH plays a role in central sensitization and inflammatory hypersensitivity (Duan et al., 2007). Blocking ASIC_{Ca} by intrathecal injection of PcTX1 significantly attenuated both thermal and mechanical hypersensitivity induced by peripheral inflammation with the standard complete Freund's adjuvant (CFA) treatment. Moreover, suppression of ASIC_{Ca} expression in rat SDH using specific antisense oligonucleotides also reduced the thermal and mechanical hypersensitivity following the CFA treatment. Interestingly, blocking ASIC_{Ca} with PcTX1 or suppressing ASIC_{Ca} expression with antisense oligonucleotides did not affect either thermal or mechanical hypersensitivity in naïve rats without CFA treatment (Duan et al., 2007). Thus, ASIC_{Ca} in SDH neurons specifically contributes to chronic pathological rather than acute physiological pain.

4.3. Activation and central actions of ASIC_{Ca}

4.3.1. ASIC_{Ca} in synaptic plasticity

Recent evidence suggests that nociceptive hypersensitivity and chronic pain involve molecular and cellular mechanisms similar to those underlying physiological learning and memory (for review, see Ji et al., 2003; Sandkuhler, 2000). The functional significance of ASIC_{Ca} in synaptic plasticity has been studied in the context of forebrain long-term potentiation (LTP) of synaptic transmission and the regulation of synaptic strength associated with activity-dependent learning (Wemmie et al., 2002, 2003, 2004). For example, normal CA1 hippocampal LTP requires expression of ASIC_{Ca} and activation of ASIC_{Ca} (Wemmie et al., 2002). Interestingly, ASIC_{Ca} activation is also required for the hyperactivity of SDH neurons induced by repetitive C-fiber stimulation (known as the “wind-up”) or by peripheral inflammation (Duan et al., 2007). Considering the fact that C-fiber discharge rates required for

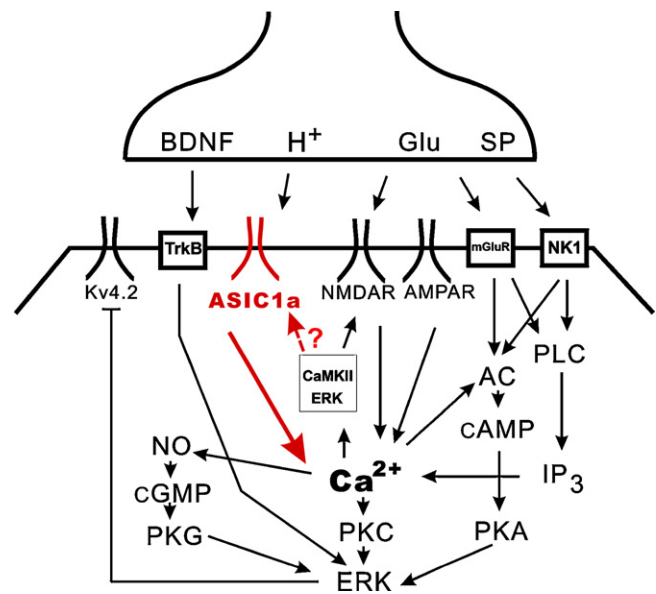


Fig. 2. Post-translational regulation in SDH neurons and induction of central sensitization. Peripheral inflammation increases release of the neurotransmitter glutamate and the neuromodulators substance P (SP) and brain-derived neurotrophic factor (BDNF), paralleled by synaptic acidosis. Activation of the Ca^{2+} -permeable ASIC_{Ca} channel (red), AMPA receptor (AMPA) and NMDA receptor (NMDAR) in the SDH leads to Ca^{2+} influx. Stimulation of group I metabotropic glutamate receptor (mGluR) and SP receptor NK1 activates phospholipase C (PLC) and stimulates Ca^{2+} release from inositol (1,4,5)-trisphosphate (IP_3)-sensitive intracellular store. Moreover, these G-protein coupled receptors also activate adenylate cyclase (AC), product 3',5'-cyclic AMP (cAMP) and then activate protein kinase A (PKA). The increase in intracellular Ca^{2+} activates CaMKII, protein kinase C (PKC) and protein kinase G (PKG), and extracellular signal-regulated protein kinase (ERK). BDNF activates TrkB receptor, which also induces ERK activation. Then activated ERK suppresses the activity (reduced open probability) of the Kv4.2 potassium channel, resulting in larger depolarization. Moreover, the activation of ERK and CaMKII also modulates the NMDA receptor and/or the Ca^{2+} -permeable ASIC_{Ca} channel. These post-translational steps result in an overall increased sensitivity of SDH neurons to afferent input, contributing to the generation of central sensitization.

inducing “wind-up” could also induce LTP at C-fiber synapses in the SDH (Ikeda et al., 2006; Sandkuhler, 2007), ASIC_{Ca} -dependent LTP-like synaptic modification is likely to be involved in the modification of spinal processing of nociceptive signals and the development of pain hypersensitivity.

4.3.2. ASIC_{Ca} in central nociceptive hypersensitivity

How does ASIC_{Ca} activation in SDH neurons contribute to the well-known glutamate receptor-dependent synaptic plasticity underlying nociceptive hypersensitivity (Ikeda et al., 2003, 2006)? Peripheral inflammation associated with tissue damage activates NMDA receptors and postsynaptic Ca^{2+} influx, which in turn triggers Ca^{2+} -sensitive intracellular signal cascades responsible for central sensitization. Consistent with the finding in hippocampal pyramidal neurons (Wemmie et al., 2002), depolarization due to opening of ASIC_{Ca} may help to release Mg^{2+} blockade of NMDA receptors in SDH neurons (Fig. 1). In addition, activation of ASIC_{Ca} itself mediates Ca^{2+} influx and further enhances Ca^{2+} -dependent cascades in SDH neurons (Fig. 2). Considering the fact that NMDA receptors are involved in both the induction and the expression of hyperalgesic and allodynic reflex hypersensitivity, ASIC_{Ca} may act synergistically with NMDA receptors in the activity-dependent central sensitization underlying pain hypersensitivity. Under physiological conditions, spinal synaptic transmission after non-damaging noxious stimuli is mediated by AMPA receptors in SDH neurons (Engelman et al., 1999; Hartmann et al., 2004; Malmberg and Yaksh, 1992). Consistent with the observa-

tion that the NMDA receptor does not mediate acutely painful stimuli (South et al., 2003), ASIC_{Ca} is not required for generating acute pain elicited by physiological, non-damaging thermal and mechanical stimuli in the spinal cord (Duan et al., 2007).

4.3.3. Mechanisms underlying ASIC_{Ca} activation

An important question to address is how ASIC_{Ca} in the second-order SDH neurons becomes activated following peripheral inflammation, which causes tissue acidosis only in the periphery. It has been proposed that extracellular acidosis associated with synaptic activity may activate central ASICs (for review, see Krishtal, 2003). Measurements with a pH-sensitive fluorescent probe showed that synaptic activation of cultured hippocampal neurons results in a transient decrease of extracellular pH to ~6.4 near the cell surface, due to exocytosis of synaptic vesicles of a low-pH content (Miesenbock et al., 1998). Under normal physiological nociceptive transmission, which may be accompanied by only a weak acidosis in the synaptic cleft, local pH reduction seems to be insufficient to activate endogenous ASIC_{Ca}. On the other hand, elevated acidification in the synaptic cleft due to peripheral inflammation-induced hyperactivity of afferent nociceptor terminals may activate postsynaptic ASIC_{Ca} in SDH neurons, as suggested by the finding that repetitive stimulation of the dorsal root evokes transient acidification in the dorsal horn (Chvatal et al., 1988). However, it remains to be established whether the pH reduction at the synaptic cleft associated with SDH neurons is indeed sufficient for activating ASIC_{Ca} in the chronic pain animal models. Measurements using pH-sensitive fluorescent proteins, e.g., pHlourin (Miesenbock et al., 1998), expressed on the surface of afferent terminals and SDH neurons may help to determine the precise level of acidification in the synaptic cleft *in vivo*. Furthermore, peripheral inflammation up-regulates the expression of ASIC1a in SDH neurons (Duan et al., 2007; Wu et al., 2004). The activity of ASIC_{Ca} may be augmented via the activation of NMDA receptors (Gao et al., 2005) in the SDH and be involved in mediating the summation of the afferent C fiber-evoked slow synaptic potentials. This implies that the induction of inflammatory pain hypersensitivity might involve ASIC_{Ca}. On the other hand, the up-regulation of ASIC_{Ca} may influence the persistent changes in excitability of SDH neurons and contribute to the expression of inflammatory pain hypersensitivity. Thus, ASIC_{Ca} in SDH neurons is well posed to serve as a mediator of inflammatory pain—both in sensing acidification associated with peripheral inflammation-induced persistent synaptic activity and in coupling nociceptive stimuli to intracellular cascades associated with the induction and expression of central sensitization. Finally, we note that recent elucidation of the first X-ray structure of ASIC1a at 1.9 Å resolution revealed a large extracellular domain with potential binding pockets for non-proton ligands (Jasti et al., 2007). While no such ligand for ASIC1a has been identified so far, the possibility exists that ligands or modulators for this channel produced under inflammatory conditions may also be responsible for stimulating the SDH neurons via specific activation of the ASIC_{Ca} in these neurons.

4.3.4. Potential role of ASIC2a-containing ASICs

Although ASIC_{Ca} is the dominant type of ASICs in the SDH (Duan et al., 2007), ASIC2a-containing ASICs were also found in the spinal cord and ASIC2a was also up-regulated following peripheral inflammation (Baron et al., 2008; Wu et al., 2004). Since intrathecal injection of PcTX1 or ASIC1a antisense oligonucleotides did not completely reverse inflammatory pain hypersensitivity in rats, the ASIC2a-containing ASICs in the spinal cord may also contribute in a minor way to nociceptive sensitization in the spinal cord. Further specific blocker of ASIC2a-containing ASICs will be necessary for more definitive determination of its function.

4.4. Regulation of ASIC_{Ca}

4.4.1. Transcriptional and translational regulation

Although molecular signaling pathways responsible for ASIC1a up-regulation in SDH neurons following peripheral inflammation remain to be elucidated, several lines of evidence support Ca²⁺- and activity-dependent mechanisms. First, in DRG neurons, inflammatory mediators such as serotonin, nerve growth factor, bradykinin, and interleukin 1, all increase ASIC subunit expression and acidosis-induced currents (Mamet et al., 2002). Second, P11, a member of the S100 small phospholipid- and Ca²⁺-binding protein family, is up-regulated by inflammatory mediators, including nerve growth factor (NGF) (Masiakowski and Shooter, 1988) and nitric oxide (Pawliczak et al., 2001) and promotes surface expression of ASIC1a (Donier et al., 2005). Third, the transcriptionally active region of ASIC1 gene is preceded by the cAMP response element (CRE) that binds to cAMP response element binding protein (CREB), which could couple intracellular Ca²⁺ elevation to gene transcription (Impey et al., 2004). Furthermore, nociceptor activation causes the release of BDNF (Michael et al., 1997), which is known to modulate chronic pain (for review, see Pezet and McMahon, 2006). Thus, BDNF may regulate ASIC1a expression via BDNF-dependent CREB activation. Besides transcriptional regulation, local protein synthesis may be also important for central sensitization and chronic pain. Emerging evidence shows that treatment with neurotrophins BDNF and NT-3 (Kang and Schuman, 1996), or increasing intracellular cAMP by forskolin (Frey et al., 1993; Huang et al., 1994), can induce long-lasting protein synthesis and LTP in the hippocampus. Whether BDNF- and NGF-dependent ASIC_{Ca} translational regulation participates in the development of central sensitization and inflammatory pain awaits further investigations.

4.4.2. Post-translational regulation

Aside from the contribution of elevated protein expression, post-translational regulation of ASIC_{Ca} may also participate in nociceptive sensitization. Indeed, several ASIC_{Ca} regulators have been reported in various brain regions, including Ca²⁺ (Babini et al., 2002; de Weille and Bassilana, 2001; Waldmann et al., 1997), Zn²⁺ (Chu et al., 2004), Pb²⁺ (Wang et al., 2006), nitric oxide (Cadiou et al., 2007), lactate (Allen and Attwell, 2002), arachidonic acid (Allen and Attwell, 2002; Smith et al., 2007), and redox reagents (Andrey et al., 2005; Chu et al., 2006). These regulators may modify ASIC_{Ca} channel functions as well as its trafficking and membrane insertion. Although postsynaptic insertion of GluR1-containing AMPA receptors in SDH neurons has been suggested to underlie LTP associated with central sensitization and inflammatory hyperalgesia (Galan et al., 2004), capsaicin-induced acute inflammatory hyperalgesia is accompanied by increased postsynaptic density of GluR1 only in lamina II non-peptidergic noxious synapses but not in lamina I peptidergic noxious synapses (Larsson and Broman, 2008). There is also a distinct possibility that other changes such as phosphorylation and translocation of ASIC_{Ca} may contribute to the observed LTP in lamina I peptidergic noxious synapses after inflammatory hyperalgesia (Ikeda et al., 2003). In the CA1 hippocampal neurons, NMDA receptor activation indeed sensitizes cells to acidic conditions by inducing ASIC1a phosphorylation (Gao et al., 2005). It is thus of interest to determine whether phosphorylation and translocation of ASIC_{Ca} to SDH neuronal synapses contribute directly to inflammatory pain hypersensitivity.

4.4.3. Regulation of ASIC_{Ca} by pro-nociceptive neuropeptides

Although proton is the only known agonist so far for the activation of ASICs, a variety of extracellular and intracellular signaling molecules can modulate the activities of ASICs and thus

profoundly influence the function of these channels in both physiological and pathological processes (for review, see Xu and Xiong, 2007). For example, FMRF-amide and structurally related peptides are abundant in invertebrate nervous systems where they have been proposed to function as neurotransmitters and neuromodulators. In the mammalian CNS, two FMRF-amide like peptides including FLQPQRF-NH₂ (neuropeptide FF, NPFF) and AGEGLSSPFWSLAAPQRF-NH₂ (neuropeptide AF, NPAF) have been found (Yang et al., 2008). Among many biological roles suggested for the NPFF system, the possible modulatory role of NPFF in nociceptive processing and opiate analgesia has been widely investigated. Existing evidence indicates that FMRF-amide and NPFF modify the response to nociceptive stimuli (Raffa and Connelly, 1992; Tang et al., 1984; Yang et al., 1985), and NPFF expression is increased in the spinal cord during chronic inflammation (Kontinen et al., 1997; Vilim et al., 1999; Yang and Iadarola, 2003). Although, it has been generally assumed that NPFF exerts its actions via G-protein coupled receptors (NPFF receptor 1 and NPFF receptor 2), a second set of targets relevant to potential pro-nociceptive actions are the ASICs. The idea is supported by the following observations: (1) RF-amide peptides (peptides with RF-amide at their C-terminus) including FMRF-amide, FRRF-amide and their mammalian homologues NPFF, delay the rate of ASIC desensitization and increase the peak current amplitude in some cases following a low pH stimulus, thereby potentiating acid-evoked currents (Lingueglia et al., 2006; Yang et al., 2008). (2) Endogenous RF-amide peptide NPFF decreases the steady-state desensitization of ASIC_{Ca}. During conditions that normally induced steady-state desensitization, this peptide profoundly enhances ASIC_{Ca} activity (Sherwood and Askwith, 2008), suggesting that NPFF modulation of ASIC_{Ca} desensitization could contribute to the development of chronic pain.

5. ASIC_{Ca} in neuropathic pain

Damage to the nervous system can cause chronic neuropathic pain. A recent study showed that PcTX1 had analgesic effects in rodent models of chronic constriction injury of the sciatic nerve and vincristine-induced neuropathy. These PcTX1 effects were related to the endogenous enkephalin pathway, since the PcTX1 treatment increased the Met-enkephalin level and these PcTX1 analgesic effects were lost in mice lacking the *preproenkephalin* gene (Mazzuca et al., 2007). This study indicates an interesting link between ASIC_{Ca} and the opioid system. While the mechanistic detail underlying this phenomenon remains unknown, ASIC_{Ca} in the CNS may also represent a target for the development of safer painkillers for the neuropathic pain.

6. ASIC_{Ca} in supraspinal regions

Synaptic plasticity phenomena associated with hyperalgesia and allodynia in pathological pain states are not restricted to the synapse between nociceptive afferents and second-order SDH neurons, but are potentially operational in higher brain regions that process the sensory and affective components of pain, including the brainstem, thalamus, dorsal insular cortex, anterior cingulate cortex, and amygdala (for review, see Craig, 2003; Zhuo, 2008). It has been reported that ASIC_{Ca} is functionally expressed in amygdala and cortex (Wemmie et al., 2003, 2004). However, it is still unknown whether supraspinal ASICs are engaged in the modulation of chronic pain hypersensitivity. Based on the observations that peripheral inflammation and nerve injury cause plastic changes or LTP in the cortical and subcortical synapses underlying behavior sensitization (Wei and Zhuo, 2001; Wu et al., 2005a, 2005b), chronic pain is likely to involve cortical areas (Zhuo, 2008). Thus, treating chronic pain requires understanding of

plastic changes in somatosensory pathways at multiple levels. At the cortical level, in addition to encoding pain, cortical neuronal plasticity contributes to the emotional aspect of pain (Carrasquillo and Gereau, 2007; Neugebauer et al., 2004; Tracey and Mantyh, 2007). Mice with disruption of the *ASIC1* gene show reduced fear responses to both cue- and context-specific Pavlovian fear conditioning (Wemmie et al., 2003). In contrast, overexpression of ASIC1a in the amygdala increases acidosis-evoked currents and enhances context fear conditioning (Wemmie et al., 2004). Thus, ASIC_{Ca} may play a role in the emotional-affective component of pain.

7. ASIC_{Ca} in peripheral sensory neurons

7.1. Role of ASIC1a and 2a

Tissue acidosis is an important feature of inflammation or nerve injury in the PNS. Protons directly gate depolarizing cationic channels on sensory neurons (Bevan and Yeats, 1991; Krishtal and Pidoplichko, 1981), which correspond to the now well-characterized ASICs (Waldmann et al., 1997). All ASIC subunits are expressed in DRG neurons, with 1a, 1b, 2b and 3 present in small DRG neurons, i.e. nociceptors (Chen et al., 1998; Garcia-Anoveros et al., 2001; Voilley et al., 2001). This localization strongly suggests that ASIC channels in DRG neurons play a role in nociception. Indeed, expression of ASIC subunits in DRG neurons is facilitated by inflammation (Voilley et al., 2001) and pro-inflammatory molecules (Mamet et al., 2002). In contrast, ASIC1a mRNA levels decrease in DRG neurons in spared nerve injury and spinal nerve ligation models. Consistent with the down-regulation of the excitatory peptides SP and CGRP and the up-regulation of the inhibitory peptides, neuropeptide tyrosine and galanin, following peripheral axotomy (Hokfelt et al., 1994), the ASIC1a down-regulation in the neuropathic pain model may represent an adaptive response to relieve the consequences of peripheral nerve damage and to protect the injured neuron.

The involvement of peripheral ASICs in pain sensation has been investigated using gene-targeted mice. However, the reports to date give an inconsistent picture. Although ASIC_{Ca} is most sensitive to protons and ASIC1a is abundantly expressed in DRG neurons, *ASIC1*-null mice do not differ from wild-type animals either in the functioning of cutaneous mechanoreceptors or in mechanical hyperalgesia produced by repeated intramuscular acid injections (Page et al., 2004; Sluka et al., 2003). Studies with *ASIC2* gene deletion mice showed that these mice have normal mechanical sensation in the noxious range (Drew et al., 2004; Price et al., 2000; Roza et al., 2004).

7.2. Role of ASIC3

Three separate studies have evaluated the behavioral phenotype of *ASIC3* knockout mice, with disparate results. Price et al. (2001) showed a small but significant increase in the sensitivity to mechanical stimulation after hindpaw inflammation, and a reduced mechanical hypersensitivity after injection of acid (pH 4.0 saline) into the gastrocnemius muscle. Sluka et al. (2003) later found that the hypersensitivity induced by injection of acidic solution into the muscle is specific to ASIC3-containing but not to ASIC1a/1b-containing channels. In contrast, Chen et al. (2002) found an increased sensitivity to thermal, mechanical, and acid stimuli in *ASIC3* knockout mice but detected no alterations in hypersensitivity after capsaicin or carrageenan treatment. More recently, in transgenic mice overexpressing a dominant-negative form of ASIC3, it has been shown that the response to thermal stimuli was normal, but the animals showed an increased response to intraperitoneal acid injection and mechanical

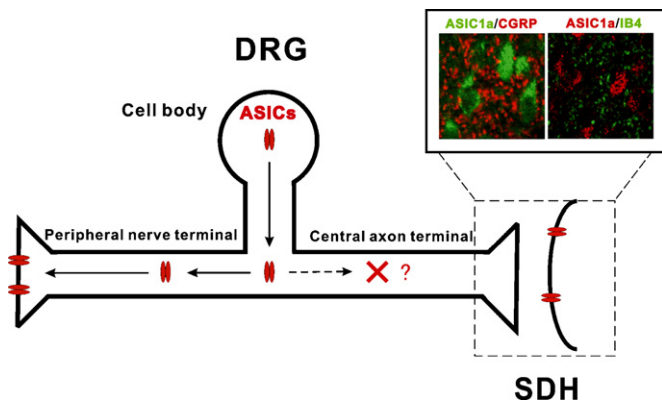


Fig. 3. Schematic representation of unidirectional transport of ASICs from the cell body of DRG neurons toward the peripheral nerve terminal. However, CRGP and IB4 do not co-localize with ASIC1a in the central axon terminals (based on Duan et al., 2007).

hypersensitivity after inflammatory stimuli (Mogil et al., 2005). To date, no satisfactory explanations for these discrepancies have been provided, but possible causes are the variability in genetic background or species, differences in testing paradigms, environmental variability and other complementary effects. For example, Mogil et al. (2005) reported an elevated function of TRPV1 channels in ASIC-transgenic mice. This increased TRPV1 activity may contribute to the observed hypersensitivity to painful stimuli in mice with impaired functional ASICs. In addition, mice express relatively low levels of ASICs in their DRG neurons (Leffler et al., 2006; Lin et al., 2008) as compared with other species such as rats (Deval et al., 2008). As a consequence, the activation of peripheral ASIC3 by acidosis and inflammatory mediators contributes to inflammatory pain in rats (Deval et al., 2008). However, consistent with the results from ASIC1 knockout mice (Sluka et al., 2003), peripheral ASIC_{Ca} seems not mediate acidic and inflammatory pain in rats (Deval et al., 2008). Interestingly, ASIC_{Ca} is specifically involved in visceral mechanosensation in mice (Page et al., 2004).

7.3. Transport of ASIC subunits

While ASIC proteins have been detected in the soma and in the peripheral nerve endings of DRG neurons (Garcia-Anoveros et al., 2001; Price et al., 2001), the presence of ASIC subtypes in the central projections in the dorsal horn of the spinal cord is less clear. A recent study uncovered the absence of ASIC1a in either peptidergic (e.g. CGRP) or non-peptidergic (e.g. IB4) central terminals (Fig. 3). Thus it is likely that ASIC1a is transported from DRG cell bodies to sensory terminals in the periphery, but not to the spinal cord. A similar distribution pattern was previously observed for the ASIC2a subunit (Garcia-Anoveros et al., 2001). The unidirectional transport of ASICs from the cell body of DRG neurons toward the periphery is, as far as we know, unique. Other sensory receptor channels such as P2X₃ (Vulchanova et al., 1998) and TRPV1 (Guo et al., 1999) are transported both to the periphery and central terminals. The peripheral transport of ASICs suggests a selective sorting mechanism at the branch point of peripheral and central axons of DRG neurons, previously unnoticed for any other proteins. Such unique peripheral ASIC location further supports a specific role of ASIC_{Ca} of SDH neurons in regulating central sensitization underlying chronic pain.

8. Concluding remarks

ASICs are highly expressed in neurons of the CNS, where they sense changes of extracellular proton concentrations under both physiological and pathological conditions. Enhanced Ca²⁺ signaling in SDH neurons following peripheral inflammation, through the up-regulation of ASIC_{Ca}, may contribute to hypersensitivity of SDH neurons and inflammatory pain (Fig. 1). Therefore, specific blockade of ASIC_{Ca} exerts anti-nociceptive effects by reducing or preventing the development of spinal sensitization induced by inflammation (Duan et al., 2007). Through membrane depolarization, activation of ASIC_{Ca} may also indirectly exert an effect *via* the relief of Mg²⁺ blockade of NMDA receptors (Fig. 1), which is essential for spinal nociceptive plasticity underlying chronic pain. Because blockers of NMDA receptors used for the treatment of chronic pain have severe side-effects (South et al., 2003), drugs

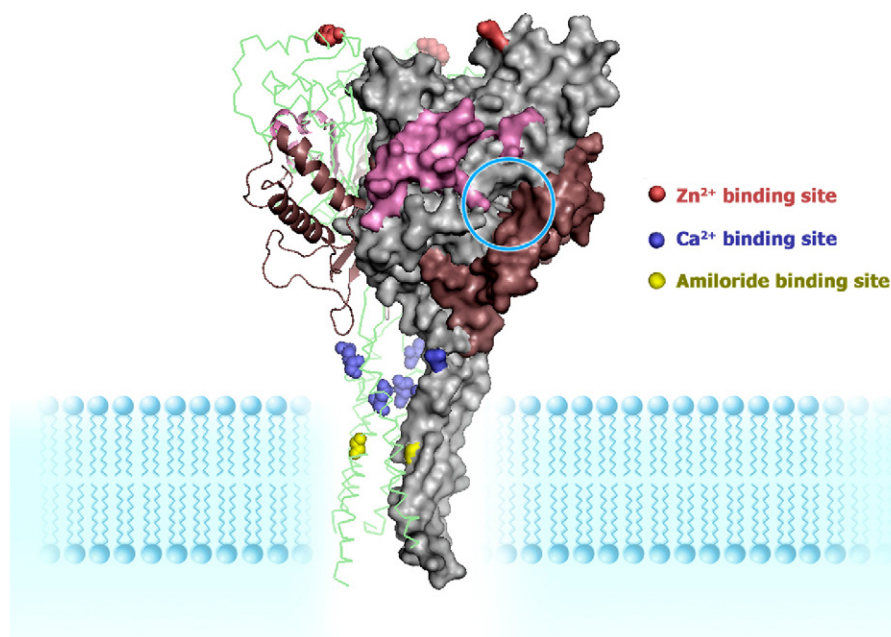


Fig. 4. Structure and regulatory sites of ASIC_{Ca}. Blue circle indicates the putative binding motif of PcTX1 (based on Jasti et al., 2007; Salinas et al., 2006).

specifically targeting non-classical Ca²⁺-permeable channels such as ASIC_{Ca} might offer new analgesic approaches, similar to that proposed for ischemic neuroprotection (for review, see [Besancon et al., 2008](#)). Furthermore, ASIC_{Ca} also might be activated indirectly by glutamate in a NMDA receptor-dependent manner ([Gao et al., 2005](#)). The link between NMDA receptors and ASIC_{Ca} could make this approach especially important in interfering the induction and expression of chronic pain.

Although ASIC_{Ca} appears to be an attractive drug target for chronic pain ([Duan et al., 2007](#); [Mazzuca et al., 2007](#)), stroke ([Gao et al., 2005](#); [Pignataro et al., 2007](#); [Xiong et al., 2004](#)) and neurodegeneration ([Friese et al., 2007](#)), no successful drug against ASIC_{Ca} has been developed ([Diochot et al., 2007](#); [Xiong et al., 2008](#)). This is due in part to the paucity of structural information on these channels. Recent elucidation of the first three-dimensional structure of chicken ASIC1a by [Jasti et al. \(2007\)](#) showed a large extracellular region that may form the binding site for other molecules and play a crucial role in channel gating ([Fig. 4](#)). Such new structural information, aided by computational modeling of ligand docking dynamics and experimental assays of channel functions, may help the development of small-molecule drugs that act specifically on ASIC_{Ca}, with the aim of developing therapies for chronic pain and other pathological conditions involving excessive glutamate release and acidosis.

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