Treating pathological pain: is KCC2 the key to the gate?

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Nicolas Doyon[1,2], Francesco Ferrini[3], Martin Gagnon[1,2] and Yves De Koninck[1,2]

[1]Institut Universitaire de santé mentale de Québec, Québec, G1J 2G3, Canada
[2]Department of Psychiatry and Neuroscience, Université Laval, Québec, Québec, G13 7P4, Canada
[3]Department of Veterinary Sciences, University of Turin, 10095, Grugliasco, Turin, Italy

Despite intensive efforts, development of novel treatments of neuropathic pain has encountered mitigated success. While common and incapacitating, neuropathic pain remains poorly treated, with few patients experiencing greater than 50% symptom relief [(1)]. As an example, Pregabalin, which targets the $\alpha_2\delta$ subunit of the voltage-dependent calcium channel and which is the current gold standard for neuropathic pain, reduces the pain score significantly in only one out of four patients [(2)]. Targeting TrpV1 channel, which has attracted considerable resources, has only yielded the weak-selling capsaicin patch and has recently been denied indication expansion into HIV-related neuropathic pain by the FDA. In addition to weak efficacy issues, most approved treatments cause common and significant central side effects such as sedation and dizziness, diminishing the quality of life of affected patients. Opiate-based treatments, for example, are plagued with several side effects, beyond sedation, including psychomotor issues, abuse liability, tolerance and even paradoxical hyperalgesia [(3)]. Recent promising alternatives, such as anti-nerve growth factor (NGF) antibodies have demonstrated very strong analgesic efficacy, yet, these strategies were put on hold due to severe side effects; many patients developing joint damage [(4)].

The unmet needs of improved efficacy and better adverse event profile will require a different approach. One potential way of treating neuropathic pain is to restore proper inhibitory pathways in the central nervous system. Indeed, central disinhibition has long been known to play a major role in the pathophysiology of neuropathic pain states [(5)]. After nerve injury, there is a marked decrease in the inhibitory efficacy of $GABA_A$ and glycine receptor-mediated transmission which results in aberrant transmission of sensory and nociceptive information to the brain [(6)]. A first step to develop therapeutics that would restore proper inhibition is to understand the underlying mechanism of disinhibition. While several causes have been proposed [(6)], a key mechanism that has emerged involves the disruption of Cl^- homeostasis resulting from loss of activity of the K^+-Cl^- co-transporter KCC2, a molecule responsible for maintaining efficient Cl^- mediated inhibition in central neurons [(7)]. This mechanism appears implicated across several pathological pain syndromes with diverse aetiologies, including spinal cord

injury [8], inflammation [9], painful diabetic neuropathy [10], trigeminal pain [11] and more recently even morphine-induced paradoxical hyperalgesia [3].

Several components of the cellular signalling cascade underlying impaired Cl^- homeostasis in spinal nociceptive pathways in pathological conditions have been identified, uncovering several potential therapeutic targets [12,3]. Enhanced BDNF release either from microglia [12,3,13] or from sensory nerves [14], acting on TrkB receptors to cause downregulation of KCC2, appears as a common culprit in several conditions. In processes involving microglia, a key event is the *de novo* expression of the purinergic P2X4 receptors (P2X4R) in these cells at the spinal level [15,12,3,13]. Each step of the P2X4R-BDNF-TrkB-KCC2 pathway thus emerges as a potential druggable target for combating pathological pain symptoms. Indeed, the ablation of P2X4R prevents the development of neuropathic pain [13]. Importantly, ablating microglia, chelating BDNF or blocking P2X4R or TrkB receptors reverses established pain hypersensitivity, indicating that the identified pathway plays an active role in maintaining pathological pain [12,3]. Targeting signalling events upstream of KCC2 may thus serve to reverse the pathology, not only prevent its development. Yet, altering microglial function may be detrimental as they are involved in essential support and immune activities. Similarly, interfering with BDNF-TrkB signalling is likely to impact on a multitude of trophic and survival mechanisms in neurons. An obvious alternative approach to counter disinhibition is to increase $GABA_A$ receptor-mediated function. However, a fundamental limitation of drugs acting on $GABA_A$ receptors such as benzodiazepines or barbiturates may be found in the ionic mechanisms underlying their action.

Indeed, since KCC2 is responsible for Cl^- extrusion, disruption of its function causes a collapse of the transmembrane Cl^- gradient and a depolarizing shift in $GABA_A$ reversal potential (E_{GABA}). This in turn leads to a decrease in inhibitory efficacy [16]. The strategy of compensating the decrease in Cl^- driving force by an increase in Cl^- conductance to restore inhibitory current may be at first met by success. Yet, it inherently has its limitations because it may exacerbate the collapse of the Cl^- gradient. This appears to be the case whereby low dosage of midazolam is effectively anti-hyperalgesic in neuropathic pain models, while the efficiency of the drug is reduced at high doses [17]. The mechanistic explanation for this paradoxical effect may have an ionic basis. For hyperpolarization to result from Cl^- currents, it is mandatory that the Cl^- reversal potential (E_{Cl}) be more hyperpolarized than the membrane potential which is only possible at low intracellular Cl^- concentration ($[Cl^-]_i$). The value of $[Cl^-]_i$ results from a dynamical equilibrium between Cl^- influx through channels and its efflux through KCC2. It follows

that KCC2 hypofunction does not only lead to depolarization of the $GABA_A$ reversal potential (E_{GABA}), it also renders E_{GABA} more labile [18]. For example, large Cl^- loads exacerbate E_{GABA} depolarization [19] and repeated or sustained activity can cause dynamic collapse of inhibition, especially in dendrites [20,18]. Thus, increased $GABA_A$ receptor-mediated activity will have two opposing effects in cells with depleted KCC2 activity: first, an increase in Cl^- mediated inhibition, followed by an activity dependant collapse of Cl^- transmembrane gradient. This negative feedback implies that increasing $GABA_A$ activity will rapidly reach a limit.

This phenomenon in itself is not sufficient however to explain the decrease in drug efficacy occurring at high dose. The latter paradoxical effect can be explained by the depolarizing flow of HCO_3^- to which $GABA_A$ and glycine channels are also permeable [21]. In contrast to E_{Cl}, E_{HCO3} is maintained relatively stable by pH buffering mechanisms and rapid diffusion of CO_2 across the membrane allowing efficient $[HCO_3^-]_i$ replenishment by conversion of H_2O and CO_2 via a carbonic anhydrase (CA) mediated reaction [21]. Accordingly, upon collapse of Cl^- currents due to a drug-induced heightened $GABA_A$ function, the opposing HCO_3^- current becomes dominant, effectively counteracting the pro-inhibitory action of the drug.

One strategy to mitigate the negative effects of HCO_3^- currents through $GABA_A$ channels is to block the CA activity, for example using acetazolamide, enabling activity-dependant depletion of $[HCO_3^-]_i$. The decrease in HCO_3^- current will parallel the collapse of the Cl^- current [3] extending the efficacy of pro-$GABA_A$ drugs [17]. However, this strategy also has its limits because it does not prevent the dynamic collapse in Cl^- gradient, meaning that a certain amount of hyperexcitability will remain uncompensatable. Furthermore, impaired Cl^- extrusion can cause a positive feedback loop between excitation and Cl^- accumulation in which case sustained inputs can lead to catastrophic failure of inhibition [18]. In addition, potential problems associated with impaired intracellular pH regulation in absence of normal CA activity may limit the usefulness of targeting HCO_3^- currents.

For the reasons exposed above, a promising analgesic strategy is to enhance KCC2 activity not only to restore E_{GABA} to its normal value, but also to prevent activity dependant Cl^- accumulation and its associated side effects. Furthermore, since KCC2 already operates near its equilibrium point [22], potential excess activity caused by treatment aiming to restore its normal function is unlikely to have adverse effects on neuron dynamics [18]. How can KCC2 function be restored? The most

straightforward strategy is to directly target KCC2 function, expression and/or turnover. Thus, enhancing KCC2 function can be achieved by promoting its synthesis and/or reducing its degradation, but also potentially by modulating transporter function. For example, it has been suggested that the quaternary structure of KCC2 is an important determinant of its function. Indeed, KCC2 oligomerization has been suggested as a rapid mechanism by which KCC2 activity is modulated during development [23], the oligomeric form being the active one. Consistent with these findings, BDNF-dependent pain hypersensitivity appears to be associated with an increase in the monomer/oligomer ratio [3]. Compounds stabilizing the oligomeric state may thus be effective KCC2 enhancers. Proof of principle that positive modulation of KCC2 function can have therapeutic benefits has recently been provided for analgesia [24] and treatment of motor spasticity [25].

Because KCC2 expression is restricted to central neurons [26], targeting this transporter may reduce the risk of unwanted effects in peripheral nervous system or in other tissues. And because disrupted KCC2 activity appears to be involved in several neurological and psychiatric disorders including epilepsy, motor spasticity, stress and schizophrenia [27,28,20,29,30], KCC2 enhancing drugs may have broad therapeutic potential.

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