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REVIEW
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Chloride – The Underrated Ion in Nociceptors

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In contrast to pain processing neurons in the spinal cord, where the importance of chloride conductances is already well established, chloride homeostasis in primary afferent neurons has received less attention. Sensory neurons maintain high intracellular chloride concentrations through balanced activity of Na^+-K^+-$2Cl^-$ cotransporter 1 (NKCC1) and K^+-Cl^- cotransporter 2 (KCC2). Whereas in other cell types activation of chloride conductances causes hyperpolarization, activation of the same conductances in primary afferent neurons may lead to inhibitory or excitatory depolarization depending on the actual chloride reversal potential and the total amount of chloride efflux during channel or transporter activation. Dorsal root ganglion (DRG) neurons express a multitude of chloride channel types belonging to different channel families, such as ligand-gated, ionotropic γ-aminobutyric acid (GABA) or glycine receptors, Ca^{2+}-activated chloride channels of the anoctamin/TMEM16, bestrophin or tweety-homolog family, CLC chloride channels and transporters, *cystic fibrosis transmembrane conductance regulator* (CFTR) as well as volume-regulated anion channels (VRACs). Specific chloride conductances are involved in signal transduction and amplification at the peripheral nerve terminal, contribute to excitability and action potential generation of sensory neurons, or crucially shape synaptic transmission in the spinal dorsal horn. In addition, chloride channels can be modified by a plethora of inflammatory mediators affecting them directly, via protein-protein interaction, or through signaling cascades. Since chloride channels as well as mediators that modulate chloride fluxes are regulated in pain disorders and contribute to nociceptor excitation and sensitization it is timely and important to emphasize their critical role in nociceptive primary afferents in this review.

Keywords: anoctamin 1, glycine receptor, $GABA_A$ receptor, NKCC1, KCC2

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INTRODUCTION

In the past, excitation of primary afferent neurons has been associated mainly with cation fluxes across the cell membrane, setting the neurons' excitability, responsiveness to tissue damaging stimuli as well as action potential (AP) generation and propagation. However, the dynamic of Cl^- homeostasis and the role of Cl^- fluxes across the cell membrane in primary

Abbreviations: 5-HT, serotonin; Ano, anoctamin; AP, action potential; Best, bestrophin; CCC, cation-chloride cotransporter; CF, cystic fibrosis; CFTR, cystic fibrosis transmembrane conductance regulator; ClCA, chloride channel accessory; IP_3, Inositol-1,4,5-trisphosphate; SDH, dorsal horn of the spinal cord; GABA, γ-aminobutyric acid; GlyR, glycine receptor; GPCR, G-protein coupled receptor; KCC, K^+-Cl^- Cotransporter; MAC, maxi-anion channel; NKCC, Na^+-K^+-$2Cl^-$ cotransporter; PGE_2, prostaglandin E2; PIP_2, phosphatidylinositol-4,5-bisphosphate; S1P, sphingosine-1-phosphate; VGCC, voltage-gated calcium channels; VRAC, volume-regulated anion channel; VSOAC, volume-sensitive organic osmolyte anion channel.

afferents and their spinal connections are gaining increasing attention and turn out to be of critical importance in particular for the development and maintenance of neuropathic pain. In the following review we will summarize which chloride conductances are expressed in DRG neurons and what is currently known about their contribution to nociception and chronic pain development.

Cl^- ION HOMEOSTASIS IN DRG NEURONS

The intracellular Cl^- concentration of neurons is maintained by cell membrane transporters including Na^+-K^+-$2Cl^-$ cotransporter 1 (NKCC1), or K^+-Cl^- cotransporter 2 (KCC2), the latter becomes prominent in mature neurons (Payne et al., 2003; Ben-Ari et al., 2012; Kaila et al., 2014). Both transporters mediate electroneutral transport of Cl^- and utilize the electrochemical gradient generated by the Na^+-K^+ ATPase. KCCs in general extrude Cl^- from the cell leading to low intracellular Cl^- concentration and a negative equilibrium potential for Cl^-, while NKCCs increase cytoplasmic Cl^- concentration by shuffling Cl^- into the cell resulting in a more depolarized Cl^- equilibrium potential. When the intracellular Cl^- concentration is very low and the Cl^- equilibrium potential (E_{Cl-}) is below the resting membrane potential (V_M) of the respective neuron, Cl^- influx leads to hyperpolarization mediated by net inward flux of negative charge. In contrast, at high intracellular Cl^- concentration, activation of a Cl^- conductance will depolarize the cell due to Cl^- efflux. However, this may still cause inhibition of the respective neuron via two mechanisms: (1) slow membrane depolarization by Cl^- efflux causes inactivation of voltage-gated Na^+ channels that results in reduced excitability and (2) increased Cl^- conductance reduces the input resistance of the membrane 'shunting' electrical input upon stronger depolarization (Price et al., 2009; Kaila et al., 2014). Strong and rapid depolarization occurs if E_{Cl-} is near the AP threshold resulting in AP discharge (Prescott et al., 2006; Price et al., 2009).

In contrast to other cell types, sensory neurons, whose cell bodies reside in the DRG, express sustained NKCC1, KCC1 and KCC3 activity but low or even undetectable KCC2 (Rivera et al., 1999; Sung et al., 2000; Kanaka et al., 2001; Coull et al., 2003; Gilbert et al., 2007; Price et al., 2009; Pieraut et al., 2011; Lucas et al., 2012). Because KCC1 and -3 activity is increased by cell swelling but is low under isoosmotic conditions, these cation-chloride cotransporters (CCCs) do not significantly reduce the intracellular Cl^- concentration (Gamba, 2005). This is supported by the finding that KCC3 ablation in nociceptors does not affect heat sensitivity (Ding and Delpire, 2014). Therefore, mainly NKCC1 determines the high intracellular Cl^- levels in mature DRG neurons in normal isotonic situation. In addition to CCCs, also the Na^+-independent Cl^--HCO_3^--anion exchanger AE3 might contribute to the intracellular Cl^- accumulation (Pfeffer et al., 2009). This transporter is expressed in ∼60% of peptidergic and ∼30% of non-peptidergic DRG neurons (Barragan-Iglesias et al., 2014). The actual reversal potential for Cl^- in DRG neurons is subject to regulation and varies between −20 mV and −70 mV (Gilbert et al., 2007; Funk et al., 2008). Therefore, activation of Cl^- channels usually will cause depolarization, and the degree and velocity of depolarization determines whether this has inhibitory or excitatory consequences (Sung et al., 2000; Prescott et al., 2006; Gilbert et al., 2007; Funk et al., 2008). Furthermore, localized signaling and intracellular Cl^- diffusion additionally increase heterogeneity and complexity of anion currents (Kuner and Augustine, 2000; Gulledge and Stuart, 2003; Doyon et al., 2011; Raimondo et al., 2012). Thus, depending on spatial and temporal fluctuations of Cl^- levels, Cl^- ion channel activation can have different effects on the overall activity of primary afferents.

CCCs, like NKCC, KCC and the Na^+-Cl^--cotransporter NCC, and anion transporters such as the Na^+-dependent Cl^--$2HCO_3^-$-exchanger and the Na^+-independent Cl^--HCO_3^--anion exchanger, link different ion species including H^+ and HCO_3^- and even the membrane potential to Cl^- level regulation (Doyon et al., 2011, 2016). In addition, Cl^- channels are permeable to other anions, of which HCO_3^- is physiologically most important since sustained HCO_3^- outward flux through anion channels is ensured by free diffusion of CO_2 across the membrane (Price et al., 2009). This allows for constant replenishment of HCO_3^- catalyzed by carbonic anhydrase which is detectable in ∼30% of DRG neurons (Prabhakar and Lawson, 1995; Price et al., 2009). The contribution of HCO_3^- to the total anion current through the open anion channels can change when the driving force for Cl^- is altered due to a reduction in Cl^- gradient (Cordero-Erausquin et al., 2005). A collapse of the Cl^- gradient can lead to an increased proportion of HCO_3^- to the total anion current or even to a biphasic anion current response. The E_{Cl-} can change rapidly, particularly during strong activation and in small compartments, or on a long time range during pain conditions due to regulation of CCCs (Coull et al., 2003; Funk et al., 2008; Wright et al., 2011; Doyon et al., 2016; Li et al., 2016).

As a consequence of the high intracellular Cl^- concentration mediated by the active accumulation of Cl^- by NKCC1, for example GABA-evoked depolarizing currents are observed in primary afferents at resting membrane potential (Sung et al., 2000); likewise, activation of G protein-coupled receptors, e.g. by lysophosphatidic acid (LPA) and sphingosine-1-phosphate (S1P), has been shown to activate excitatory chloride conductances (Ponsioen et al., 2009; Camprubi-Robles et al., 2013; Qi et al., 2018).

In contrast to the well-established contribution of deregulated CCCs with disturbed Cl^- homeostasis and rising Cl^- levels in spinal cord neurons to the pathophysiology of pain disorders (Coull et al., 2003), the regulation of CCCs in primary sensory neurons in inflammation and pain is still controversially discussed. In an arthritis model, NKCC1 is downregulated, whereas sciatic nerve injury and inflammatory mediators increase NKCC1 expression and activity (Morales-Aza et al., 2004; Funk et al., 2008; Chen et al., 2014; Modol et al., 2014). At the same time, these mediators reduce expression of KCC2 in DRGs, which leads together with NKCC1 activation to increased intracellular Cl^- levels and nociceptor excitability (Funk et al., 2008; Pieraut et al., 2011). Thus, persistent inflammation

can increase GABA-induced depolarization by affecting Cl^- homeostasis in DRG neurons (Zhu et al., 2012), while ablation of NKCC1 or pharmacological inhibition by bumetanide increases the latency in the hot plate or tail flick tests and alleviates thermal and mechanical hypersensitivity after sciatic nerve lesion (Modol et al., 2014). Similarly, the Cl^--HCO_3^- exchanger AE3 is upregulated in a formalin-induced pain model and a Cl^--HCO_3^- anion exchange inhibitor blocks the evoked allodynia and hyperalgesia (Barragan-Iglesias et al., 2014). These reports highlight the significance of Cl^- homeostasis and Cl^- conductances in nociceptors.

CHLORIDE CHANNELS/TRANSPORTERS EXPRESSED IN PRIMARY SENSORY AFFERENTS

Cl^- conductances in primary afferents can be carried by different members of the chloride channel superfamily (**Figure 1**) and are of importance at the peripheral nerve terminal in the target tissue, along the peripheral axon, on the cell soma, as well as at synaptic terminals within the spinal dorsal horn. However, neuromorphological evidence regarding the subcellular localization of the chloride channels is sparse, as most evidence is derived from the cell body of DRG neurons. Besides the pentameric ligand-gated ionotropic $GABA_A$ and glycine receptors, the large class of Cl^- channels and transporters comprises several structurally unrelated families. These include Ca^{2+}-activated Cl^- channels of the anoctamin (Ano or TMEM16), bestrophin (Best) and tweety homolog (Ttyh) families, the CLC channel/transporter family, the *cystic fibrosis transmembrane conductance regulator* (CFTR), volume-regulated anion channels (VRAC) that are formed by LRRC8 proteins, and SLCO2A1 as the molecular correlate of maxi-anion channels. Of those, $GABA_A$, Ano1, Best1, Thyh1, CLC-3 and CLC-6 have been associated with nociception: either their expression is altered in pain states or their activity modulates pain (see **Figure 1**, marked in red), however other candidates may also contribute and can be addressed by an increasing number of genetic, chemogenetic, optogenetic and pharmacological tools (Bormann, 2000; Enna and McCarson, 2006; Poet et al., 2006; Boudes et al., 2009; Zeilhofer et al., 2009; Liu et al., 2010; Cho et al., 2012).

Ligand-Gated Chloride Channels

$GABA_A$ Receptors

γ-aminobutyric acid (GABA) is the main inhibitory neurotransmitter of the central nervous system (Olsen, 2002). It binds to two different receptor types, the ionotropic $GABA_A$ receptors and the metabotropic Gi/o protein-coupled $GABA_B$ receptors. $GABA_A$ receptors are members of the Cys-loop receptor family of ligand-gated ion channels that share a pentameric structure with a large N-terminal extracellular domain for ligand binding, four transmembrane regions including the pore-forming segment, and one large cytoplasmic loop for intracellular modifications (Galaz et al., 2015). To date 19 different subunits have been identified (six α-, three β-, three γ-, three ρ- and one of each ε-, δ-, θ-, and π-subunits) (Sigel and Steinmann, 2012). The major $GABA_A$ channel isoform in adult DRG neurons is composed of two α1- and β2-subunits and one γ2-subunit (Sigel and Steinmann, 2012). Alternative subunit assemblies define different functional and physiological properties.

GABA has been implicated as an important modulator at different levels of the pain pathway, although mainly micro-circuitries within the spinal dorsal horn (SDH) and supraspinal brain regions have been investigated (Enna and McCarson, 2006). Already in the 1970s it was established that primary sensory neurons respond to GABA stimulation with depolarization (De Groat et al., 1972). Since then, a multitude of studies link this enigmatic response to a possible chloride conductance (Desarmenien et al., 1979, 1980, 1981; Gallagher et al., 1983a,b). In embryonic DRG neurons, GABA generates an inward current, which is inhibited by the $GABA_A$ antagonists bicuculline, picrotoxin and TBPS (Valeyev et al., 1999). The attributes of these GABA-induced currents depend on the primary afferent cell type, with TTX-sensitive and capsaicin-insensitive neurons generating larger currents than capsaicin-sensitive nociceptors (White, 1990). Accordingly, unique developmental expression patterns of different $GABA_A$ subunits are reported: α2 and β3 subunit mRNA is expressed in all embryonic and adult DRG neurons, while β2 mRNA is only present in adult ones; 37% of DRG neurons express the $GABA_A$ γ- subunit (Furuyama et al., 1992; Maddox et al., 2004); $GABA_A$ subunits α1, α6, β1, γ2L, and ρ2 are absent in DRG, and the δ-subunit is only weakly expressed (Ma et al., 1993; Maddox et al., 2004; Du et al., 2017).

By now, a role for $GABA_A$-mediated currents has been established in all morpho-functional nociceptor compartments, where they have different roles in pain regulation and distinct GABA sources: GABA released at an injury site sensitizes nociceptors at the peripheral terminal, while GABA acting in a paracrine mode on the cell bodies within the DRG has mostly antinociceptive effects (Du et al., 2017). $GABA_A$ responses can be evoked both in the dorsal root as well as in the sciatic nerve, demonstrating the presence of these receptors on the central and peripheral axon (Bhisitkul et al., 1987). $GABA_A$ receptors located at central terminals of primary afferents in the spinal dorsal horn respond to GABA-release from spinal interneurons and induce presynaptic inhibition of synaptic inputs, which in most sensory systems is responsible for contrast enhancement and gain control (Zimmerman et al., 2019). Activation of these $GABA_A$ receptors by GABA released at axo-axonic synapses induces Cl^- efflux that causes primary afferent depolarization. Paradoxically, this depolarization leads to a reduced transmitter release from afferent terminals by either shunting inhibition, inactivation of voltage-gated Na^+ channels or inactivation of voltage-gated Ca^{2+} channels and is thus antinociceptive (Rudomin and Schmidt, 1999; Kullmann et al., 2005; Lidierth, 2006; Witschi et al., 2011). Sensory afferents synapse to different dorsal horn inhibitory GABA interneurons that are further modulated by descending projections from cortex and brainstem. These interneurons then affect the transmission of painful signals from the nociceptor to the secondary neuron. Loss of presynaptic,

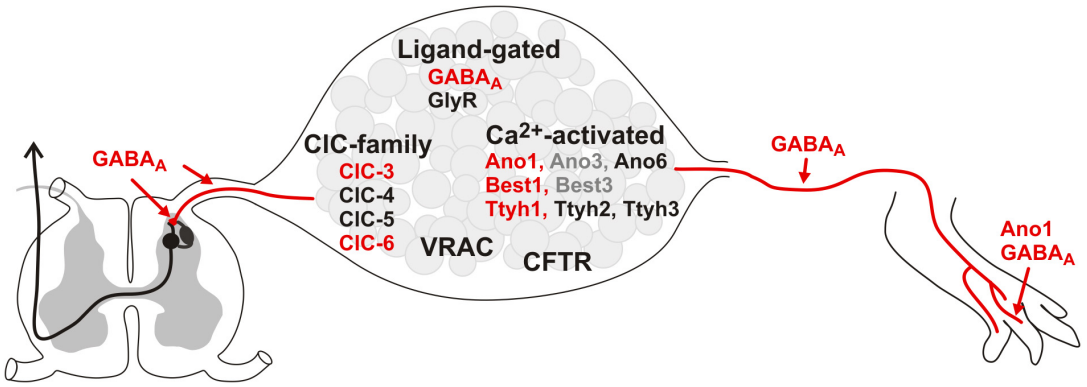

FIGURE 1 | Cl^- conductances expressed in DRGs. Expression of the depicted Cl^- channels/transporters has been demonstrated in DRGs. The channels in red have been associated with pain: Their activity in nociceptors affects pain sensation and/or their expression is modulated in primary afferents in pain conditions. In contrast, for the conductances written in black neither a clear contribution to pain behavior, nor a regulation in pain models has been demonstrated so far. The gray candidates show low expression and no particular role in nociceptors has been assigned to them yet. As most data exists on the mRNA/protein level within the DRG, little is known about the distribution of the Cl^- conductances within the central or peripheral axons and their terminals. So far, only Ano1 and $GABA_A$ have been functionally established at the peripheral axon terminal, while $GABA_A$ is also involved in presynaptic inhibition of the primary afferents in the dorsal horn of the spinal cord.

$GABA_A$-mediated inhibition leads to tactile hypersensitivity and impaired texture discrimination (Zimmerman et al., 2019). The descending projections can set pain thresholds based on internal and emotional states, with acute stress and expected pain producing analgesia, while chronic stress and anxiety facilitate pain (Porreca et al., 2002; Basbaum et al., 2009; Jennings et al., 2014; Francois et al., 2017). The main source for descending pain modulation is the rostroventral medulla (RVM), which harbors both glutamatergic ON neurons that facilitate nociception by excitation of primary afferent terminals and/or excitatory neurons within SDH (Heinricher et al., 2009) and antinociceptive OFF cells that provide inputs onto nociceptive primary afferents and thus suppress pain transmission (Budai and Fields, 1998). Interestingly, the majority of RVM-derived descending input is GABAergic, with indirect GABAergic projections via SDH GABAergic interneurons that, when inhibited, lead to mechanical hyposensitivity (Francois et al., 2017), as well as GABAergic inputs to sensory afferents that, when inhibited, increase both heat and mechanical sensitivity (Zhang et al., 2015).

In inflamed tissue, blood and immune cells actively release GABA, which may directly act on primary afferent nerve terminals and induce excitation and sensitization (Bhat et al., 2010; Bravo-Hernandez et al., 2014). The $GABA_A$ subunits α1 and β2/3 have been detected by immunohistochemistry in 10–14% of unmyelinated peripheral axons in the cat glabrous skin (Carlton et al., 1999). Peripheral administration of the selective $GABA_A$ receptor agonist muscimol evokes nocifensive behavior whereas the $GABA_A$ blockers picrotoxin or bicuculline inhibit formalin-induced pain-like behavior (Bravo-Hernandez et al., 2014; Jang et al., 2017). GABA-activated currents are modified by inflammatory mediators, like prostaglandin E2 (PGE_2), bradykinin, histamine, ATP or interferon gamma (Sokolova et al., 2001; Vlachova et al., 2001; Labrakakis et al., 2003; Vikman et al., 2003; Toulme et al., 2007). The neurotransmitter serotonin (5-HT) or a 5-HT_2 receptor agonist potentiate, while dopamine D1 receptor agonists, *N*-methyl-D-aspartate (NMDA) – but not kainic acid – as well as adenosine and caffeine inhibit GABA currents in DRG neurons (Xi and Akasu, 1996; Hu and Li, 1997; Li et al., 2004). Likewise, neuropeptides such as substance P, neurokinin A, and neurokinin B inhibit $GABA_A$-induced currents, likely mediated by protein kinase C, but not protein kinase A (Guan et al., 1994; Wu et al., 1994; Yamada and Akasu, 1996; Akasu and Yamada, 1997; Yang et al., 2003; Si et al., 2004; Li L. et al., 2015).

Thus, sustained inflammation or neuropathic alterations appear to strongly affect the function and expression of GABA currents and receptors. Twenty-four hours after complete Freund's adjuvant (CFA)-induced inflammation, retrogradely-labeled DRG neurons innervating the inflamed knee joint show increased GABA sensitivity and a decreased AP threshold (Chakrabarti et al., 2018). Following nerve ligation or chronic constriction injury, GABA-induced conductances and depolarization in small, medium, and large DRG neurons are attenuated (Bhisitkul et al., 1990; Chen et al., 2014; Ran et al., 2014). This is accompanied by respective downregulation of the $GABA_A$ α2-subunit by 30% in ipsilateral DRGs and reduction of the number of γ2-subunit mRNA expressing neurons (Obata et al., 2003; Obradovic et al., 2015). Consequently, injections of the GABA agonists muscimol or gaboxadol into the DRG immediately after nerve injury attenuate, whereas the GABA antagonists bicuculline or picrotoxin aggravate pain-like behavior (Naik et al., 2008, 2012; Ran et al., 2014). Upregulation of endogenous GABA within the sensory ganglion via GABA uptake inhibition alleviates thermal hyperalgesia, whereas knockdown of the α2 subunit further decreases pain thresholds (Obradovic et al., 2015). In contrast to the reduction of GABA currents described so far, GABA-induced conductances increase in axotomized cutaneous neurons and this is attenuated by BDNF, whereas NGF has no effect (Oyelese and Kocsis, 1996; Oyelese et al., 1997). DRG neuron cultures, which to a

certain extent represent an axotomy model *per se,* also develop increased $GABA_A$ current densities with time in culture (Lee et al., 2012). Other pain models such as formalin or reserpine injections are associated with upregulated α5 mRNA and protein expression in DRGs and spinal cord, and peripheral or intrathecal administration of an α5 antagonist prevents and reverses mechanical hypersensitivity (Bravo-Hernandez et al., 2016; De la Luz-Cuellar et al., 2019). Albeit there is an increasing body of data, the contribution of $GABA_A$ receptors in nociceptors to pathological pain is still controversial.

Glycine Receptors

The ligand-gated ionotropic glycine receptor (GlyR) is activated by amino acid ligands, with glycine, taurine and beta-alanine being the most common agonists, whereas the alkaloid strychnine acts as a high affinity antagonist (for reviews see Lynch, 2009; Dutertre et al., 2012; Galaz et al., 2015). Four different types of alpha subunits (GlyR α1-4) and one beta subunit (GlyR β) form heteropentameric ion channels in a 2α:3β stoichiometry (Dutertre et al., 2012). Homomeric receptors composed solely of α subunits have been observed in recombinant expression systems whereas β subunits are retained in the endoplasmic reticulum and are thus unable to form functional channels (Dutertre et al., 2012; Galaz et al., 2015).

The role of glycine receptors in pain modulation mainly relates to the SDH, where α3β heteromeric GlyRs are important anion channels of glycinergic inhibitory neurotransmission in the superficial SDH (Lynch, 2009). α3 subunits are mainly clustered by gephyrin at postsynaptic membranes in lamina 2, and colocalize with α1 subunits in around half the cases (Harvey et al., 2004). These α3 containing GlyRs are phosphorylated by proteinkinase A activated downstream of PGE_2 receptor EP_2, thereby diminishing glycinergic inhibitory input to lamina 2 neurons (Ahmadi et al., 2002; Harvey et al., 2004). In GlyR $\alpha3^{-/-}$ mice, this PGE_2-mediated reduction of inhibitory postsynaptic potentials of lamina 2 neurons is abolished, together with alleviated pain sensitization in response to chronic peripheral inflammation, while acute inflammatory pain stimuli are not affected (Harvey et al., 2004). In addition, analgesic effects of cannabinoids in different models of chronic inflammatory and neuropathic pain are absent in these mice (Xiong et al., 2012). Tissue damage during the neonatal period decreases GlyR-mediated input onto SDH GABAergic and glutamatergic neurons in adulthood and both mRNA and protein levels of the GlyR β subunit are upregulated in spinal cord in animals subjected to prolonged pain (Li et al., 2013; Galaz et al., 2015). Thus a major role for GlyRs in SDH circuits is well documented whereas the reports on glycinergic effects on primary afferents are sparse and inconsistent. In chicken embryo DRGs, uptake of [14C]2-deoxyglucose as a marker of excitatory activity is facilitated in response to glycine stimulation, and taurine – a GlyR (but also $GABA_A$) agonist - causes depolarizing responses in frog primary afferents (Saji and Obata, 1981; Padjen et al., 1989). In contrast, GlyRs are not involved in presynaptic modulation of transmitter release in rat spinal cord sections and neither taurine nor glycine show any detectable agonist activity in mammalian DRG neurons (Robertson, 1989; Valeyev et al., 1996, 1999; Betelli et al., 2015). Forty four percent of all rat DRG neurons express GlyR β subunit mRNA (Furuyama et al., 1992), L5 DRG neurons express both α3 and α1 GlyR subunits, and protein expression is decreased after intrathecal PGE_2 injection (Wang et al., 2018). DRG and SDH neuron co-cultures develop inhibitory glycinergic synapses which are able to generate inhibitory synaptic transmission at DRG neurons (Shypshyna and Veselovs'kyi, 2010). This suggests that GlyR in primary afferent neurons are predominantly involved in setting transmission efficacy at SDH synapses but are not involved in nociceptive transduction.

Calcium-Activated Chloride Channels

Anoctamins

Ano1 was identified in 2008 as the first member of the anoctamin protein family (Ano; TMEM16) (Caputo et al., 2008; Schroeder et al., 2008; Yang et al., 2008). This protein family consists of ten members, Ano1-10, and the recent structural data revealed ten transmembrane domains (Paulino et al., 2017). The subunits assemble as dimers with two distinct pores to serve divers functions ranging from calcium level regulation to lipid scramblase and ion channel activity (Caputo et al., 2008; Schroeder et al., 2008; Yang et al., 2008; Pifferi et al., 2009; Wanitchakool et al., 2017). Alternative splicing further increases the number of isoforms with different biophysical properties and expression (Ferrera et al., 2009; Mazzone et al., 2011; Ertongur-Fauth et al., 2014). Ano1, 2, and 6 have been shown to convey Ca^{2+}-activated Cl^- conductances, although Ano6 can conduct both anions and cations depending on intracellular Ca^{2+} concentration and membrane potential (Caputo et al., 2008; Schroeder et al., 2008; Yang et al., 2008; Pifferi et al., 2009; Ye et al., 2019). Ano conductances are synergistically regulated by intracellular Ca^{2+}, membrane depolarization, membrane lipids and heat (Cho et al., 2012; Schreiber et al., 2018; Le et al., 2019; Lin et al., 2019). The Ca^{2+} sensitivity represents a hallmark of Ano function: Cryo-EM structures of mouse Ano1 reveal two Ca^{2+} binding sites within the inner vestibule of the hour-glass shaped pore (Paulino et al., 2017). Ca^{2+} binding leads to conformational changes particularly in the α6 transmembrane domain rendering the pore conductive (Paulino et al., 2017). The Ca^{2+} sensitivity is dependent on membrane voltage and temperature and varies strongly between the different splice variants ranging from approximately 100 to 400 nM for Ano1 and from <1 μM up to 100 μM for Ano6 (Ferrera et al., 2009; Grubb et al., 2013; Ertongur-Fauth et al., 2014; Strege et al., 2015; Lin et al., 2019). In native cells, the organization of Ano1 in microdomains/signaling complexes together with caveolin-1 allows for very specific signaling downstream of Ca^{2+}, despite the various sources of this ion, like TRP channels, voltage-gated calcium channels (VGCCs), Inositol-1,4,5-trisphosphate (IP_3)-receptors on the endoplasmic reticulum, or store-operated calcium entry (Jin et al., 2013, 2016). In nociceptors, Ano1 is specifically activated by local Ca^{2+} signals mediated by TRPV1 and inflammatory mediators like serotonin or bradykinin via G-protein coupled receptors (GPCRs) activating the IP_3-receptor cascade (Liu et al., 2010; Jin et al., 2013; Salzer et al., 2016) (see **Figure 2**). This activation of Ano1 has not only been demonstrated at the soma by electrophysiological recordings, but was also shown

in behavioral tests, confirming its functional importance at the peripheral nerve ending (Liu et al., 2010). Ano1 channels localize to microdomains in the membrane and interact with the GPCR and IP_3R (Jin et al., 2013). This compartmentalization is lipid raft-dependent and might (1) allow for a sufficient local Ca^{2+} concentration for channel activation and (2) shield Ano1 from global Ca^{2+} waves (Jin et al., 2013, 2016). This may impede the direct translation of results obtained from expression systems to native cells and vice versa. Furthermore, the loop between transmembrane domain 2 and 3, which is involved in forming the bradykinin/IP_3-receptor signaling unit, contains two differentially-spliced segments (Ferrera et al., 2009; Jin et al., 2013). In nociceptors neither the expression pattern of the splice variants nor the potential regulation of splicing in (chronic) pain states have been analyzed, but might affect the interaction of the anoctamins with other proteins as well as their Ca^{2+}-sensitivity and thus bear an additional mode of channel regulation. Anoctamin channel activity also responds to changes in the membrane environment: upon heterologous expression, inhibition of phospholipase A_2 suppresses human Ano1- and Ano6-mediated currents (Schreiber et al., 2018). Furthermore, binding of the phospholipid phosphatidylinositol-4,5-bisphosphate (PIP_2) is necessary for Ano1 channel activity under submaximal Ca^{2+} conditions, while it prevents fast channel desensitization under Ca^{2+} saturation (Le et al., 2019). Activation of Gq-PCR could thus activate Ano1 via Ca^{2+}-release from the endoplasmic reticulum by IP_3, but would counteract Ano1 activation due to PIP_2 depletion (Le et al., 2019). This is of particular interest in chronic pain states, because PIP_2/Ca^{2+}-signaling is altered under these conditions demonstrated by elevated IP_3-levels in chronic constriction injury of the sciatic nerve (Zhang et al., 2018).

This complex activation points to two roles of Ano channels in nociceptors: Firstly, they serve as primary sensory channels to detect noxious heat (Cho et al., 2012), and secondly, they are involved in the amplification of intracellular signals affecting membrane voltage (Jin et al., 2013). mRNA of all three anoctamins is expressed in mouse and human trigeminal and dorsal root ganglia (Boudes et al., 2009; Flegel et al., 2015). Ano1 is found primarily in small-diameter nociceptors and upregulated in formalin and neuropathic pain models and associated with increased excitability of sensory neurons (Yang et al., 2008; Liu et al., 2010; Cho et al., 2012; Jin et al., 2013; García et al., 2014; Deba and Bessac, 2015; Pineda-Farias et al., 2015; Takayama et al., 2015; Zhang et al., 2018; Chen et al., 2019). Depletion of Ano1 increases the response latency of mice to noxious heat stimuli and this stresses the importance of Ano1 as a physiological heat sensor (Cho et al., 2012). However, Ano1 does not only complement the role of TRPV1 due to their redundant function as a primary temperature sensor, but also associates with TRPV1 channels and thus amplifies TRPV1-mediated currents in expression systems and DRG neurons (Takayama et al., 2015) (see **Figure 2**). This coupling between TRPV1 and Ano1 also seems to be relevant *in vivo*: Activation of Ano1 induces nocifensive behavior in mice, although this effect could be largely due to direct TRPV1 activation (Deba and Bessac, 2015; Liu et al., 2016). Pharmacological inhibition or conditional knockout of Ano1 alleviates thermal and mechanical hyperalgesia both in neuropathic and inflammatory pain models (Lee et al., 2014; Pineda-Farias et al., 2015; Chen et al., 2019).

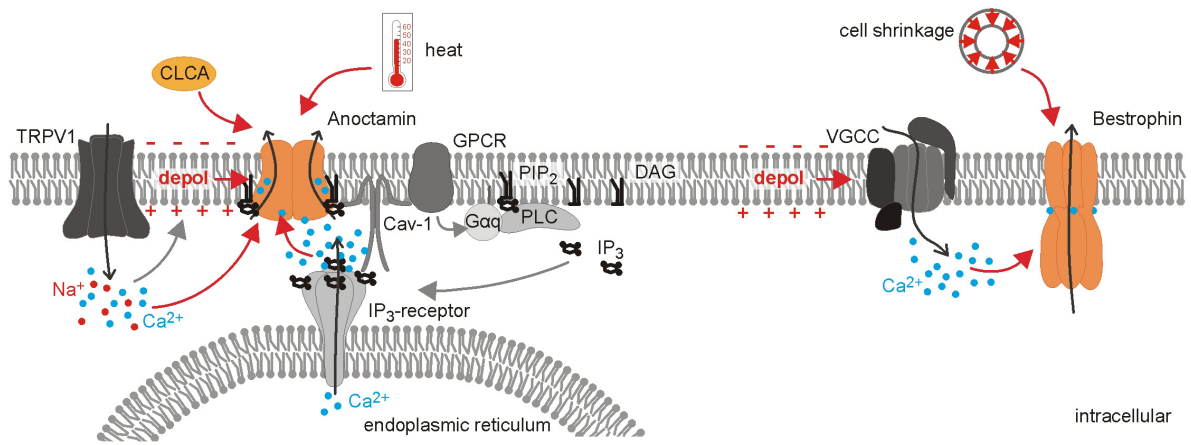

FIGURE 2 | Differential activation of Ca^{2+}-gated Cl^- channels in nociceptors. Overview of the distinct regulation of the Ca^{2+}-gated Cl^- channels anoctamin and bestrophin. **(A)** Ano1 is found in a signaling complex together with GPCR, Caveolin-1 (Cav-1) and the Inositol-1,4,5 trisphosphate (IP_3) receptor in the membrane of the endoplasmic reticulum. This proximity of signaling molecules allows for a localized Ca^{2+} rise which is sufficient to activate the Cl^- channel Ano1, while the Ca^{2+} increase mediated by VGCCs does not affect anoctamin currents. Binding of phosphatidylinositol -4,5-bisphosphate (PIP_2) to anoctamin is necessary for channel opening under low Ca^{2+} concentration and prevents channel desensitization when Ca^{2+} concentration is high. Ano1 is further activated by heat and membrane depolarization. Direct interaction of TRPV1 and Ano1 leads to subsequent activation of anoctamin mediated by the TRPV1-induced depolarization and Ca^{2+} influx. Ano1 and TRPV1 act as peripheral heat sensors, but whether the full signaling complex exists at the peripheral nerve terminals has not been unequivocally shown so far. Additionally, the soluble N-terminus of chloride channel accessory (CLCA) increases surface availability of anoctamin. **(B)** In contrast to anoctamin, bestrophins can be activated by the Ca^{2+} influx through VGCCs and cell shrinkage.

Bestrophins

Four members of the Bestrophin family, Best1-4, have been identified in humans and three paralogs are found in mice (Hartzell et al., 2008). The X-ray structure of the chicken and bacterial bestrophin paralog has revealed that the subunits assemble as homo- or heteropentamers forming a central pore, which can conduct anions as large as glutamate and GABA (Sun et al., 2002; O'Driscoll et al., 2008, 2009; Park et al., 2013; Bharill et al., 2014; Kane Dickson et al., 2014; Yang et al., 2014). Both cell volume as well as intracellular Ca^{2+} regulate the largely voltage-independent bestrophin currents: cell shrinkage by hyperosmotic solution inhibits hBest1 and mBest2 by 50–70%, while cell swelling causes a smaller and less robust current increase (Fischmeister and Hartzell, 2005). The channel displays a high Ca^{2+} sensitivity (EC_{50} ∼150–200 nM), which renders it partially conductive under resting Ca^{2+} conditions, and can be activated by VGCCs (Hartzell et al., 2008; O'Driscoll et al., 2008; Boudes et al., 2009; Lee et al., 2010). As for anoctamins, the Ca^{2+} sensitivity of bestrophins is attributable to direct interaction of the divalent cation with an acidic amino acid cluster below the membrane-cytosol interface found in each subunit (Kane Dickson et al., 2014; Paulino et al., 2017). This Ca^{2+}-clasp is formed by the proximal C-terminus and the N-terminus of the adjacent subunit (Kane Dickson et al., 2014). Interestingly, a splice variant of Best1 lacking the conserved N-terminal domain, including the first transmembrane domain, still produces a Cl^- conductance that is activated by Ca^{2+} when heterologously expressed (Kuo et al., 2014). Just as for Best1, several splice variants are also known for Best3 (Golubinskaya et al., 2019). The role of bestrophins in nociception has not been fully established yet, but both Best1 and Best3 mRNA are detected in mouse DRGs and Best1 mRNA and protein expression is shown in rat DRGs (Al-Jumaily et al., 2007; Boudes et al., 2009; Pineda-Farias et al., 2015). Expression of bestrophin and the corresponding conductance seems to be limited to medium-sized DRG neurons (André et al., 2003). Upregulation of Best1 in mice after sciatic nerve transection is compensated for in Best1 knockout animals by upregulation of Best3 (Boudes et al., 2009). Although an increase in Best1 expression is not observed after spinal nerve ligation in rats, intrathecal injection of Best1 antibody reduces Best1 protein in DRG and spinal cord and attenuates spinal nerve ligation-induced tactile allodynia, indicating a contribution of bestrophin to neuropathic pain (Pineda-Farias et al., 2015). In addition to the regulation of bestrophins themselves, these channels may be affected by altered Ca^{2+} signaling in nociceptors, for example as a consequence of VGCC upregulation in inflammation (Bourinet and Zamponi, 2005).

Tweety-Homolog

Three genes, Ttyh1-3, form the Ca^{2+}-activated Tweety-homolog Cl^- channel family and strong mRNA expression of all three members is detected in mouse and human trigeminal ganglia and DRG (Flegel et al., 2015). Each member is sufficient to produce a swelling-induced volume-regulated Cl^- conductance ($VRAC_{Cl,swell}$) with very similar properties to native $VRAC_{Cl,swell}$ which is predominantly found in astrocytes (Han et al., 2019). However, Ttyh2 and Ttyh3 have originally been introduced as Ca^{2+}-sensitive channels with a large single-channel conductance, while only the C-terminally spliced Ttyh1 is activated in a Ca^{2+}-independent fashion by hypertonic solution (Suzuki and Mizuno, 2004). The outwardly rectifying native $VRAC_{Cl,swell}$ current with significant glutamate permeability is independent of Ca^{2+}, but sensitive to inhibitors for tyrosine kinase and mitogen-activated protein kinase (Han et al., 2019). Therefore, the assignment of tweety homologs to Ca^{2+}-activated, volume-activated or even Maxi-chloride conductances is inconsistent and needs further investigation. Of note, Ttyh1 is downregulated in DRGs after sciatic nerve transection (Al-Jumaily et al., 2007; Boudes et al., 2009; Flegel et al., 2015). However, since RNA-sequencing of mouse cerebral cortex revealed stronger expression of Ttyh1 and 3 in astrocytes compared to neurons, while expression of Ttyh2 in neurons is very low but rather appears in oligodendrocytes, it can be assumed that the high expression in DRGs is attributable to a function in non-neuronal cells rather than a relevant anion conductance in nociceptors (Zhang et al., 2014).

Chloride Channel Accessory (ClCA)

Chloride channel accessory has previously been described as another distinct family of Ca^{2+}-activated Cl^- channels with four orthologs identified in humans and eight orthologs in mice, of which one might be a pseudogene (Gandhi et al., 1998; Gruber et al., 1998, 1999; Evans et al., 2004; Loewen and Forsyth, 2005; Patel et al., 2009). Yet, based on bioinformatic analysis, further studies revealed that ClCA genes encode soluble proteins with or without a transmembrane domain or GPI anchor that do not yield a Cl^- conductance *per se*, but rather modulate other Cl^- channels (Gibson et al., 2005; Mundhenk et al., 2006; Patel et al., 2009). Secreted ClCA1 enhances Ano1 surface expression which in turn leads to increased current density (Sala-Rabanal et al., 2015) (see **Figure 2**). mRNA transcripts of ClCA1, 2, 3, and 5 are expressed in mouse DRGs, and ClCA5 is downregulated after axotomy (Al-Jumaily et al., 2007; Imhof et al., 2011). In contrast, a 1724-fold increase of ClCA3 mRNA transcripts is found after induction of inflammatory pain in an antigen-induced model for arthritis, but a role for this protein could so far not be corroborated in a follow-up study, where ClCA3 knockout mice show a minor reduction in joint swelling but no pain phenotype (Ebbinghaus et al., 2014). Nonetheless, CICAs may be functional regulators of nociception due to their paracrine modulation of other Cl^- channels in neighboring neurons in the DRG and thus deserve to be further addressed.

CLC Family of Chloride Channels and Transporters

Jentsch et al. (1990) cloned the first voltage-gated Cl^- channel from *Torpedo,* CLC-0, as the founding member of the CLC family and only one year later they identified and characterized the first mammalian homolog from rat skeletal muscle (Steinmeyer et al., 1991). To date, the mammalian CLC family comprises nine members that can be subdivided into plasma membrane Cl^- channels (CLC-1, CLC-2, CLC-Ka, and CLC-Kb) and secondary active $2Cl^-/H^+$ exchangers (CLC-3, CLC-4, CLC-5, CLC-6, and CLC-7). They are generally located within intracellular

(lysosomal and endosomal) membranes. CLC family members have 18 α-helices located within the membrane and share homodimeric co-assembly into functional channels/transporters, with one ion conduction path per subunit (Jentsch and Pusch, 2018). Some members (CLC-2, CLC-3, CLC-5, and CLC-7) are widely expressed whereas others exhibit strict tissue-specific expression (Jentsch et al., 2005; Jentsch and Pusch, 2018). Lumbar DRGs of mice express mRNA transcripts encoding CLC-3, CLC-4, CLC-5, and CLC-6 (Qi et al., 2018) and protein expression in DRGs is demonstrated for CLC-3 and CLC-6 (Poet et al., 2006; Pang et al., 2016). Only few studies address the importance of CLC proteins in the peripheral nervous system, but these demonstrate that CLC proteins are utterly important for determining excitability of DRG neurons under (patho)physiological conditions.

CLC-3 and CLC-5

CLC-3 is expressed in both isolectin-B4-binding (IB4) non-peptidergic, and peptidergic nociceptors (Bali et al., 2013; Pang et al., 2016). Although resting membrane potentials and voltage-dependent currents are unaltered after knockout or knockdown of CLC-3 and CLC-5, ablation of CLC-3 increases the excitability of DRG neurons as indicated by decreased AP thresholds and decreased rheobase (Pang et al., 2016; Qi et al., 2018). This is in line with the hypersensitivity to mechanical sensory stimulation in neuropathic and tumor pain models in rodents with a knockdown or genetic knockout of CLC-3 (Bali et al., 2013; Pang et al., 2016). Furthermore, CLC-3 and CLC-5 are involved in the downstream signaling of the biologically active sphingolipid S1P (Pang et al., 2016; Qi et al., 2018). S1P is released by platelets after surgery, trauma or blood vessel damage and induces nocifensive behavior in animal models *in vivo* and signatures of nociceptor activation in humans (Zhang et al., 2006; Mair et al., 2011; Camprubi-Robles et al., 2013; Li C. et al., 2015). As the cellular mechanisms underlying these S1P effects, we found that S1P directly depolarizes nociceptors through an excitatory inward current, which is significantly attenuated by siRNA-mediated CLC-3 or CLC-5 knockdown (Camprubi-Robles et al., 2013; Qi et al., 2018). The acute excitation of nociceptive neurons and the activation of CLC transporters involve S1P receptor type 3 ($S1PR_3$)-dependent activation of the Rho GTPase signaling cascade, however, not Rho-associated protein kinase (ROCK) (Camprubi-Robles et al., 2013; Quarta et al., 2017; Qi et al., 2018; Kalpachidou et al., 2019). Importantly, these studies not only establish a role of CLC-3 and CLC-5 transporters in nociception, but whole-cell patch-clamp recordings also provide evidence that they affect conductances in the plasma membrane of neurons (Camprubi-Robles et al., 2013; Pang et al., 2016; Qi et al., 2018). Currently there is no data available whether CLC-3 and CLC-5 interact with accessory proteins and partially locate to the plasma membrane as found for certain splice variants in heterologous expression systems, or induce another Cl^- conductance (Kawasaki et al., 1995; Huang et al., 2001; Okada et al., 2014; Guzman et al., 2015; Jentsch and Pusch, 2018). After spared nerve injury, CLC-3 mRNA and protein levels surprisingly decrease in lumbar DRGs (Pang et al., 2016). In contrast to its acute excitatory action, the persisting suppression of CLC-3 expression correlates well with mechanical hypersensitivity which is rescued through intrathecal delivery of CLC-3 adenoviral vector (Pang et al., 2016). CLC-3 mRNA expression is also significantly reduced in DRGs in a cancer pain model, and siRNA knockdown of CLC-3 in DRGs further increases tumor-induced mechanical hyperalgesia (Bali et al., 2013).

CLC-6

CLC-6 is a $2Cl^-/H^+$ exchanger in the membrane of (late) endosomes almost exclusively expressed in the nervous system (Poet et al., 2006). Its abundance is exceptionally high in trigeminal ganglia, DRGs and spinal cord suggesting a relevance of the transporter in the somatosensory system (Poet et al., 2006). Mice with a global depletion of CLC-6 exhibit dramatically increased tail-flick latencies in response to painful stimuli indicating severe deficits in nociception (Poet et al., 2006). As CLC-6 deficient mice do not show significant loss of neurons and only moderate other behavioral deficits, the nociceptive deficits most likely appear to be related to a disruption of neuronal functions through intracellular lysosomal deposition (Poet et al., 2006). However, the pathophysiological mechanisms causing the prominent impairment of nocifensive behavior caused by genetic deletion of CLC-6 are currently not understood.

Cystic Fibrosis Transmembrane Conductance Regulator (CFTR)

Cystic fibrosis (CF) is a life-limiting autosomal recessive disorder caused by mutations in the gene encoding CFTR. CFTR is a member of the family of ATP binding cassette transporters, but is an anion channel that hydrolyzes ATP during the transport cycle of anions (e.g., Cl^-, HCO_3^-), i.e., CFTR is an ATP-gated anion channel allowing for Cl^- flux along the electrochemical gradient (reviewed in Hwang and Kirk, 2013). CF-associated mutations mostly reduce CFTR channel function, giving rise to a multiplicity of symptoms in several organ systems and severe respiratory disease that is the major cause of death of CF patients. Although the main CF disease strains are non-neuronal, relevance of CFTR in neurons has been proposed for years, as the channel is expressed in the central and peripheral nervous system of several species, including rodents, pigs and humans (Mulberg et al., 1994, 1995, 1998; Rogan et al., 2010; Kanno and Nishizaki, 2011; Reznikov et al., 2013; Marcorelles et al., 2014; Reznikov, 2017). CFTR may be exclusively expressed in neurons but not glial cells in humans (Guo et al., 2009; Niu et al., 2009; Marcorelles et al., 2014). Importantly, evidence for CFTR-mediated anionic currents is not available for most cell types. However, there are some indications for functional expression of CFTR in DRG neurons and for its relevance in the development of mechanical allodynia (Kanno and Nishizaki, 2011). Indirect evidence suggests that noradrenalin may stimulate ATP release through CFTR after β3-adrenergic receptor stimulation as a mechanism to promote neuropathic pain (Kanno et al., 2010; Kanno and Nishizaki, 2011). Following activation of AMPA receptors in cultured spinal cord microglia, ATP release is strongly attenuated by pharmacological inhibition and genetic knockout of CFTR, indicating that CFTR may contribute to ATP

release in spinal cord and probably also in DRGs (Liu et al., 2006; Kanno and Nishizaki, 2011). However, currently it is unknown whether these mechanisms are relevant for pain processing in nociceptors despite the undoubted significance of purinergic signaling in the entire pain pathway (e.g., Jahr and Jessell, 1983; reviewed in Burnstock, 2013). Functional, biophysical and pharmacological properties of CFTR in DRG and spinal cord neurons/glia remain elusive at present and pain phenotypes have not been conclusively reported in CF patients.

Volume-Regulated Anion Channels (VRAC or VSOAC)

VRAC, also referred to as volume-sensitive organic osmolyte anion channel (VSOAC), mediates fluxes of anions and organic osmolytes (e.g., amino acids and their derivatives or methylamines). The channels accordingly control regulatory volume decrease (RVD) to compensate for cell swelling, changes of tonicity or intracellular ionic strength (for a comprehensive review see Jentsch et al., 2016). LRRC8 family members are the molecular correlates of VRAC, and LRRC8A constitutes an essential subunit which co-assembles with one or more other LRRC8 member(s) to give rise to VRAC (Qiu et al., 2014; Voss et al., 2014). DRG neurons express mRNA transcripts encoding LRRC8A and a VRAC current can be induced both by hypotonic extracellular solution as well as by hypertonic pipette solution suggesting that LRRC8A contributes to VRAC in DRG neurons, albeit a role in nociception has not been shown unequivocally (Wang et al., 2017; Liu et al., 2019). Interestingly, Ca^{2+}-activated Cl^- channels might contribute to VRAC: Ano1 can also be activated by cell swelling and members of the tweety-homolog family (see Ca^{2+}-activated Cl^- channel section) constitute essential subunits for swelling-induced VRAC in cultured cortical astrocytes (Han et al., 2019; Liu et al., 2019). As mRNA transcripts encoding Ttyh1/2/3 are apparently abundant in lumbar DRGs, and as expression levels of Ttyh1 appear to be slightly regulated in axotomized DRG neurons, it is tempting to speculate that tweety proteins may contribute to VRAC currents in DRG neurons (L4/L5; Al-Jumaily et al., 2007; Boudes et al., 2009). However, functional expression of these proteins has not been explored in the somatosensory system yet.

Maxi-Anion Channels (Also Maxi-Cl^- Channels)

Maxi-anion channels (MACs) exhibit extraordinarily large unitary conductances (200–500 pS), and probably are ubiquitously expressed in virtually every cell type (reviewed in Sabirov et al., 2016). Apart from providing transfer routes for anions, MACs mediate release pathways for ATP and are associated with purinergic signaling (e.g., Bell et al., 2003; Sabirov and Okada, 2005). In resting cells, MACs are usually inactive, but can be activated by a multitude of stimuli including cell stress (osmotic, ionic strength, mechanical, heat, oxidation, etc.). Further, MAC activity is modulated in context of GPCR signaling through several ligands such as endothelin-1, adenosine, and bradykinin, and inhibited by PGE_2 (summarized in Sabirov et al., 2016, 2017). The prostaglandin transporter SLCO2A1 has been identified as the molecular correlate of MACs (Kanai et al., 1995; Sabirov et al., 2017). As SLCO2A1/MAC activity is modulated by a large number of pain-initiating and pro-inflammatory stimuli (e.g., cell damage, ligands of GPCRs, prostaglandins, etc.) and given its involvement in purinergic signaling, these channels may be of relevance in peripheral pain mechanisms. However, the abundance and function of SLCO2A1 in the peripheral nervous system has not been addressed yet.

CLINICAL POTENTIAL OF CHLORIDE CHANNELS/TRANSPORTERS FOR PAIN THERAPY

As nociceptors are the primary sensors for noxious stimuli and the site of peripheral sensitization before subsequent changes in the entire pain pathway manifest chronification, they remain an attractive target site for pain therapies. Multiple studies demonstrate that painful conditions lead to changes in various Cl^- channels, transporters and homeostasis in primary afferent nociceptors, while *vice versa* altered activity of Cl^- channels and transporters affects pain perception. This draws attention to nociceptor chloride channels and transporters as potential target for the development of analgesic drugs. Bumetanide, a loop diuretic which targets the kidney-specific NKCC2, also prevents the accumulation of phosphorylated NKCC1 in DRGs, as well as the concomitant downregulation of KCC2 in the spinal cord, and alleviates mechanical and thermal hypersensitivity in a model for neuropathic pain (Modol et al., 2014). Similarly, compensating the CCC changes by viral delivery of KCC2 into DRG and spinal neurons reverses the depolarizing shift of the reversal potential for GABA-mediated currents and abolishes hypersensitivity induced by nerve injury (Li et al., 2016). Although these studies indicate great potential for targeting Cl^- homeostasis in pain conditions, they have to be considered carefully since Cl^- homeostasis mechanisms and nociceptor function may not be fully identical in rodents and humans (Funk et al., 2008; Rocha-Gonzalez et al., 2008; Zhang et al., 2018).

Besides, Cl^- channels expressed in primary afferent nociceptors can be targeted for potential pain treatment. However, this is complicated first by the previously described variability of the Cl^- reversal potential, which can switch the effect of Cl^- channel opening from inhibitory to excitatory and thus profound control of Cl^- concentration is indispensable, and second by the involvement of Cl^- conductances in other physiological functions. Notably, Best1 and Ano1 play an important role in retina and secretory epithelia function, respectively (Hartzell et al., 2008; Oh and Jung, 2016). To overcome the problems of potential unwanted effects, splice variant- or subunit-specific drug development may offer promising perspectives: α1-sparing $GABA_A$ receptor agonists alleviate hyperalgesia induced by inflammation or chronic nerve constriction through pre- and postsynaptic action at nociceptive synapses in the SDH without a sedating effect (Knabl et al., 2008; Di Lio et al., 2011; Witschi et al., 2011). Likewise, α3 subunits of GlyRs moved into the focus of analgesic drug discovery, since they are targeted by cannabinoids and mediate analgesic properties. Although their primary action seems to be at the SDH, α3 GlyRs

are also expressed in DRG neurons, and these might contribute to the analgesic effect (Lynch et al., 2017). Thus, Cl^- channels on primary afferents can be targeted to achieve analgesia also in chronic pain conditions, but warrant further developments for pain relief in humans.

SYNOPSIS

Cl^- homeostasis is tightly regulated in nociceptive primary afferents and essential for the effect of activation of Cl^- channels on the excitability of these neurons. In contrast to the 'static' action of cation flux on cellular membrane potential and excitability, the Cl^- reversal potential in primary afferent neurons typically lies between the resting membrane potential and the AP threshold and is subject to fluctuation during persisting pain. This can convert the normally inhibitory action of Cl^- channel opening into an excitatory one contributing to pain, allodynia, and hyperalgesia. Not only Cl^- concentrations but also Cl^- channels and transporters expressed in DRG neurons are deregulated in pain disorders (see **Figure 1**). A distinct role and mode of activation in nociceptors is already established for some of them, like $GABA_A$ receptors or Ano1, while the importance of others such as tweety homologs or CICA has not been explored although they are detectable in DRGs. Altogether, Cl^- ions are emerging as relevant components of nociceptor function and should no longer be underrated as important players in acute pain processing and the pathogenesis of chronic pain disorders.

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All authors listed have made a substantial contribution to writing the manuscript, revised it and approved it for publication. BW designed the figures.

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