J Pharmacol Sci **92**, 79 – 83 (2003)

Journal of Pharmacological Sciences

Current Perspective

Regulation of Presynaptic Calcium Channels by Synaptic Proteins

Gerald W. Zamponi*

Department of Physiology and Biophysics, Cellular and Molecular Neurobiology, Research Group, University of Calgary, 3330 Hospital Dr. NW, Calgary T2N 4N1, Canada

Received April 28, 2003

Abstract. Calcium entry into nerve termini via voltage gated calcium channels is an essential step in neurotransmission. Consequently, second messenger regulation of calcium channel activity modulates synaptic activity. It has been suggested that calcium channels must physically couple to the release machinery, and a physical interaction between a synaptic protein interaction (*synprint*) site contained within mammalian presynaptic calcium channels and synaptic proteins such as syntaxin 1, SNAP-25, and synaptotagmin has been demonstrated. Interestingly, synaptic calcium channels in invertebrates lack this region. In invertebrates, synaptic transmission is instead dependent on a presynaptic calcium channel splice variant that can physically associate with the adaptor proteins Mint-1 and CASK. We suggest that in the absence of a *synprint* region, these proteins may localize calcium channels to the synaptic release machinery. The interactions between synaptic proteins and mammalian N-type calcium channels serves to regulate calcium channel activity directly, as well as indirectly by altering second messenger regulation of the channels. This provides for a feedback mechanism that allows the fine-tuning of calcium channel activity during various steps in neurotransmitter release. This does not occur with invertebrate synaptic calcium channel homologs, suggesting that the regulation of calcium channel activity by synaptic proteins is a mechanism unique to vertebrates.

Keywords: syntaxin, N-type channel, SNAP-25, cysteine string protein, G protein

Presynaptic calcium channels associate with synaptic proteins

Calcium channel mediated calcium entry into presynaptic nerve terminals is an essential step in the release of neurotransmitters (1, 2). As a consequence, regulation of calcium channel activity through pharmacological means or by activation of second messenger cascades modulates synaptic transmission. In most vertebrate neurons, synaptic transmission is mediated by members of the Ca_v2 calcium channel family, in particular N-type and P/Q-type channels (1, 3). The α_1 subunits of both N-type and P/Q-type calcium channels contain a long intracellular linker region connecting domains II – III (see Fig. 1), which associates with key synaptic proteins such as syntaxin 1, SNAP-25, synaptotagmin 1, and cysteine string protein (CSP), and has been termed the synaptic protein interaction (*synprint*) site (4, 5; for review, see Ref. 6). In many cases, the binding stability is critically dependent on intracellular calcium concentration, thus allowing for a dynamic rearrangement of calcium channel-SNARE protein complexes in response to calcium influx (7). Such a physical docking of the vesicle release machinery to the source of extracellular calcium influx would allow the synaptic vesicles to be spatially localized to calcium microdomains, thus presumably enhancing the efficiency of neurotransmitter release. If so, then competitive inhibition of the calcium channel SNARE protein interaction would be expected to antagonize synaptic transmission. Indeed, injection of synthetic N-type calcium channel *synprint* peptides into superior cervical ganglion (SCG) neurons depressed postsynaptic responses by as much as 50% (8). Based on such studies, it is thus reasonable to conclude that a tight physical interaction between presynaptic calcium channels and the release machinery is essential for optimal synaptic release. Two considerations, however, may provide reason for pause. First,

*Corresponding author. FAX: +1-403-210-8106
E-mail: Zamponi@ucalgary.ca

Invited article

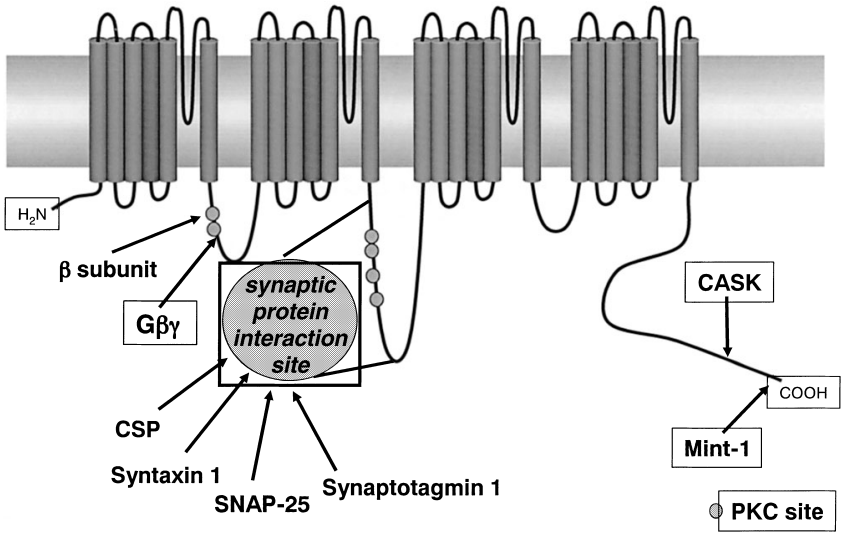

Fig. 1. Transmembrane topology of the N-type calcium channel α_1 subunit illustrating major protein interactions sites. The α_1 subunit contains four homologous transmembrane domains (each comprised of six transmembrane spanning helices plus a reentrant pore forming loop), which are connected by large cytoplasmic linker regions. The calcium channel β subunit and G$\beta\gamma$ predominantly bind to the domain I – II linker region (with possible additional interactions in the C-terminal region, not shown). The domain II – III linker region of vertebrate N-type channels contains a synaptic protein interaction site that interacts with CSP, syntaxin 1, synaptotagmin 1, and SNAP-25. The C-terminus region interacts with the adaptor proteins Mint-1 and CASK. PKC-dependent phosphorylation sites in the I – II linker and the II – III linker regulate channel activity and/or the ability of regulatory proteins to interact with the channel (see Ref. 15).

splice variants of human N-type calcium channels lacking the *synprint* region have been identified recently (9). These variants are widely expressed throughout the CNS, and are incapable of association with syntaxin 1A and likely other synaptic proteins. Although it is at this point not clear as to whether these variants are located presynaptically, a putative contribution of these channels to synaptic transmission would therefore occur independently of an interaction with the vesicle release machinery. More strikingly, invertebrates express only a single representative of the Ca_v2 calcium channel family, and detailed sequence analysis of these channels in *Drosophila*, *C. elegans*, squid, and mollusks reveals that they all lack the synaptic protein interaction site (10). Yet, invertebrate neurons are certainly capable of neurotransmitter release, thus suggesting the possibility that a physical association of synaptic proteins and presynaptic calcium channels may not be fundamentally essential for this process. This raises the questions as to how invertebrate calcium channels couple to the neurotransmitter release machinery? By answering these questions, one may uncover fundamentally important processes that may well also apply to vertebrate synaptic transmission.

The calcium channel structural determinants of invertebrate synaptic transmission were recently examined by Spafford et al. (10) using the freshwater mollusk *Lymnaea stagnalis* as a model system. As with other invertebrates, *Lymnaea stagnalis* neurons express a single representative of the three different Ca_v families. The LCa_v2 homolog that corresponds to vertebrate N and P/Q-type calcium channels functionally behaves like an N-type calcium channel with regard to its activation, inactivation, and selectivity properties (11). Knockout of this gene via RNA interference blocks *Lymnaea* synaptic transmission, indicating that this channel is necessary for synaptic activity. At the same time, as expected, cleavage of syntaxin and SNAP-25 via botulinum toxins also blocks synaptic transmission. Yet, although synaptic transmission therefore depends on both the LCa_v2 calcium channel, and a functional complement of synaptic proteins, due to the absence of a synprint region, there is no physical interaction between vesicle release proteins and the channels. More surprisingly, the injection of rat *synprint* peptides was nonetheless capable of disrupting neurotransmitter release, which suggests a non-specific action of these peptides and may thus require us to re-evaluate conclusions based on earlier experiments in SCG neurons. At the same time, it is widely accepted that synaptic vesicles must be localized in some fashion to the source of calcium entry to allow for efficient and rapid synaptic transmission (12). A mechanism which may provide such a co-localization independently of direct synaptic

protein binding to the channels was independently proposed by two laboratories. Maximov and Bezprozvanny (13) showed that targeting of N-type calcium channels to presynaptic nerve terminals in hippocampal neurons requires the presence of a C-terminus splice region that is capable of interacting with the adaptor proteins Mint-1 and CASK. Spafford et al. (10) reached a similar conclusion in their *Lymnaea stagnalis* neuron model and showed that peptides that competitively inhibit Mint binding to the LCa_v2 channel acutely block synaptic transmission. In addition, knockdown of CASK via RNAi prevented synaptic activity. Taken together, these groups proposed that CASK and/or Mint may contribute to the appropriate targeting of presynaptic calcium channels and proposed their colocalization with the synaptic release machinery. Yet, the presence of Mint and CASK interaction domains may not be sufficient for synaptic localization of mammalian P/Q-type calcium channels, as the removal of the *synprint* site in rat $Ca_v2.1$ channels results in inappropriate subcellular targeting (8, 14). From an evolutionary point of view, it is interesting to note that the appearance of a Mint and CASK interacting domain on Ca_v2 calcium channels occurred at a much earlier evolutionary stage than that of the *synprint* region, which is only found in vertebrates. This suggests that the synaptic protein interaction site is a vertebrate specialization rather than a fundamental requirement for synaptic transmission (14, 15).

Modulation of presynaptic calcium channel activity by synaptic proteins

In addition to tightening the coupling between calcium influx and the synaptic vesicle release machinery, the association between presynaptic calcium channels and synaptic proteins may provide an avenue for regulating channel activity. Indeed, as first reported by Bezprozvanny et al. (16), coexpression of syntaxin 1A with N-type and P/Q-type calcium channels in *Xenopus* oocytes results in a hyperpolarizing shift in the midpoint of the steady state inactivation curve of these channels, thus resulting in a reduced availability for opening and consequently an inhibition of channel activity. In subsequent studies, syntaxin 1A, syntaxin 1B and SNAP-25 were found to reduce N-type channel availability in tsA-201 cells (17 – 19). In the concomitant presence of both syntaxin 1 and SNAP-25, channel availability became restored (19). This may suggest that calcium channels that are associated with only one of the t-SNARE proteins and can thus not contribute to synaptic transmission would be less likely to open, whereas channels associated with the entire t-SNARE complement are capable of participating in vesicle release, and thus require an increased availability for opening. Such a mechanism would ensure selective opening of calcium channels that can contribute to synaptic transmission and prevent unnecessary calcium overload of the presynaptic terminal. A similar effect is observed with P/Q-type calcium channels; however, in this case the presence of synaptotagmin 1 is also required before channel availability is normalized (20). It is important to note that the ability of synaptic proteins to regulate presynaptic calcium channel function has now been demonstrated in intact neurons (21, 22).

Three additional factors modulate the ability of syntaxin to regulate channel activity. The synaptic protein interaction site contains numerous protein kinase C (PKC) consensus sites and can be phosphorlyated by PKC in vitro (23). In biochemical assays, this results in a dramatic reduction of syntaxin 1 binding (23). Consistent with these biochemical data, activation of PKC abolishes the ability of syntaxin 1A to modulate N-type channel availability in tsA-201 cells, thus providing a mechanism of second messenger control over syntaxin mediated effects (18). A perhaps more curious finding is the observation that the effects of syntaxin on N-type channel availability are syntaxin state dependent. A point mutation that locks syntaxin 1A permanently in the "open" conformation renders this protein incapable of regulating the steady state inactivation behavior N-type channels (19). Together with observations that nSec-1 coexpression prevents syntaxin regulation of N-type channels, this provides a mechanism by which N-type calcium channel could be dynamically regulated during various steps in the exocytosis process during which syntaxin cycles between open and closed conformations.

Crosstalk between syntaxin and G protein regulation of N-type channels

Stanley and Mirotznik reported that G protein regulation of N-type calcium channels in a chick calyx preparation is abolished following the cleavage of syntaxin with botulinum toxin C1 (24). Although this was suggestive of the possibility that the presence of syntaxin may be required to permit G protein inhibition of N-type channels, this finding appeared at odds with the observation that in expression systems that do not endogenously express syntaxin, G protein modulation of N-type channels could readily be observed. Recent work of Jarvis et al. (17) provided interesting insights into this issue. Upon coexpression of N-type calcium channels with syntaxin 1A, but not with syntaxin 1B, N-type channels appeared to undergo a tonic G protein inhibition that occurred in the absence of G protein coupled

receptor activation. Biochemical evidence suggests that syntaxin 1A acts as a chaperone to colocalize endogenous G_β subunits with the N-type calcium channel in the plasma membrane, thus facilitating G protein regulation (for review, see Ref. 25). Subsequent experiments revealed that the *synprint* region and the G_β subunit interact with distinct portions of the syntaxin molecule and that this interaction does not require the presence of G_γ (19). Moreover, unlike with the syntaxin effects on N-type channel availability discussed in the previous section, activation of PKC did not affect syntaxin mediated G protein inhibition of the channel (18). Finally, expression studies performed with chick DRG neurons indicate that this effect is a physiological feature (26). Similar effects were reported for the action of CSP (i.e., a CSP induced G protein inhibition mediated by G proteins recruited to the channel via CSP; 27). More recently it was shown that the cysteine string domain of CSP was responsible for the chaperone effect, whereas the J-domain of the CSP molecule independently promoted G protein inhibition of the channel though a G_α pathway (28).

Not only do synaptic proteins appear to regulate G protein pathways converging on the N-type channels, but the direct binding of G_β subunits to synaptic proteins appears to have the propensity to regulate synaptic transmission directly (i.e., independently of modulating calcium influx through the N-type channel; 29). This adds further complexity to the interplay between second messenger pathways, N-type calcium channels, and the synaptic release machinery.

Conclusions

We have come a long way in our understanding of the molecular interactions between presynaptic calcium channels and the synaptic vesicle release machinery (for reviews, see Refs. 15, 25, 30). What was originally thought of purely as a mechanism that anchors the vesicle release apparatus to the vicinity of the channels now appear to serve an important regulatory mechanism of calcium channel function per se, thus providing multiple avenues for fine tuning calcium influx and hence, neurotransmitter release.

Acknowledgments

This work was supported by operating grants from the Canadian Institutes of Health Research (CIHR). GWZ is a CIHR Investigator and a Senior Scholar of the Alberta Heritage Foundation for Medical Research and holds the Novartis Chair for Schizophrenia Research.

References

1 Wheeler DB, Randall A, Tsien RW. Roles of N-type and Q-type Ca^{2+} channels in supporting hippocampal synaptic transmission. Science. 1994;264:107–111.
2 Atwood HL, Karunanithi S. Diversification of synaptic strength: presynaptic elements. Nat Rev Neurosci. 2002;3:497–516.
3 Wu LG, Westenbroek RE, Borst JG, Catterall WA, Sakmann B. Calcium channel types with distinct presynaptic localization couple differentially to transmitter release in single calyx-type synapses. J Neurosci. 1999;19:726–736.
4 Sheng Z, Rettig T, Takahashi M, Catterall WA. Identification of a syntaxin-binding site on N-type calcium channels. Neuron. 1994;13:1303–1313.
5 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;15:4100–4110.
6 Catterall WA. Structure and regulation of voltage-gated Ca^{2+} channels. Annu Rev Cell Dev Biol. 2000;16:521–555.
7 Sheng Z-H, Rettig J, Cook T, Catterall WA. Calcium-dependent interaction of N-type calcium channels with the synaptic core complex. Nature. 1996:379:451–454.
8 Mochida S, Sheng ZH, Baker C, Koayashi H, Catterall WA. Inhibition of neurotransmission by peptides containing the synaptic protein interaction site of N-type Ca^{2+} channels. Neuron. 1996;17:781–788.
9 Kaneko S, Cooper CB, Nishioka N, et al. Identification and characterization of novel human $Ca_v2.2$ (α_{1B}) calcium channel splice variants lacking the synaptic protein interaction site. J Neurosci. 2002;22:82–92.
10 Spafford JD, Munno DW, Van Nierop P, et al. Calcium channel structural determinants of synaptic transmission between identified invertebrate neurons. J Biol Chem. 2003;278:4258–4267.
11 Spafford JD, Chen L, Feng ZP, Smit AB, Zamponi GW. Expression and modulation of an invertebrate presynaptic calcium channel α_1 subunit homolog. J Biol Chem. In press 2003. (Published online)
12 Augustine GJ. How does calcium trigger neurotransmitter release? Curr Opin Neurobiol. 2001;11:320–326.
13 Maximov A, Bezprozvanny I. Synaptic targeting of N-type calcium channels in hippocampal neurons. J Neurosci. 2002;22: 6939–6952.
14 Mochida S, Westenbroek RE, Yokoyama CT, et al. Requirement for the synaptic protein interaction site for reconstitution of synaptic transmission by P/Q-type calcium channels. Proc Natl Acad Sci USA. 2003;100:2819–2824
15 Spafford JD, Zamponi GW. Functional interactions between presynaptic calcium channels and the neurotransmitter release machinery. Curr Opin Neurobiol. In press 2003.
16 Bezprozvanny I, Scheller RH, Tsien RW. Functional impact of syntaxin on gating of N-type and Q-type calcium channels. Nature. 1995;378:363–366.
17 Jarvis SE, Magga J, Beedle AM, Braun JEA, Zamponi GW. G protein modulation of N-type calcium channels is facilitated by physical interactions between syntaxin 1A and $G\beta\gamma$. J Biol Chem. 2000;275:6388–6394.
18 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–

2948.
19 Jarvis SE, Barr W, Feng ZP, Hamid J, Zamponi GW. Molecular determinants of syntaxin 1 modulation of N-type calcium channels. J Biol Chem. 2002;277:44399–44407.
20 Zhong H, Yokoyama C, Scheuer T, Catterall WA. Reciprocal regulation of P/Q type Ca^{2+} channels by SNAP-25 syntaxin and synaptotagmin. Nat Neurosci. 1999;2:939–941.
21 Bergsman JB, Tsien RW. Syntaxin modulation of calcium channels in cortical synaptosomes as revealed by botulinum toxin C1. J Neurosci. 2000;20:4368–4378.
22 Stanley EF. Syntaxin I modulation of presynaptic calcium channel inactivation revealed by botulinum toxin C1. Eur J Neurosci. 2003;17:1303–1305.
23 Yokoyama CT, Sheng Z, Catterall WA. Phosphorylation of the synaptic protein interaction site on N-type calcium channels inhibits interactions with SNARE proteins. J Neurosci. 1997;17:6929–6938.
24 Stanley EF, Mirotznik RR. Cleavage of syntaxin prevents G-protein regulation of presynaptic calcium channels. Nature. 1997;385:340–343.
25 Jarvis SE, Zamponi GW. Interactions between presynaptic Ca^{2+} channels, cytoplasmic messengers and proteins of the synaptic vesicle release complex. Trends Pharmacol. 2001;22:519–525.
26 Lü Q, AtKisson MS, Jarvis SE, Feng ZP, Zamponi GW, Dunlap K. Syntaxin 1A supports voltage-dependent inhibition of a1B Ca^{2+} channels by Gbg in chick sensory neurons. J Neurosci. 2001;21:2949–2957.
27 Magga JM, Jarvis SE, Arnot MI, Zamponi GW, Braun JEA. Cysteine string protein regulates G protein modulation of N-type calcium channels. Neuron. 2000;28:195–204.
28 Miller LC, Swayne LA, Kay JG, et al. Distinct modes of action by the J domain and cysteine string domain of CSP modulate G protein inhibition of N-type calcium channels. J Cell Sci. In press 2003.
29 Blackmer T, Larsen EC, Takahashi M, Martin TF, Alford S, Hamm HE. G protein betagamma subunit-mediated presynaptic inhibition: regulation of exocytotic fusion downstream of Ca^{2+} entry. Science. 2001;292:293–297.
30 Atlas D. Functional and physical coupling of voltage-sensitive calcium channels with exocytotic proteins: ramifications for the secretion mechanism. J Neurochem. 2001;77:972–985.