Peptides as invaluable tools in the search for innovative analgesics

Rosario González-Muñiz

Instituto de Química Médica (IQM-CSIC). Juan de la Cierva 3, 28006 Madrid, Spain. E-mail: rosario.gonzalezmuniz@iqm.csic.es

In recent years, peptides are undergoing a renaissance in pharmaceutical research and development for diverse medical applications, due on one hand to their high selectivity, efficacy and relative safety, and on the other owing to great improvements in specific delivery systems [1]. Within the still not well resolved medical needs we can cite PAIN, which involves extremely complex/interrelated series of signaling and modulatory pathways of the nervous system. Hence, *a priori* all mediators involved in pain signaling are potential targets for therapeutic intervention and pain management [2]. While most research in this field during the last 30 years was focused on opioid peptide receptors, most recently the attention is being centered on other targets involved in pain sensation and transmission, especially on the contribution of different ion channels. In fact, one out of the four analgesic agents approved in the new century is a peptide, Ziconotide (Prialt®), acting on a subtype of voltage gated Ca^{2+} channels [3].

Ziconotide is the synthetic equivalent of the naturally occurring ω-conotoxin MVIIA (ω-MVIIA), isolated from the marine snail, *Conus magus*. It is a 25 amino acid, basic polypeptide containing three disulfide bridges (Figure 1), which blocks N-type calcium channels, inhibiting excitatory neurotransmitter release from primary afferent nerve terminals leading to antinociception [4]. Ziconotide was commercialized in 2004 as an intrathecal therapy for the symptomatic management of severe chronic pain, especially in patients refractory to morphine treatment. Therefore, the use of Prialt® is not general and needs an appropriate microinfusion device for direct delivery into the spinal horn. Concerning secondary effects, Ziconotide has been associated with CNS-related adverse events, including psychiatric symptoms, cognitive impairment, and decreased awareness.

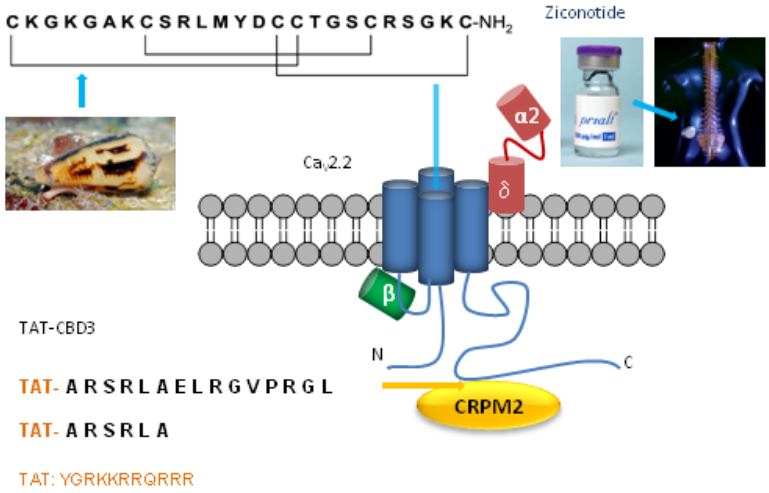

Figure 1. Analgesic peptides targeting N-type $Ca_V2.2$ channels

A few years ago, Khanna and coworkers described that inhibition of the $Ca_V2.2$ channel interaction with the collapsing response mediator protein 2 (CRMP2) is a promising approach to manage pain. It is expected that targeting the regulators of $Ca_V2.2$ channel trafficking could result in safer analgesics than those directly block the channel pore, which are limited by side effects due to inhibition of the physiological function of these channels in the CNS. Thus, a cell penetrating CRMP2-derived peptide, TAT-CBD3 (Figure 1), displayed antinociceptive responses in different animal models, including the formalin behavior test, mechanical hypersensitivity, and painful neuropathy coupled to AIDS therapy, while as expected it did not cause significant neurobehavioral deficits [5,6]. Recently, the study of truncated analogues and homology-guided modifications of TAT-CBD3 peptide underscore that the activity of CBD3 peptide relies on the *N*-terminal six residues (Figure 1), and that Ala1 and Arg4 residues are crucial for binding to CaV2.2 channels [7]. In addition, mutation of Leu5 to Met led to a potent peptide in a rat model of HIV-induced sensory neuropathy. This antinociceptive scaffold could be the starting point towards the future design and development of small-molecule CBD3 mimetics.

Transient Receptor Potential (TRP) channels are important players under physiopathological conditions, with subtypes responsible of temperature sensation (thermoTRPs) implicated in inflammatory, acute and chronic pain states. Direct blockade of TRPV1 by peptides, toxins and small-molecules led to pain relief, but normally also results in hyperthermia and decreased sensitivity to painful stimulus. Therefore, in route to new analgesic agents, the modulation of TRPV1

through the inhibition of specific PPIs was also explored. Thus, the group of McNaugthon published that hindering the interaction of TRPV1 and A kinase anchoring protein 79 (AKAP79) through peptides derived from key residues of the PPI interface led to analgesic activity [8,9]. On one hand, peptides from the TRPV1 structure, like the 736-745 (KDDYRWCFRV) fragment (Figure 2), conjugated to TAT to promote uptake across cell membranes, displayed antinociceptive properties in three mice models of inflammatory hyperalgesia, without interfering with the normal gating of the channel [8]. Reciprocally, some peptides extracted from the AKAP79 structure involved in the interaction with TRPV1, like TAT-326-336 fragment (Figure 2), showed *in vivo* analgesic activity in the same animal models, while the TAT scramble peptide was ineffective [9]. Therefore, antagonizing the TRPV1-AKAP79 interaction is a new strategy to treat inflammatory hyperalgesia.

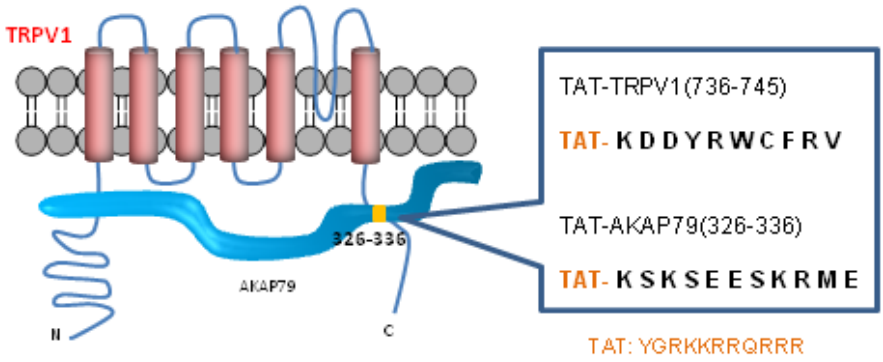

Figure 2. Analgesic peptides disrupting TRPV1-AKAP interaction

The group of X. Dong established that transient receptor potential channel subtypes TRPA1 and TRPV1 co-express in DRG neurons together with a regulatory protein, Tmem100, forming ternary complexes [10]. Tmem100 is able to bind simultaneously to both TRPA1 and TRPV1, interacting through different positions. In the ternary complex Tmem100 selectively inhibits the TRPA1 activity. It was also found that mutation of the C-terminal charged KRR fragment by a neutral QQQ sequence (Tmem100-3Q) prevented the binding to TRPA1 but increased binding to TRPV1. More importantly, a 28-mer peptide (T100-mut, Figure 3), containing the *C*-terminal sequence of Tmem100-3Q and myristoylated at *N*-terminal to increase cell permeability, was able to abolish the TRPA1-induced activity in the complex, while did not exhibit any effect on the capsaicin-induced TRPV1 activity. Accordingly, this permeable peptide alleviates TRPA1-mediated mechanical and inflammatory hyperalgesia in mice models, after mustard oil, paclitaxel or CFA injection. Further studies with truncated analogues of T100-mut, indicated that the *N*-terminal 18-mer sequence also inhibited the TRPA1-mediated activities *in vitro* and *in vivo*, and suggest a key role for the first four amino acid residues and the QQQ fragment. This work and the future prospect of this project could probably provide alternative promising pain therapies.

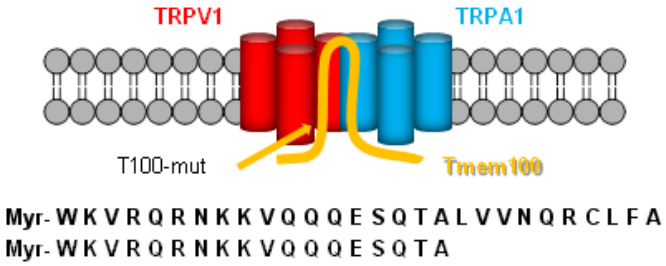

Figure 3. Analgesic peptides targeting TRPV1-TRPA1-Tmem100 ternary complex

Taking into account that the number of protein-protein interactions related to pain pathways is constantly growing, and to the extent that the intercommunication between receptors, channels and pain regulatory proteins (receptome) is better known at the molecular level, the search for new analgesic compounds, in general, and analgesic peptides, in particular, is an open field to the scientific community.

GLOSSARY OF KEY TERMS

Pain: unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage
Nociception: process by which noxious stimulation is communicated through the peripheral and central nervous system
Hyperalgesia: increased pain from a stimulus that usually provokes pain
Allodynia: pain due to a stimulus that does not usually provoke pain
Neuropathy: condition that occurs when peripheral nerves become damaged or disrupted

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