Role of Cav3.2 Low-Threshold Calcium Channel in Mechanosensitivity and Neuropathic Pain*

Les rôles des canaux calciques Cav3.2 à faible seuil d'activation dans la sensibilité mécanique et la douleur neuropathique

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Résumé Les fibres nerveuses peuvent être divisées en trois catégories, jouant chacune leur rôle dans différents types de sensations. Ces fibres ainsi que les récepteurs qu'elles expriment jouent un rôle crucial dans les pathologies liées aux perceptions thermiques, chimiques, nociceptives et mécaniques. Dans cette étude, les auteurs ont porté leur intérêt sur les fibres nerveuses Aδ et C, connues pour leurs rôles dans la mécanocception, et une sous-famille de ces fibres plus particulièrement, les fibres exprimant des récepteurs mécaniques à faible seuil d'activation (*low-threshold mechanoreceptor* or LTMRs). Sachant que les canaux calciques Cav 3.2, de type T, sont connus pour leur rôle dans la transmission d'informations sensorielles, leur prévalence ainsi que leur activité dans les LTMRs ont été évaluées via l'utilisation de souris génétiquement modifiées. Cette étude montre que

les canaux Cav3.2 sont impliqués dans la transmission d'informations mécanique et thermique au froid, et donc potentiellement dans les douleurs neuropathiques. En outre, les canaux Cav3.2 ont été détectés le long des axones des fibres LTMRs, suggérant un rôle important de ces canaux dans la vitesse de conduction.

Mots clés Douleur · LTMR · Cav3.2 · Allodynie · Nociception · Mécanocception

Introduction

Neuropathic pain is defined as pain caused by damage or disease affecting the somatosensory nervous system [1] and can be a challenging issue for clinicians and their patients. With a global prevalence of 8%, which increases up to 40% in HIV patients, neuropathic pain is a hot topic in the field of pain. A wide range of treatment is available to help decrease the discomfort of patients, from medication to active alternative strategies but they are ineffective in numerous patients. A biopsychosocial approach to respond to neuropathic pain is now accepted as most pertinent, but spontaneous pain and allodynia are still widely present [2,3]. Nerve fibres are key players in nociception and are divided into three subtypes that contribute to the conduction of sensory information (Fig. 1A). Aδ and C fibres are involved in noxious and mechanical stimuli detection and transmission. Other major players in the development of pathological pain are the low-threshold activated mechanoreceptors (LTMRs), which convert tactile signaling into the sensation of pain. These receptors are found in both A? and C fibres, and a subpopulation of neurons found in the dorsal root ganglia (DRG) has been found to be involved in hypersensitivity to light touch in chronic pain. These neurons predominantly express low-voltage-activated calcium channels Cav3.2, which participate in the modification of the cells' excitation [4]. Despite these findings, the anatomical and functional roles of Cav3.2 have yet to be addressed. In their recent

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publication Amaury et al. describe the role of Cav3.2 channels via genetically modified mice in allodynic symptoms of neuropathic pain.

Action potential propagation and mechanosensitivity in C-LTMRs

To understand Cav3.2 channels' function within sensory neuron subtypes, the authors created a genetically engineered mouse (knock In or KI) with a fusion of green fluorescent protein (GFP) allowing a visual detection of Cav3.2 channels (Fig. 1B). The results showed Cav3.2 channels are expressed in LTMR fibres, more precisely in A δ fibres innervating hair follicles and in C fibres in DRG. They are found

from the periphery and all along the axon, with a stronger density in parts of the axon specifically involved in cell excitability. Then, to investigate the role of Cav3.2 channels in mechanosensation and AP propagation, an in vitro skin nerve preparation was used to study the electrophysiological properties of C-LTMRs. Light mechanical stimulation was applied, and the channels responded at very low application of stimulation (below 10 mN), allowing Cav3.2 channels to be identified as low-threshold receptors.

In the next experiment, authors used TTA-A2, a selective T-type calcium channel antagonist to block the Cav3.2 locally, in fibre receptive fields in the hairy skin. The results revealed an elevation of the activation threshold of C-LTMRs confirming that Cav3.2 controls the mechanosensitivity threshold in these receptors. Additionally, a reduction of AP firing frequency was observed in fibres with inactivated Cav3.2. Furthermore, the authors used TTA-2 to block the entire skin-nerve preparation to target the axonal Cav3.2 and investigate their role in AP conduction velocity (CV). A significantly slowed CV was observed, suggesting the necessity of functional Cav3.2 channels in the axons.

Fig. 1 A. A δ and C fibres are known to have a role in both nociception and mechanoreception. Low-threshold mechanoreceptor fibres (LTMRs) are a subfamily of these fibres. B. Insertion of green fluorescent protein (GFP) was made in mice to investigate the localisation of Cav3.2 channel. Moreover, deletion of this channel was assessed to study its pathological role in perception of noxious and innocuous pain. C. Behavioral tests like cold plate and dynamic Von Frey were made to analyse both thermosensation and nociception

Pathological pain and noxious perception

Since the previous experiment showed that Cav3.2 controls the mechanosensitivity threshold, the authors decided to assess the consequence of the absence of the channels in different pain models by using genetically modified mice. They induced suppression of Cav3.2 specifically in small-diameter DRG neurons found to be involved in hypersensitivity to light touch in chronic pain (mice furtherly named SNS KO). Using von Frey filaments, mice reactions were tested to innocuous, intermediate, and noxious stimuli, as a control. The SNS KO mice responded less strongly to the intermediate stimulus, suggesting a reduced sensitivity to light touch.

They were then subjected to two tests: the dynamic von Frey test and the Randall–Selitto test, designed to assess the capacity of the animal to respond to a mechanical noxious stimulus. SNS KO mice showed a reduced response to noxious stimulation (Fig. 1C).

Then, the authors assessed the thermoception of the animals (hot and cold perception) by exposing them to different tests, including a dynamic cold-plate test. This test allows studying both analgesia and hyperalgesia on freely moving mice at once. Escaping behaviour (number of jumps) was assessed, and SNS KO mice had a lower tendency to try to escape than wild types [5]; whereas, the other tests confirmed this propensity to decreased cold sensitivity in SNS KO mice.

To evaluate the role of Cav3.2 channels in pathological (continuous) pain, a formalin test was made, allowing the

observation of pain in both the short and long term. In these two conditions, SNS KO mice had significantly reduced responses to pain, pointing out the crucial role of Cav3.2 in both acute and inflammatory pain.

To mimic mechanical allodynia a spared nerve injury (SNI) was completed, in which the sciatic nerve is partially ligated, leaving the mouse with hypersensitivity in one of the paws [6]; this model is used to simulate neuropathic pain [7] and in this case, the authors measured the times where an innocuous stimulus leads to a painful response. The control SNI mice had a reduced threshold to initiate a painful response, which was expected due to the injury. However, SNS KO mice which underwent SNI had a less significant reduction of this threshold.

Therefore, these results point out that Cav3.2 channels play a critical role in the perception of cold and noxious stimulation in health and disease settings.

Conclusion

The study reported that the role of Cav3.2 channels in LTMRs fibres is essential to detect, conduct, and transfer tactile and noxious information. LTMR fibres are central to many chronic pain syndromes and are associated with allodynia, hypersensitivity, and spontaneous pain, all of them still very present in current treatment. This study suggests that Cav3.2 channel inhibitors could represent an interesting strategy for neuropathic pain treatment, but the proof of concept is still to be done.

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References

1. Merskey H, Bogduk N (1994) Classification of chronic pain, 2nd edn, IASP Task Force on Taxonomy. IASP Press, Seattle
2. Murnion BP (2018) Neuropathic pain: current definition and review of drug treatment. *Aust Prescr* 41:60–3
3. Finnerup NB, Attal N (2015) Pharmacotherapy for neuropathic pain in adults: a systematic review and meta-analysis. *Lancet Neurol* 14:162–73
4. Perez-Reyes E (2003) Molecular physiology of low-voltage-activated t-type calcium channels. *Physiol Rev* 83:117–61
5. Yalcin I, Charlet A, Freund-Mercier MJ, et al (2009) Differentiating thermal allodynia and hyperalgesia using dynamic hot and cold plate in rodents. *J Pain* 7:767–73
6. Richner M, Bjerrum OJ, Nykjaer A, et al (2011) The spared nerve injury (SNI) model of induced mechanical allodynia in mice. *J Vis Exp* 18:pii3092
7. Shields DS, Eckert AW 3rd, Basbaum AI (2003) Spared nerve injury model of neuropathic pain in the mouse: a behavioral and anatomic analysis. *J Pain* 4:465–70