SNARE complexes mediate TNFα-induced co-trafficking of TRPV1 and TRPA1 on pain peptide-containing vesicles to the surface of sensory neurons

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Abstract

Transient receptor potential (TRP) A1 and V1 channels play pivotal roles in the transduction of pain signal, yet little is known about the molecular machinery underlying their transport to the plasmalemma during chronic inflammation. Herein, TRPA1 and TRPV1 were found on large dense core vesicles containing calcitonin gene-related peptide (CGRP) and co-localised on fibres and cell bodies of cultured sensory neurons expressing both. Co-trafficking of TRPV1 with TRPA1 was visualised directly in sensory neurons. TNFα enhanced their co-trafficking and elevated their surface content. Syntaxin 1–interacting protein, Munc18–1, proved necessary for the response to TNFα, and for TRPV1-triggered CGRP release. TNFα-induced trafficking of TRPV1 and TRPA1 to the surface required a synaptic vesicle associated membrane protein 1 (VAMP1) (but not 2/3). VAMP1 assembled into stable functional large complexes (104–288 k) upon stimulation to mediate CGRP release with the participation of SNAP-25 and syntaxin 1. Inactivating one or two of the latter proteins by botulinum neurotoxin (BoNT)/A or /C1 inhibited the TNFα- elevated delivery. Accordingly, enhancement by TNFα of Ca^{2+} influx through the upregulated surface expressed TRPV1 and TRPA1 channels was abolished by BoNT/A. In contrast, Ca^{2+} influx mediated by TRPV1 or TRPA1 in TNFα untreated control cells was not affected by BoNT/A. Thus, in addition to the neurotoxins' known inhibition of the release of neuropeptides, their therapeutic potential is augmented by lowering the exocytotic delivery of pain transducing channels and the resultant hyper-sensitisation in inflammation.

Introduction

Considerable research has focused on the TRPV1 and A1 channels in sensory neurons, because of their pivotal roles in the transduction of pain signals. TRPV1 detects heat (> 43 °C), acid and various chemicals including capsaicin, whereas, TRPA1 is activated by various pungent chemicals or cold temperature (< 15 °C) (Fig .1A). TRPV1 and A1 are expressed on distinct, but overlapping, populations of sensory nerves. Activities of both channels are upregulated during chronic pain. Pro-algesic cytokines, such as TNFα stimulates the expression and release of CGRP and substance P (SP) from sensory neurons. TNFα participates in the genesis of inflammatory and neuropathic pain, and elicits long-term mechanical allodynia in both naïve and nerve injury models. On the other hand, activation of peripheral TRPA1 plays a critical role in the development of TNFα -induced mechanical hyperalgesia. These data suggest that negating TRPV1 and A1 may reduce neuropathic and arthritic pain. Such an important goal could be achieved using BoNTs that inactivate different SNAREs, but their involvement in the exocytotic surface delivery of each channel must first be demonstrated.

Results

As a prelude to monitoring the channels' trafficking in sensory neurons, it was pertinent to identify the type of vesicle in which they reside. Using confocal microscopy, TRPA1 was visualized on the cell bodies of cultured rat trigeminal ganglion neurons (TGNs) (not shown) and co-localized on neurites with large dense-core vesicle (LDCV) markers [such as secretogranin II (SgII), CGRP and SP] (Fig. 1B). Likewise, TRPV1 displayed striking co-localisation with SP and SgII (Fig. 1B). In contrast, the distribution of the classic marker of small synaptic vesicles [vesicular glutamate transporter 1/2 (vGlut1/2)] very rarely coincided with TRPV1 (Fig. 1B). Thus, TRPA1 and TRPV1 localise on LDCVs in sensory neurons.

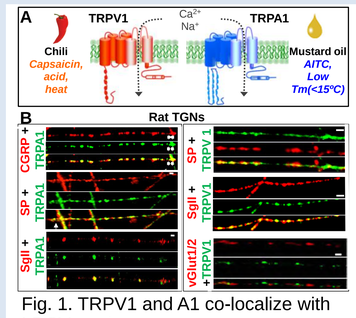

Fig. 1. TRPV1 and A1 co-localize with LDCV-markers

Cognizant of the report that TRPA1 is involved in the development of TNFα-induced mechanical hyperalgesia, the ability of TNFα to induce the surface delivery of TRPA1 was examined. TNFα (100 ng/ml) was applied to live TGNs for 24 h, before incubation for 10 min with TRPA1-ecto to label the TRPA1 delivered to their surface. After washing and incubation with Alexa 488-conjugated secondary antibody, the surface expression of TRPA1 could be visualised under the resting condition using the TRPA1-ecto antibody (Fig. 2). Treatment with TNFα resulted in a significantly (~3 fold) increased TRPA1 labelling on the cell surface over the basal level (Fig. 2). Likewise, TRPV1-ecto antibody readily detected the surface expressed TRPV1-DsRed fusion protein under the resting condition. Treatment with TNFα induced ~3-fold increment in the fluorescence intensity on the cell surface, similar to the increase observed for TRPV1 (Fig.2).

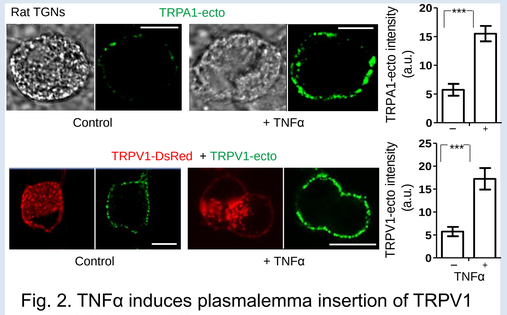

Fig. 2. TNFα induces plasmalemma insertion of TRPV1 and TRPA1 in rat TGNs.

Involvement of SNAREs in TNFα-enhanced insertion of TRPA1 and V1 into the plasmalemma was investigated using different BoNTs to inactivate SNAP-25 (/A, /C1), syntaxin 1 (/C1) or rat VAMP (isoform 1, /D; 2/3, /B, /D). TGNs were incubated with each of the BoNT at 100 nM for 24 h at 37°C followed by TNFα treatment. Notably, BoNT/A, /C1 or /D cleaved ≥ 80% of their respective SNARE substrates (not shown) and each greatly reduced TRPV1 and TRPA1 insertion into the cell surface (Fig. 3A). In contrast /B, which truncated > 80% of VAMP 2 and 3 but did not cleave VAMP1 (not shown), failed to significantly affect trafficking of TRPV1 and A1 to the surface (Fig.3A). Notably, VAMP1 assembled into stable functional large complexes (104–288 k) upon stimulation to mediate CGRP release with the participation of SNAP-25 and syntaxin 1.

As BoNT/D additionally cleaved VAMP1, our data suggest that VAMP1 rather than 2 or 3 is involved in the surface delivery of these two channels. This notion was further confirmed by the fact that TNFα induced more TRPV1-DsRed to the surface by over-expression of VAMP1 than 2 (Fig. 3B). Thus, SNAREs in sensory neurons are essential for TNFα-induced membrane insertion of TRPV1 and A1.

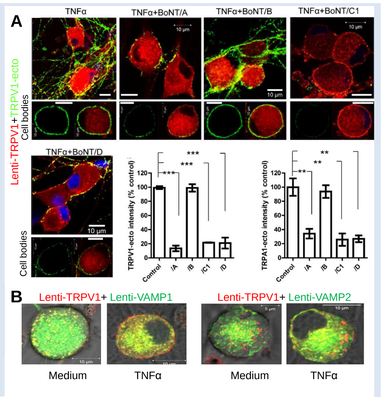

Fig.3. SNAREs are essential for TNFα-induced membrane insertion of TRPV1 and A1.

Knock down of Munc18–1, a protein essential for SNARE complex formation, resulted in ~70% blockade of capsaicin-stimulated CGRP release compared to the non-targeted control and ~80% reduction in the content of surface-associated TRPV1 and TRPA1 in TGNs stimulated with TNFα (Fig. 4).

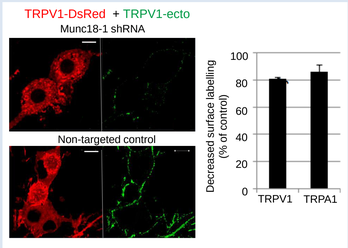

Fig. 4. Munc18–1 is essential for trafficking of TRPV1 and TRPA1 to the cell surface of TGNs.

Confocal images revealed that lentiviral-expressed TRPA1-GFP co-localizes with TRPV1-DsRed in TGNs (Fig. 5A), supporting the idea that these channels might reside in the same transporting vesicles. This hypothesis was evaluated using a proximity ligation assay which is a sensitive measurement of two proteins that reside within< 40 nm. Numerous positive fluorescent spots were detected on fibres of the neurons pre-treated with TNFα; in contrast, only a few spots could be observed on the neurites of the control cells (Fig. 5B). These collective data provide the first evidence that TRPV1 and A1 are transported together in VAMP1-containing LDCVs to the plasma membrane after stimulating neurons express both with TNFα.

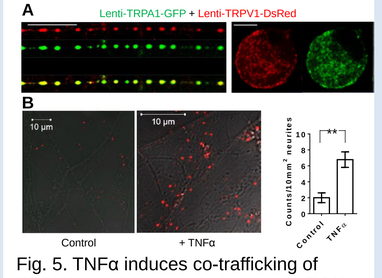

Fig. 5. TNFα induces co-trafficking of TRPV1 and A1 to the surface in rat TGNs.

To determine the functional consequences of blocking the TNFα-induced membrane trafficking of TRPV1 and A1 by BoNTs, Ca^{2+} influx through TRPV1 and A1 was measured with Fluo-4-AM using 1 µM capsaicin or 50 µM allyl isothiocyanate (AITC) as their respective agonist. Pre-treatment with TNFα for 24 h significantly increased the Ca^{2+} influx via TRPV1 and A1 relative to the control. In stark contrast, TNFα -enhanced Ca^{2+} influx was normalized to the agonist induced control level by pre-incubation with BoNT/A (Fig. 6). Interestingly, TRPV1-and A1 mediated Ca^{2+} influx in TNFα untreated control cells was not affected by BoNT/A (Fig. 6). In conclusion, TNFα-mediated potentiation of the response of TRPV1 and A1 is returned to the non-treated control value by prior exposure to BoNT/A whereas the basal level of Ca^{2+} influx remains unaltered.
These results confirm that TNFα-induced elevation of TRPA1 and V1 activities in mediating Ca^{2+} influx are due to the increased surface delivery of these channels via a SNARE-dependent mechanism.

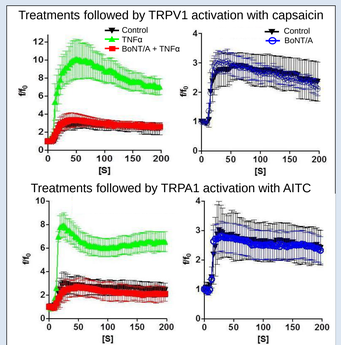

Fig. 6. TNFα enhances Ca^{2+} influx in cultured TGNs which is blocked by BoNT/A.

Figure 7. During injury and inflammation, inflammatory cells release TNFα which binds to its receptor (TNFR) on sensory neurons. This results in activation of intracellular cascades to increase CGRP synthesis. TNFα also elevates Ca^{2+} influx. These culminate in the enhancement of trafficking of CGRP containing vesicles, and release of CGRP and others. TRPA1 and V1 channels are packaged into CGRP- and VAMP1-containing vesicles and delivered to the plasma membrane involving the formation of SNARE complexes composed of SNAP-25, syntaxin 1, and VAMP1, as well as Munc18–1. Under such conditions, the delivery of these channels to the neuronal surface via such processes is elevated; this cascade likely contributes to the genesis of hyperalgesia and allodynia.

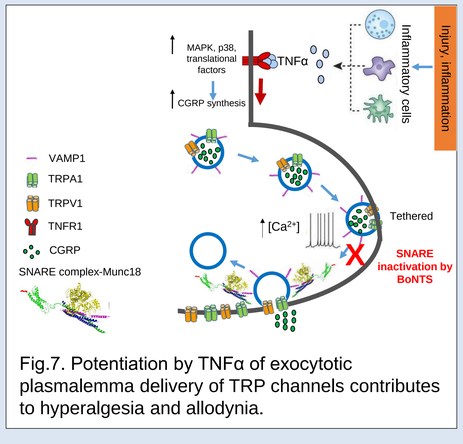

Fig.7. Potentiation by TNFα of exocytotic plasmalemma delivery of TRP channels contributes to hyperalgesia and allodynia.

Conclusions

- Pain transducing channels, TRPA1 and TRPV1 are found on VAMP-1 containing LDCVs in sensory neurons.
- TNF-α induced trafficking of TRPV1 and TRPA1 to the surface of sensory neurons requires SNAP-25, VAMP-1, syntaxin1 and Munc18–1.
- TNF-α induces co-trafficking of TRPV1 and TRPA1 in sensory neurons which express both.
- The ability of BoNTs to normalize excessive channel trafficking and transmitter release supports their potential as safe therapeutics to treat chronic neurogenic/inflammatory disorders.

References

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