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Inactivation kinetics of voltage-gated calcium channels in glutamatergic neurons are influenced by SNAP-25

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SNAP-25 forms part of the SNARE core complex that mediates membrane fusion. Biochemical and electrophysiological evidence supports an accessory role for SNAP-25 in interacting with voltage-gated calcium channels (VGCCs) to modulate channel activity. We recently reported that endogenous SNAP-25 negatively regulates VGCC activity in glutamatergic neurons from rat hippocampal cultures by shifting the voltage-dependence of inactivation of the predominant P/Q-type channel current in these cells. In the present study, we extend these findings by investigating the effect that manipulating endogenous SNAP-25 expression has on the inactivation kinetics of VGCC current in both glutamatergic and GABAergic cells recorded from 9–13 DIV cultures. Silencing SNAP-25 in glutamatergic neurons significantly slowed the inactivation rate of P/Q-type VGCC current whereas alterations in SNAP-25 expression did not alter inactivation rates in GABAergic neurons. These results indicate that endogenous SNAP-25 plays an important role in P/Q-type channel regulation in glutamatergic neurons.

Key words: voltage-gated calcium channels, SNAP-25, inactivation, kinetics, hippocampal neurons, glutamatergic, GABAergic

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Introduction

The SNARE proteins syntaxin 1A, SNAP-25 and VAMP-2 interact to form the SNARE core complex that drives membrane fusion.[1] An additional role of SNAP-25 is that it can bind to diverse classes of VGCCs, generally causing an inhibition of channel function.[2-5] Although these studies performed in heterologous expression systems support a role for SNAP-25 in regulating VGCC activity, the extent to which the endogenously expressed protein influences channel function is not so clear.

Recently, we described how endogenous SNAP-25 in rat hippocampal glutamatergic neurons inhibits native VGCC currents.[6] By silencing SNAP-25 expression, we revealed that total VGCC current density was increased. This was associated with a depolarizing shift in the voltage-dependence of inactivation of the dominant P/Q-type current in glutamatergic cells. These results indicated that physiological levels of SNAP-25 can downregulate P/Q-type channel activity in hippocampal-cultured neurons at 9–13 DIV.

Together with the voltage-dependence of steady-state inactivation, the rate of open-state current inactivation is an important intrinsic property that contributes to VGCC function. Furthermore, several VGCC regulatory proteins have been shown to alter open state inactivation.[7-9] This includes a role for SNARE proteins where both syntaxin 1A and SNAP-25 have been shown to affect inactivation rates of various classes of VGCCs.[4,10,11]

In order to further understand the regulatory effects of SNAP-25 on neuronal VGCCs, we extend our previous results in this present study by analyzing whether silencing of endogenous SNAP-25 in glutamatergic neurons alters VGCC inactivation kinetics. Furthermore, since reduction of SNAP-25 expression does not affect VGCC properties in GABAergic cells,[6] we investigated the effect of

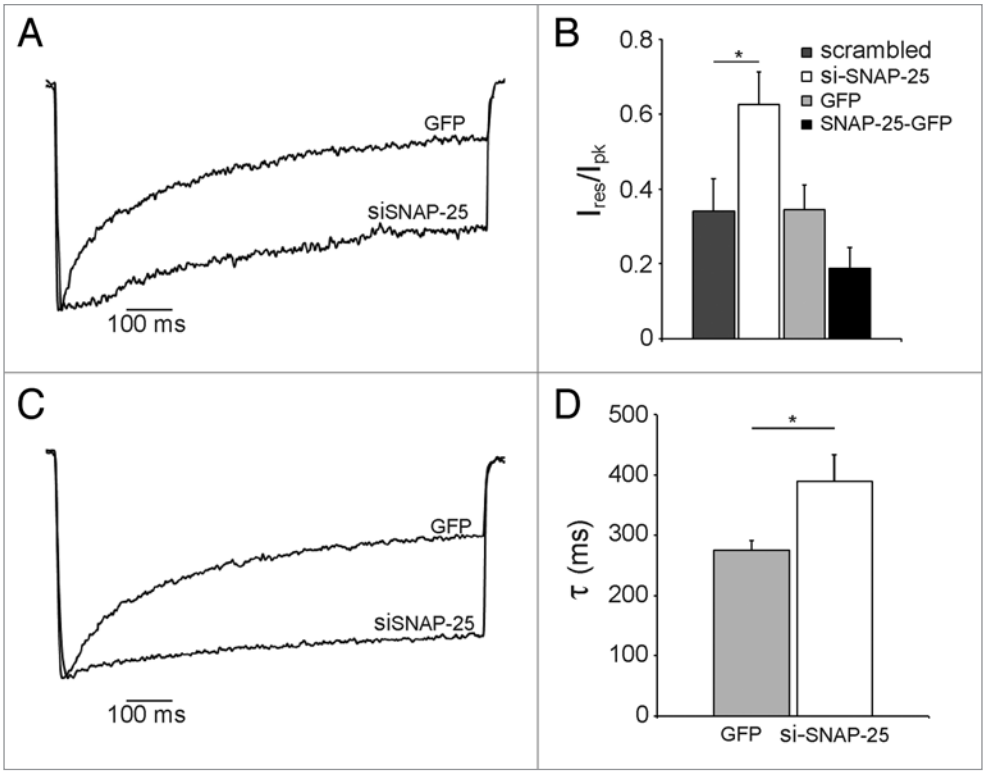

Figure 1. SNAP-25 silencing decreases the inactivation rate of VGCC current in glutamatergic neurons. (A) Superimposed representative traces of the time course of total I_{Ba} elicited by a 1s voltage step from -80 to 0 mV in glutamatergic neurons transfected with SNAP-25 siRNA plus GFP or with GFP alone. (B) Fraction of residual I_{Ba} remaining at the end of the depolarizing step normalized to peak I_{Ba} in glutamaterigic neurons transfected with a scrambled SNAP-25 siRNA control plus GFP, SNAP-25 siRNA plus GFP, GFP alone or SNAP-25 GFP. Data are expressed as the mean ± SEM. (n = 6–9). (C) Superimposed representative traces of the time course of P/Q-type I_{Ba} elicited by a 1s voltage step from -80 to 0 mV in glutamatergic neurons transfected with pSuper SNAP-25 siRNA plus GFP or with GFP alone. P/Q-type currents were isolated pharmacologically by recording in the presence of nifedipine (1 μM), ω-conotoxin-GVIA (1 μM) and SNX-482 (250 nM). (D) Time constants for the inactivation of P/Q-type VGCC currents in glutamatergic neurons transfected with pSuper SNAP-25 siRNA plus GFP or with GFP alone. Data are expressed as the mean ± SEM. (n = 7). Current decay was fitted with a single exponential function.

SNAP-25 overexpression in this neuronal subtype.

Results

SNAP-25 silencing enhances VGCC inactivation rates in glutamatergic neurons. To determine whether SNAP-25 modulates the inactivation rates of VGCC current in glutamatergic neurons, we knocked down endogenous SNAP-25 using siRNA before measuring the kinetics of VGCC current inactivation in response to a 1s depolarizing voltage step. We observed that glutamatergic cells transfected with the SNAP-25 siRNA construct exhibited a significantly slower rate of total VGCC inactivation compared to cells transfected with GFP (**Fig. 1A**) or a scrambled siRNA construct. As a result, the fraction of residual current remaining after the depolarizing voltage step was significantly greater in SNAP-25 silenced cells compared to controls (**Fig. 1B**). We also examined the effect of exogenous SNAP-25-GFP expression on inactivation since we have shown that this reduces total VGCC current density.[12] Although the fraction of residual current was reduced in SNAP-25-GFP transfected neurons, this was not significantly different from controls (**Fig. 1B**) suggesting that endogenous levels of SNAP-25 exert a near maximal effect on VGCC inactivation.

Since the major component of VGCC current in glutamatergic neurons under these conditions is P/Q-type current[6] we used a combination of other VGCC blockers to pharmacologically isolate this component in order to investigate the effect of SNAP-25 silencing on P/Q-type current inactivation constants. **Figure 1C** illustrates that P/Q-type inactivation was slower in SNAP-25 silenced cells compared to GFP expressing controls, significantly decreasing the average inactivation time by over 100 ms (**Fig. 1D**). These results suggest that endogenous SNAP-25 accelerates the open-state inactivation of P/Q-type VGCCs in glutamatergic neurons.

Alterations in GABAergic SNAP-25 expression do not affect VGCC inactivation rates. In contrast to glutamatergic neurons, total VGCC current in GABAergic neurons inactivates slowly and is dominated by L-type channel current with a relatively small P/Q-type channel component. We have also shown that overexpression of SNAP-25 in GABAergic cells reduces total VGCC current.[6] Having demonstrated that endogenous SNAP-25 enhances the inactivation rate of P/Q-type channels, we were interested to determine if SNAP-25-induced decreases in GABAergic VGCC current

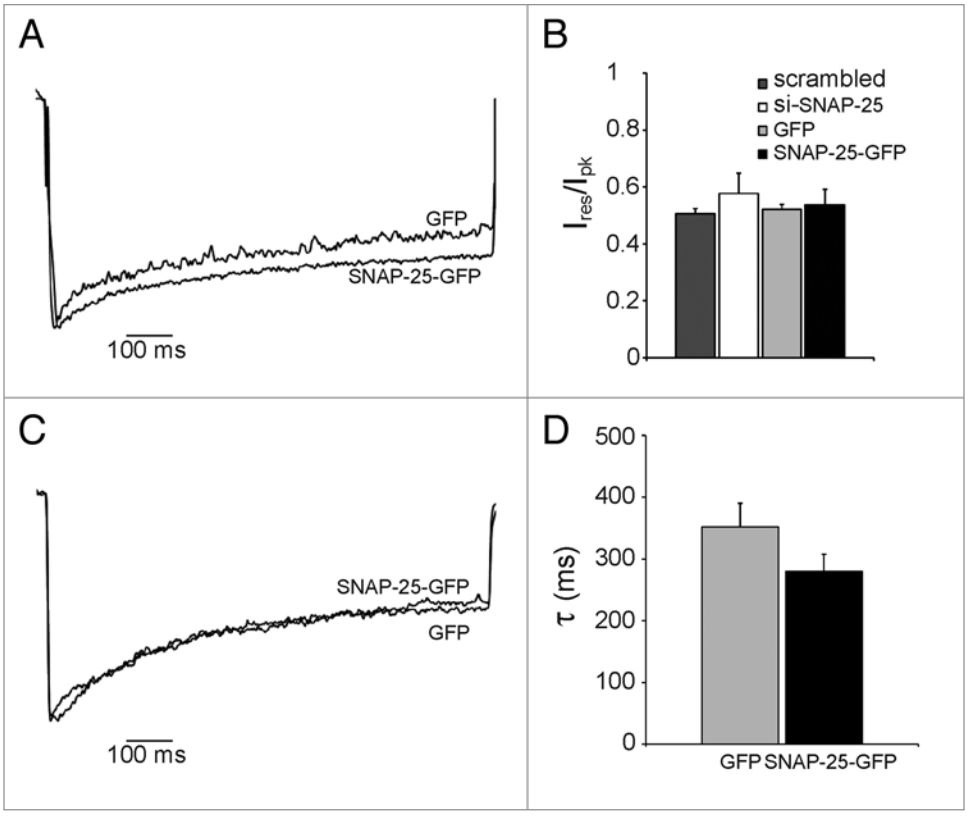

Figure 2. SNAP-25 overexpression does not alter the inactivation rate of VGCC current in GABAergic neurons. (A) Superimposed representative traces of the time course of total I_{Ba} elicited by a 1s voltage step from -80 to 0 mV in GABAergic neurons transfected with SNAP-25-GFP or with GFP alone. (B) Fraction of residual I_{Ba} remaining at the end of the depolarizing step normalized to peak I_{Ba} in GABAergic neurons transfected with a scrambled SNAP-25 siRNA control plus GFP, SNAP-25 siRNA plus GFP, GFP alone or SNAP-25 GFP. Data are expressed as the mean ± SEM. (n = 6–7). (C) Superimposed representative traces of the time course of L-type I_{Ba} elicited by a 1s voltage step from -80 to 0 mV in GABAergic neurons transfected with SNAP-25-GFP or with GFP alone. L-type currents were isolated pharmacologically by recording in the presence of, ω-agatoxin-IVA (250 nM), ω-conotoxin-GVIA (1 μM) and SNX-482 (250 nM). (D) Time constants for the inactivation of L-type VGCC currents in glutamatergic neurons transfected with GFP alone or SNAP-25-GFP. Data are expressed as the mean ± SEM. (n = 6–8). Current decay was fitted with a single exponential function.

were associated with an alteration in inactivation kinetics.

Overexpressing SNAP-25-GFP in GABAergic neurons did not significantly alter the inactivation rate of total VGCC current compared to GFP expressing controls (**Fig. 2A**). Also, neither SNAP-25 knockdown nor overexpression had any significant effect on the fraction of residual current compared to controls (**Fig. 2B**). To determine if exogenous SNAP-25 altered the predominant L-type inactivation kinetics, L-type VGCC current was isolated by recording from GFP or SNAP-25-GFP expressing GABAergic neurons in the presence of ω-agatoxin-IVA (250 nM), ω-conotoxin-GVIA (1 μM) and SNX-482 (250 nM). Under these conditions, GFP expressing neurons exhibited a slowly inactivating current (**Fig. 2C**). Although SNAP-25-GFP caused a modest decrease in the inactivation rate constant, this was not significantly different to neurons transfected with only GFP (**Fig. 2D**). Therefore, although exogenous SNAP-25 expression has been found to inhibit GABAergic VGCC current density, this is not associated with an effect on the inactivation rate of the dominant L-type channel current. This is in contrast to the effect of endogenous SNAP-25 on P/Q-type current in glutamatergic cells.

Discussion

Our previous work supported a role for endogenous SNAP-25 in regulating VGCCs in glutamatergic neurons. We found that silencing SNAP-25 expression in glutamatergic neurons caused an increase in total VGCC current, associated with a depolarizing shift in the voltage dependence of inactivation.[6] In this study, we have extended these results by demonstrating that the inactivation kinetics of P/Q-type channels are influenced by SNAP-25 in glutamatergic neurons while those of L-type channels in GABAergic neurons are not affected by exogenous SNAP-25 overexpression.

The calcium independent inactivation rate of VGCCs can be modulated by a variety of intracellular proteins that directly bind to the channel. Several of these pathways overlap and includes interaction of the α_1 pore-forming subunit with the β[13] and $\alpha_2\delta$[14] auxiliary subunits, G-proteins,[15] calcium binding proteins[8,9] and 14-3-3.[7] Depending on the VGCC subtype and degree of core-complex assembly, SNAREs have also been shown to affect VGCC inactivation kinetics. Syntaxin 1A alone has been shown to accentuate the slow inactivation of N-type VGCCs,[4,10] which is not affected

by SNAP-25 co-expression.[4] Furthermore, syntaxin 1A increases G-protein inhibition of the channel, which is also partially reversed by SNAP-25 co-expression.[11] Conversely, SNAP-25 alone does not alter the inactivation rate of N-type[4] or P/Q-type[5] but significantly increases the rate of inactivation of L-type VGCC current in Xenopus oocytes.[4]

The present study investigated whether the diverse inactivation rates of VGCC current in glutamatergic and GABAergic neurons could be due to the expression of SNAP-25. The levels of endogenous SNAP-25 expression vary between these cell types[16] and overexpression in glutamatergic cells further inhibits VGCC function.[12] We have demonstrated here that the inactivation kinetics of the predominant P/Q-type current in glutamatergic cells are enhanced when endogenous SNAP-25 is knocked down. This suggests that, in addition to an effect on steady-state voltage dependent inactivation, SNAP-25 may inhibit glutamatergic P/Q-type current via enhancing the rate of inactivation. This contrasts with the lack of effect of SNAP-25 overexpression in HEK-293 cells transfected with P/Q-type VGCCs.[5] This may reflect the potential involvement of other endogenous proteins that influence how VGCCs are regulated by SNAP-25 in native cells, which could be absent in heterologous overexpression systems. A further aspect of this study was to determine if exogenous SNAP-25 expression in GABAergic neurons, which decreases total VGCC current, affected VGCC inactivation kinetics. The results indicate that SNAP-25 overexpression had an insignificant effect on the inactivation rate of the major L-type VGCC current in these cells. This could be due to differences in the way in which SNAP-25 interacts with the Ca_v2 versus the $Ca_v1\alpha$ subunits.[2]

Overall, the results described here demonstrate that the inactivation kinetics of VGCCs in glutamatergic neurons are influenced by SNAP-25 and suggest that the role of SNAP-25 as a negative regulator of VGCC activity is more potent in glutamatergic neurons where it both shifts the voltage dependence of inactivation and enhances the inactivation rate of P/Q-type channels.

Materials and Methods

Hippocampal neuronal cultures. Rat hippocampal neuron primary cultures were prepared from the hippocampi of 18-day-old fetuses as described previously in reference 12, and were plated at low density on glass coverslips. Neurons were transfected at 5–6 DIV using the calcium phosphate precipitation method. Endogenous SNAP-25 was knocked down by transfection of a pSUPER construct while a nonspecific siRNA duplex of the same nucleotides in a scrambled sequence in the pSUPER vector was used as a negative control.

Electrophysiology. The recording solutions and experimental setup used to record whole cell VGCC currents were essentially the same as described previously in references 6 and 12. Current decay was measured in response to a 1-s depolarization from -80 to 0 mV. In this study, data were acquired at 10 kHz and digitized for analysis at 100 Hz before being fit by a single exponential of the current decay to generate inactivation kinetics. Cultured neurons were recorded at 9–13 DIV and the recorded neurons glutamatergic or GABAergic phenotype was confirmed via single cell RT-PCR as described in detail previously in reference 6. Data are expressed as the mean ± SE of *n* experiments with statistical significance determined using ANOVA at the p level indicated.

Acknowledgments

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References

1. Sudhof TC, Rothman JE. Membrane fusion: grappling with SNARE and SM proteins. Science 2009; 323:474-7.
2. Rettig J, Sheng ZH, Kim DK, Hodson CD, Snutch TP, Catterall WA. Isoform-specific interaction of the alpha1A subunits of brain Ca^{2+} channels with the presynaptic proteins syntaxin and SNAP-25. Proc Natl Acad Sci USA 1996; 93:7363-8.
3. Sheng ZH, Rettig J, Cook T, Catterall WA. Calcium-dependent interaction of N-type calcium channels with the synaptic core complex. Nature 1996; 379:451-4.
4. Wiser O, Bennett MK, Atlas D. Functional interaction of syntaxin and SNAP-25 with voltage-sensitive L- and N-type Ca^{2+} channels. EMBO J 1996; 15:4100-10.
5. Zhong H, Yokoyama CT, Scheuer T, Catterall WA. Reciprocal regulation of P/Q-type Ca^{2+} channels by SNAP-25, syntaxin and synaptotagmin. Nat Neurosci 1999; 2:939-41.
6. Condliffe SB, Corradini I, Pozzi D, Verderio C, Matteoli M. Endogenous SNAP-25 regulates native voltage-gated calcium channels in glutamatergic neurons. J Biol Chem 285:24968-76.
7. Li Y, Wu Y, Zhou Y. Modulation of inactivation properties of $Ca_v2.2$ channels by 14-3-3 proteins. Neuron 2006; 51:755-71.
8. Lee A, Westenbroek RE, Haeseleer F, Palczewski K, Scheuer T, Catterall WA. Differential modulation of Ca(v)2.1 channels by calmodulin and Ca^{2+}-binding protein 1. Nat Neurosci 2002; 5:210-7.
9. Few AP, Lautermilch NJ, Westenbroek RE, Scheuer T, Catterall WA. Differential regulation of $Ca_v2.1$ channels by calcium-binding protein 1 and visinin-like protein-2 requires N-terminal myristoylation. J Neurosci 2005; 25:7071-80.
10. Degtiar VE, Scheller RH, Tsien RW. Syntaxin modulation of slow inactivation of N-type calcium channels. J Neurosci 2000; 20:4355-67.
11. Jarvis SE, Zamponi GW. Distinct molecular determinants govern syntaxin 1A-mediated inactivation and G-protein inhibition of N-type calcium channels. J Neurosci 2001; 21:2939-48.
12. Pozzi D, Condliffe S, Bozzi Y, Chikhladze M, Grumelli C, Proux-Gillardeaux V, et al. Activity-dependent phosphorylation of Ser187 is required for SNAP-25-negative modulation of neuronal voltage-gated calcium channels. Proc Natl Acad Sci USA 2008; 105:323-8.
13. Buraei Z, Yang J. The β subunit of voltage-gated Ca^{2+} channels. Physiol Rev 90:1461-506.
14. Davies A, Hendrich J, Van Minh AT, Wratten J, Douglas L, Dolphin AC. Functional biology of the alpha(2)delta subunits of voltage-gated calcium channels. Trends Pharmacol Sci 2007; 28:220-8.
15. Tedford HW, Zamponi GW. Direct G protein modulation of Ca_v2 calcium channels. Pharmacol Rev 2006; 58:837-62.
16. Matteoli M, Pozzi D, Grumelli C, Condliffe SB, Frassoni C, Harkany T, Verderio C. The synaptic split of SNAP-25: different roles in glutamatergic and GABAergic neurons? Neuroscience 2009; 158:223-30.