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Secretory carrier-associated membrane protein 2 (SCAMP2) regulates cell surface expression of T-type calcium channels

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Abstract

Low-voltage-activated T-type Ca^{2+} channels are key regulators of neuronal excitability both in the central and peripheral nervous systems. Therefore, their recruitment at the plasma membrane is critical in determining firing activity patterns of nerve cells. In this study, we report the importance of secretory carrier-associated membrane proteins (SCAMPs) in the trafficking regulation of T-type channels. We identified SCAMP2 as a novel $Ca_V3.2$-interacting protein. In addition, we show that co-expression of SCAMP2 in mammalian cells expressing recombinant $Ca_V3.2$ channels caused an almost complete drop of the whole cell T-type current, an effect partly reversed by single amino acid mutations within the conserved cytoplasmic E peptide of SCAMP2. SCAMP2-induced downregulation of T-type currents was also observed in cells expressing $Ca_V3.1$ and $Ca_V3.3$ channel isoforms. Finally, we show that SCAMP2-mediated knockdown of the T-type conductance is caused by the lack of $Ca_V3.2$ expression at the cell surface as evidenced by the concomitant loss of intramembrane charge movement without decrease of total $Ca_V3.2$ protein level. Taken together, our results indicate that SCAMP2 plays an important role in the trafficking of $Ca_V3.2$ channels at the plasma membrane.

Keywords: Ion channels, Calcium channels, T-type channels, $Ca_V3.2$ channels, Secretory carrier-associated membrane protein 2, SCAMP2, Trafficking

Through their ability to pass calcium ions (Ca^{2+}) near the resting membrane potential, low-voltage-activated T-type channels have an important physiological role in shaping firing activity patterns of nerve cells, both in the central and peripheral nervous system. The implication of T-type channels in the control of neuronal excitability is partly defined by the density of channels embedded in the plasma membrane. Therefore, a number of molecular mechanisms and signaling pathways come into play to underly precise control of cell surface expression of T-type channels [1] and defects whether genetic or acquired can lead to severe neuronal conditions [2, 3].

Secretory carrier-associated membrane proteins (SCAMPs) form a family of integral membrane proteins essentially expressed in the trans-Golgi network and recycling endosome membranes where they regulate vesicular trafficking and vesicle recycling processes [4]. Of the five known mammalian SCAMPs, SCAMP2 shows a ubiquitous expression pattern including in neuronal tissues where SCAMP2 transcripts are observed for instance in the cerebellum, thalamus, hippocampus, and spinal cord (https://www.proteinatlas.org/ENSG00000140497-SCAMP2/tissue). SCAMP2 consists of four transmembrane helices with cytoplasmic amino- and carboxy-termini and a so-called E peptide located between

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transmembrane helices 2 and 3 essential for mediating SCAMP2 function [5]. This E domain is highly conserved among SCAMP isoforms and represents an essential molecular determinant for SCAMP2-mediated inhibition of exocytosis [6]. Only a few reports have documented the role of SCAMP2 in the regulation of ion channels and transporters [7–10]. In the present study, we aimed to assess the functional role of SCAMP2 in the regulation of T-type channels.

To address this issue, we assessed whether $Ca_v3.2$ channels and SCAMP2 associate at the protein level. Co-immuniprecipitation from tsA-201 cells expressing recombinant HA-tagged $Ca_v3.2$ and Myc-tagged SCAMP2 using an anti-HA-antibody precipitated SCAMP2-Myc with $Ca_v3.2$-HA revealing the existence of a $Ca_v3.2$/SCAMP2 protein complex (Fig. 1a). We note that co-immunoprecipitation experiments from total cell lysates do not address whether this interaction is direct or not and it is a possibility that formation of $Ca_v3.2$/SCAMP2 protein complex may also involve another intermediate protein. Next, we aimed to analyze the functional effect of SCAMP2 on $Ca_v3.2$ channels. Patch-clamp recordings from tsA-201 cells expressing $Ca_v3.2$ showed that co-expression of SCAMP2 produces an almost complete drop of the whole-cell T-type current (Fig. 1b and c). For instance, the maximal macroscopic conductance (G_{max}) was reduced by 91% ($p < 0.0001$) in cells co-expressing SCAMP2 (61 ± 18 pS/pF, n = 18) compared to cells expressing $Ca_v3.2$ alone (692 ± 62 pS/pF, n = 25) (Fig. 1d). Alanine mutagenesis of the E peptide of SCAMP2 at cysteine 201 (C201A) and tryptophan 202 (W202A) reduced this effect to 64% ($p = 0.0269$) and 39% ($p < 0.0001$) inhibition, respectively, indicating that SCAMP2-induced knockdown of $Ca_v3.2$ currents is at least partly mediated by the E peptide (Fig. 1b–d). These data also indicate that the reduction in $Ca_v3.2$ current density in the presence of SCAMP2 is not merely due to the co-expression of just any protein given that the W202A mutant construct has no big effect. With regard to the effect of SCAMP2 on the other T-type channel isoforms, co-expression of SCAMP2 in cells expressing recombinant $Ca_v3.1$ and $Ca_v3.3$ reduced G_{max} by 35% ($p < 0.0001$) and 98% ($p < 0.0001$) respectively (Fig. 1e and f and Additional file 1: Fig. S1) indicative of a differential susceptibility to SCAMP2-dependent modulation ($Ca_v3.3 \approx Ca_v3.2 > Ca_v3.1$). Next, we aimed to assess the underlying mechanism by which SCAMP2 induced knockdown of the T-type conductance. The alteration of the T-type conductance in the presence of SCAMP2 could originate from an overall decreased level of $Ca_v3.2$ proteins or from a reduced expression of the channel in the plasma membrane. Western blot analysis from total cell lysates showed that $Ca_v3.2$ protein levels were not decreased by the presence of SCAMP2. Instead, we observed a non-significant trend toward higher expression levels which may have arisen from a lower rate of vesicular exocytosis therefore preventing the channel from being targeted to the proteasomal degradation machinery (Fig. 1g and h). In contrast, recording of intramembrane charge movements (Q) that provide an accurate assessment of the number of channels embedded in the plasma membrane revealed an 85% decrease ($p < 0.0001$) of Q_{max} in cells expressing SCAMP2 (from 6.1 ± 0.7 fC/pF, n = 16 to 0.9 ± 0.2 fC/pF, n = 17) (Fig. 1i and j) indicating a decreased channel expression at the cell surface. Moreover, while the kinetics of intramembrane charge movements remained unaltered (Fig. 1k), the G_{max}/Q_{max} dependency in the presence of SCAMP2 was reduced by 52% ($p < 0.0001$) (from 0.169 ± 0.007 pS/fC, n = 16 to 0.080 ± 0.014 pS/fC, n = 11) suggesting an additional alteration of the coupling between the activation of the voltage-sensor and the pore opening of the channel (Fig. 1l). This observation is consistent with a previous report showing that besides to be concentrated primarily in intracellular membranes, SCAMP2 is also found in the plasma membrane [11] and therefore could potentially modulate the gating of the channel in addition to its insertion in the membrane. We note that the reduction

(See figure on next page.)

Fig. 1 SCAMP2 regulates T-type channel expression. **a** Co-immunoprecipitation of Myc-tagged SCAMP2 (SCAMP2-Myc) from tsA-201 cells co-transfected with HA-tagged $Ca_v3.2$ channel ($Ca_v3.2$-HA). The upper panel shows the result of the co-immunoprecipitation of SCAMP2-Myc with $Ca_v3.2$-HA using an anti-HA antibody. The lower panels show the immunoblot of Cav3.2-HA and SCAMP2-Myc from total cell lysates using an anti-HA and anti-Myc antibody, respectively. *HC*, heavy chain antibody; *LC*, light chain antibody. This experiment was performed four times from independent transfections and $Ca_v3.2$/SCAMP2 interaction was consistently observed. **b** Representative T-type current traces from tsA-201 cells expressing $Ca_v3.2$ alone (black traces) and in combination with wild-type SCAMP2 (blue traces), as well as with C201A (purple traces) and W202A (orange traces) SCAMP2 mutants in response to 150 ms depolarizing steps varied from − 90 mV to + 30 mV from a holding potential of − 100 mV. **c** Corresponding mean current/voltage (*I/V*) relationships. **d** Corresponding mean maximal macroscopic conductance values (G_{max}) obtained from the fit of the *I/V* curves with the modified Boltzmann Eq. (1). **e–f** Mean G_{max} values for tsA-201 cells expressing $Ca_v3.1$ and $Ca_v3.3$ channels, respectively. **g**. Immunoblot of $Ca_v3.2$-HA expressed in tsA-201 cells in the absence (−) and presence (+) of SCAMP2-Myc. The immunoblot shows the results of three independent sets of transfections. **h** Corresponding mean expression levels of $Ca_v3.2$-HA normalized to actin. **i** Representative intramembrane charge movement traces recorded at the ionic reversal potential from cells expressing $Ca_v3.2$ alone (black trace) and in the presence of SCAMP2 (blue trace). The doted lines depict the time course of the intramembrane charge mouvement integral. **j** Corresponding mean maximal intramembrane charge movement values (Q_{max}). **k** Corresponding mean 10–90% rise time values calculated from the integral time course shown in **i**. **l** Corresponding mean G_{max}/Q_{max} values

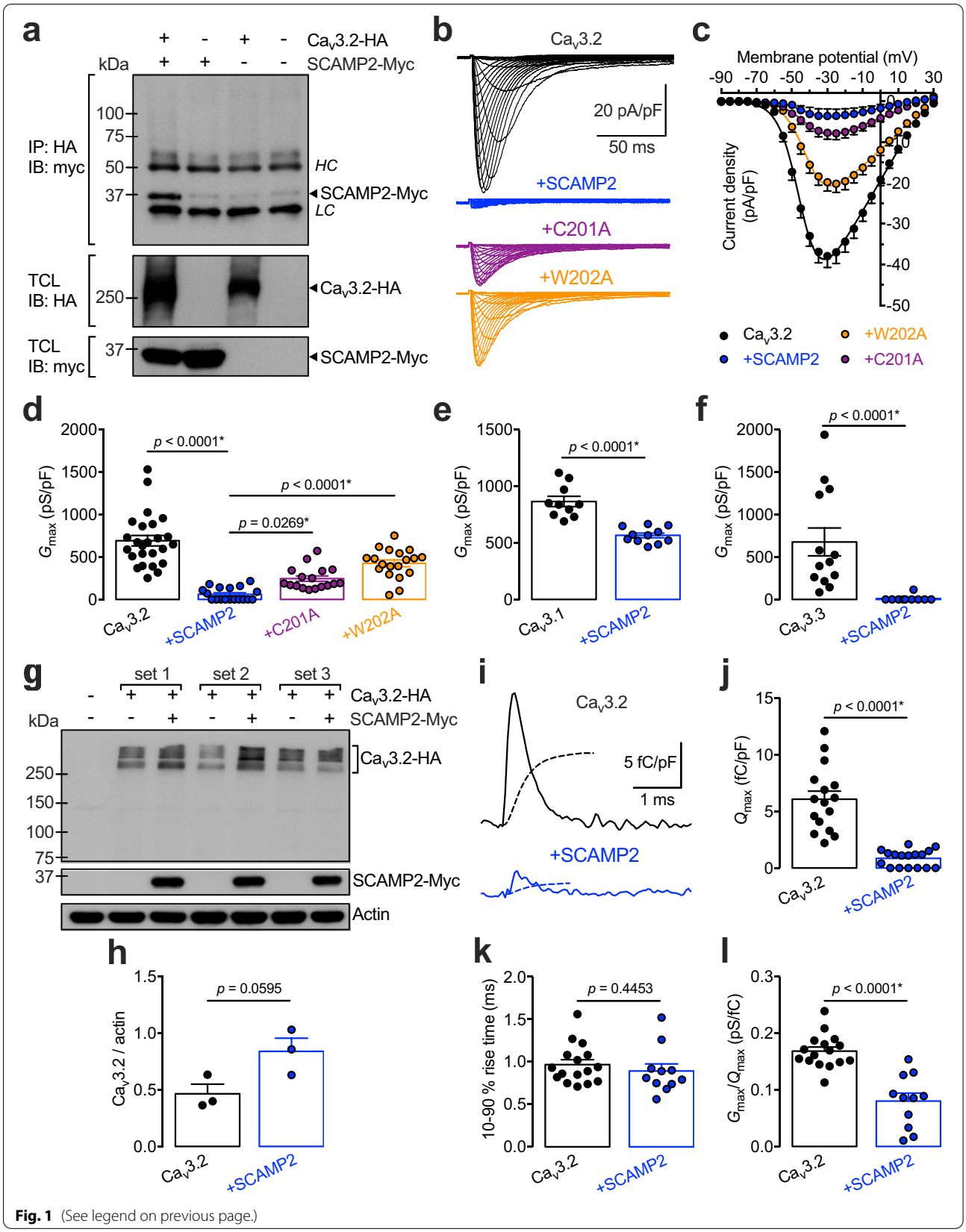

Fig. 1 (See legend on previous page.)

of Q_{max} combined with the reduction of G_{max}/Q_{max} of the small fraction of channels that still reached the plasma membrane in the presence of SCAMP2 is very similar to the reduction of the maximal T-type conductance we previously observed (91%, Fig. 1d).

Several $Ca_v3.2$ interacting proteins including KLHL1 [12], USP5 [13], Stac1 [14], calnexin [15], and Rack-1 [16] have been reported to modulate the sorting and trafficking of the channel to the plasma membrane. In this study, we reported SCAMP2 as a novel $Ca_v3.2$-interacting partner and potent repressor of the expression of the channel at the cell surface. Further investigations will be necessary to fully explore the importance of this regulation in native conditions. Importantly, altered expression of SCAMP2 has been reported in several types of cancer [17]. Given the importance of $Ca_v3.2$ channels in the development of peripheral painful neuropathies [18], it will be interesting to assess to what extent SCAMP2-mediated regulation of $Ca_v3.2$ could possibly contribute to cancer-related neuropathic pain.

Abbreviations

G_{max}: Maximal macroscopic conductance; KLHL1: Kelch-like 1; Q_{max}: Maximal intra membrane charge movement; Rack-1: Receptor for activated C kinase 1; SCAMP2: Secretory carrier membrane protein 2; Stac1: Stac adaptor protein 1; USP5: Ubiquitin-specific proteinase 5.

Supplementary Information

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Additional file 1. Fig. S1. Functional effect of SCAMP2 on $Ca_v3.1$ and $Ca_v3.3$ channels. **a** Representative T-type current traces from tsA-201 cells expressing $Ca_v3.1$ alone (black traces) and in combination with SCAMP2 (blue traces) in response to 150 ms depolarizing steps varied from -90 mV to +30 mV from a holding potential of -100 mV. **b** Corresponding mean current/voltage (*I/V*) relationships. **c** Corresponding mean maximal macroscopic conductance values (*Gmax*) obtained from the fit of the *I/V* curves with the modified Boltzmann Eq. (1). **d**–**e** Same legend as for **a**–**c** but for cells expressing $Ca_v3.3$ channel.

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Authors' contributions

LC, RNS and BJT performed experiments and analyzed the data. TV generated SCAMP2 C201A mutant cDNA. LL supervised recordings and analysis of intramembrane charge movement. NW designed and supervised the study and wrote the manuscript. All authors critically revised the manuscript and contributed significantly to this work. All authors read and approved the final manuscript.

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Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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