Cmarko and Weiss *Molecular Brain* (2020) 13:95
https://doi.org/10.1186/s13041-020-00636-y

Molecular Brain

MICRO REPORT Open Access

Selective inhibition of neuronal $Ca_v3.3$ T-type calcium channels by TAT-based channel peptide

Leos Cmarko[1,2] and Norbert Weiss[1,2*]

Abstract

Low-voltage-activated Ca_v3 calcium channels (T-type) play an essential role in the functioning of the nervous system where they support oscillatory activities that relie on several channel molecular determinants that shape their unique gating properties. In a previous study, we documented the important role of the carboxy proximal region in the functioning of $Ca_v3.3$ channels. Here, we explore the ability of a TAT-based cell penetrating peptide containing this carboxy proximal region (TAT-C3P) to modulate the activity of Ca_v3 channels. We show that chronic application of TAT-C3P on tsA-201 cells expressing Ca_v3 channels selectively inhibits $Ca_v3.3$ channels without affecting $Ca_v3.1$ and $Ca_v3.2$ channels. Therefore, the TAT-C3P peptide described in this study represents a new tool to address the specific physiological role of $Ca_v3.3$ channels, and to potentially enhance our understanding of $Ca_v3.3$ in disease.

Keywords: Calcium channel, T-type channel, $Ca_v3.3$ channel, TAT-peptide, Inhibitor

Main text

Low-voltage-activated Ca_v3 channels that generate T-type currents display unique biophysical properties that allow them to operate near the resting membrane potential of nerve cells where they generate low-threshold calcium spikes leading to burst firing of action potentials and oscillatory discharges. They play an essential role in shaping the electrophysiological properties of thalamic, olivary, and cerebellar neurons, and alteration of Ca_v3 channel activity is associated with a number of human neuronal disorders [1, 2]. However, the identification of specific physiological roles associated with each Ca_v3 isoforms ($Ca_v3.1$, $Ca_v3.2$, and $Ca_v3.3$) is often hampered for several reasons. First, Ca_v3 channels are often coexpressed in nerve cells. Second, they present a similar electrophysiological signature which renders their molecular identification problematic in native neuronal systems. And third, selective pharmacological tools are not available. Therefore, there is a need for isoform-specific modulators of Ca_v3 channels in order to better explore their respective physiological functions.

Structure-function studies have identified several channel molecular determinants that are responsible for shaping the unique gating properties of Ca_v3 channels [3–8]. Recently, we reported the importance of the carboxy terminal domain and showed that the proximal region that is highly conserved across the three Ca_v3 channel isoforms is essential for the functioning of $Ca_v3.3$ channels [9]. The question then arises as to whether an exogenous peptide corresponding to this proximal carboxy terminal region could potentially modulate the expression of Ca_v3 channels.

To address this issue, we tested the effect of a TAT-based cell penetrating peptide containing the conserved carboxy proximal region of $Ca_v3.2$ (TAT-C3P) on

* Correspondence: weiss@uochb.cas.cz
[1]Institute of Biology and Medical Genetics, First faculty of Medicine, Charles University, Prague, Czech Republic
[2]Institute of Organic Chemistry and Biochemistry, Czech Academy of Sciences, Prague, Czech Republic

recombinant Ca_v3 channels expressed in tsA-201 cells. Molecular modeling using Phyres[2] [10] predicted that this peptide may adopt a helical conformation (Fig. 1a). Cells were transfected with 5 μg of cDNA encoding for $Ca_v3.1$, $Ca_v3.2$, or $Ca_v3.3$ channels. Twelve hours after transfection, cells were treated with 10 μg / mL of TAT-C3P peptide (GenScript), or with a control peptide containing a non-conserved distal region of the carboxy terminus of $Ca_v3.2$ (TAT-C3D). The effect of the TAT peptide on T-type currents was assessed 48 h later in the whole cell configuration of the patch clamp technique. We observed that treatment of cell with the TAT-C3P produced a potent decrease of the T-type current in cells expressing $Ca_v3.3$ channels (Fig. 1b). For instance, in response to a depolarizing pulse to − 30 mV, a 2.3-fold decrease ($p < 0.0001$) in the mean peak T-type current density was observed in cells treated with TAT-C3P (− 12.1 ± 2.1 pA/pF, $n = 31$) compared to control (non-treated) cells (− 27.2 ± 2.9 pA/pF, $n = 41$) (Fig. 1c). The mean maximal slope conductance (G_{max}) was decreased by 56% ($p < 0.0001$) from 572 ± 53 pS/pF to 254 ± 39 pS/pF (Fig. 1d). This effect was not observed when cells were treated with the control TAT-C3D peptide indicating that TAT-C3P-induced inhibition of $Ca_v3.3$ was specifically mediated by the carboxy proximal peptide and not from a non-specific effect that could have resulted from TAT itself (Fig. 1d). Inhibition of T-type currents by TAT-C3P was not associated with additional alteration of the voltage-dependence of activation and inactivation, nor of the recovery from inactivation, and only a slight acceleration of the inactivation kinetics of $Ca_v3.3$ currents at hyperpolarized potentials was observed (supplemental Fig. S1). Furthermore, this inhibition was not observed when TAT-C3P was acutely infused into the cells via the patch pipette (supplemental Fig. S2) suggesting that TAT-C3P-induced inhibition of $Ca_v3.3$ is likely to have occurred via a regulatory signaling pathway controlling the expression of the channel rather than via direct alteration of the channel activity itself. Finally, we did not observe any significant effect of TAT-C3P on cells expressing $Ca_v3.1$ and $Ca_v3.2$ channels indicating that this peptide is selective for $Ca_v3.3$

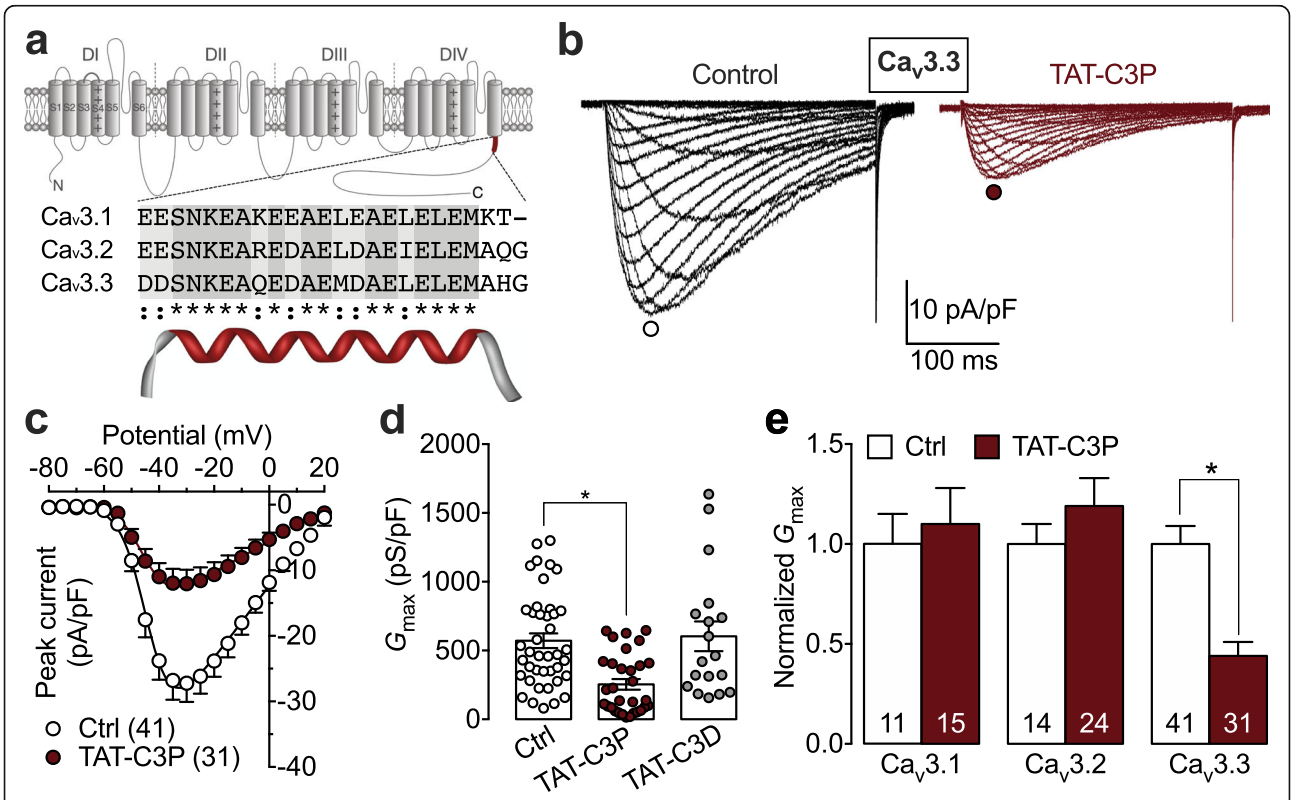

Fig. 1 Effect of TAT-C3P peptide on Ca_v3 channels. **a** Schematic representation of the membrane topology of Ca_v3 channels and protein sequence alignment showing the conserved proximal region of the carboxy terminus across all three Ca_v3 channel isoforms ($Ca_v3.1$, $Ca_v3.2$, and $Ca_v3.3$). Molecular modeling indicates that this region may adopt a helical conformation. **b** Representative T-type current traces recorded from cells expressing $Ca_v3.3$ channels in response to 300 ms depolarizing steps to values ranging from − 80 mV to + 20 mV from a holding potential of − 100 mV for control (black traces) and TAT-C3P-treated cells (red traces). **c** Corresponding mean peak current density-voltage (*I/V*) relationship. **d** Corresponding mean maximal macroscopic conductance (G_{max}) values obtained from the fit of the *I/V* curves with a modified Boltzmann equation for control (white dots), and cells treated with either TAT-C3P (red dots) or the control TAT-C3D peptide (grey dots). **e** Mean normalized G_{max} for cells expressing $Ca_v3.1$, $Ca_v3.2$, and $Ca_v3.3$ channels in response to TAT-C3P treatment

channels (Fig. 1e and supplemental Fig. S3). Considering that the proximal carboxy terminal region of Ca_v3 channels is highly conserved across the three channel isoforms, the observation that TAT-C3P was effective only on $Ca_v3.3$ channels suggests the existence of a distinct regulatory mechanism specific for $Ca_v3.3$ that may be compromised by the peptide. Additional analysis will elucidate the detailed mechanisms underlying the effect of this peptide.

While several pan Ca_v3 channel blockers have been described, there is to date no molecule selective for one particular Ca_v3 isoform [11]. This lack of selective pharmacopeia not only hampered the identification of specific physiological roles for Ca_v3 channels that in the absence of selective modulator requires the use of genetic or antisense nucleotide approaches, but also compromised the therapeutic development of Ca_v3 channel modulators. Here, we reported the first non-genetic molecular tool to selectively inhibit $Ca_v3.3$ channels in cells, and possibly in vivo. Although the molecular mechanism by which TAT-C3P inhibits $Ca_v3.3$ channels remains to be explored in detail, the observation that discreet channel molecular determinants can be harnessed to selectively target a particular channel isoform represents an appealing strategy to study specific physiological functions, and to potentially enhance our understanding of Ca_v3 channels in disease.

Supplementary information

Supplementary information accompanies this paper at https://doi.org/10.1186/s13041-020-00636-y.

Additional file 1. Extended methodology and supplemental data.

Abbreviations

TAT-C3P: TAT-based peptide containing the carboxy proximal region of Ca_v3 channels; TAT-C3D: TAT-based peptide containing the carboxy distal region of Ca_v3 channels

Acknowledgements

We thank Robin N. Stringer for copy-editing the manuscript and Charles University (Progres Q28).

Authors' contributions

LC performed electrophysiological recordings, analyzed and interpreted the results. NW designed and supervised the study, prepared figures and wrote the manuscript. All authors read and approved the final manuscript.

Funding

Not applicable.

Availability of data and materials

All data generated or analyzed during this study are included in this published article and its supplementary information files.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Received: 26 May 2020 Accepted: 12 June 2020
Published online: 19 June 2020

References

1. Zamponi GW, Striessnig J, Koschak A, Dolphin AC. The physiology, pathology, and pharmacology of voltage-gated calcium channels and their future therapeutic potential. Pharmacol Rev. 2015;67(4):821–70.
2. Weiss N, Zamponi GW. Genetic T-type calcium channelopathies. J Med Genet. 2020;57(1):1–10.
3. Staes M, Talavera K, Klugbauer N, Prenen J, Lacinova L, Droogmans G, et al. The amino side of the C-terminus determines fast inactivation of the T-type calcium channel alpha1G. J Physiol. 2001;530(Pt 1):35–45.
4. Hamid J, Peloquin JB, Monteil A, Zamponi GW. Determinants of the differential gating properties of Cav3.1 and Cav3.3 T-type channels: a role of domain IV. Neuroscience. 2006;143(3):717–28.
5. Kang HW, Park JY, Lee JH. Distinct contributions of different structural regions to the current kinetics of the Cav3.3 T-type Ca2+ channel. Biochim Biophys Acta. 2008;1778(12):2740–8.
6. Baumgart JP, Vitko I, Bidaud I, Kondratskyi A, Lory P, Perez-Reyes E. I-II loop structural determinants in the gating and surface expression of low voltage-activated calcium channels. PLoS One. 2008;3(8):e2976.
7. Karmažínová M, Baumgart JP, Perez-Reyes E, Lacinová L. The voltage dependence of gating currents of the neuronal CA(v)3.3 channel is determined by the gating brake in the I-II loop. Pflugers Arch. 2011;461(4):461–8.
8. Karmažínová M, Jašková K, Griac P, Perez-Reyes E, Lacinová Ľ. Contrasting the roles of the I-II loop gating brake in CaV3.1 and CaV3.3 calcium channels. Pflugers Arch. 2015;467(12):2519–27.
9. Jurkovicova-Tarabova B, Cmarko L, Rehak R, Zamponi GW, Lacinova L, Weiss N. Identification of a molecular gating determinant within the carboxy terminal region of $Ca_v3.3$ T-type channels. Mol Brain. 2019;12(1):34.
10. Kelley LA, Mezulis S, Yates CM, Wass MN, Sternberg MJ. The Phyre2 web portal for protein modeling, prediction and analysis. Nat Protoc. 2015;10(6):845–58.
11. Weiss N, Zamponi GW. T-type calcium channels: from molecule to therapeutic opportunities. Int J Biochem Cell Biol. 2019;108:34–9.

Publisher's Note