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REVIEW

THE SYNAPTIC SPLIT OF SNAP-25: DIFFERENT ROLES IN GLUTAMATERGIC AND GABAERGIC NEURONS?

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MOLECULAR DIVERGENCE OF THE SYNAPTIC MACHINERY IN GABAERGIC AND GLUTAMATERGIC NEURONS

Normal brain function relies on the fine balance of activity between the two most widespread fast synapse populations in the CNS, the inhibitory GABAergic and the excitatory glutamatergic terminals. In the past years, evidence has accumulated demonstrating that GABAergic and glutamatergic neurons express different molecular components involved in neuronal development, intracellular signaling, and pre-postsynaptic functions. Among the molecules differentially distributed during development, the postsynaptic adhesion molecules neuroligin-1 and neuroligin-2 are localized at excitatory or inhibitory synapses (Varoqueaux et al., 2004, 2006), where they specifically increase excitatory and inhibitory synaptic responses, respectively (Chubykin et al., 2007); N-cadherin, a transmembrane molecule that mediates calcium-dependent intercellular adhesion, becomes rapidly restricted to excitatory synapses during brain development (Benson and Tanaka 1998) and Sema4D, a protein belonging to the family of semaphorins, a class of neuronal guidance cues, is mainly involved in the formation of GABAergic but not glutamatergic synapses (Paradis et al., 2007). Other striking differences between GABAergic and glutamatergic neurons concern the selective recruitment of intracellular signal-transduction cascades that control both short and long term neuronal plasticity and use-dependent adaptation. Immunocytochemical studies showed the conspicuous absence of CaMK II from inhibitory synapses in the thalamus, cerebral cortex (Jones et al., 1994; Liu and Jones 1996) and hippocampus (Sik et al., 1998; Thiagarajan et al., 2002). Similarly, the expression of Ca^{2+}/calmodulin-dependent protein phosphatase 2B (calcineurin) (Sik et al., 1998) was found to be absent in GABAergic interneurons. As these enzymes play key roles in the cellular mechanisms underpinning short-term synaptic plasticity, their differences were proposed to account for the distinct synaptic plasticity of inhibitory and excitatory neurons (Craig and Boudin, 2001). Glutamatergic and GABAergic terminals also differ in their synaptic protein contents. Besides robust differences in anchoring proteins of postsynaptic GABA and glutamate receptors (Craig et al., 1996; Levi et al., 2004), many presynaptic proteins differentially affect GABAergic and glutamatergic synapses. For instance, loss of the presynaptic protein Munc13-1, an essential protein for priming of synaptic vesicles in hippocampal neurons, induces a dramatic reduction in the size of the readily releasable vesicle pools and in evoked neurotransmission in glutamatergic neurons, without affecting GABAergic transmission (Augustin et al., 1999; Varoqueaux et al., 2002). Similarly, the synaptic vesicle-associated proteins, synapsins, play different roles in these two neuronal cell types by determining the number of vesicles in the reserve pool at excitatory synapses, and the number of vesicles in both the readily releasable and the reserve pool at inhibitory synapses (Gitler et al., 2004).

SNAP-25: LOCALIZATION AND MOLECULAR INTERACTIONS

SNAP-25 (synaptosomal-associated protein of 25 kDa) belongs to the SNARE superfamily of small membrane proteins that participate in the regulation of synaptic vesicle exocytosis. It is a membrane bound protein anchored to the cytosolic surface of membranes via palmitoyl side

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Abbreviations: BDNF, brain-derived neurotrophic factor; SNAP-25, synaptosomal-associated protein of 25 kDa.

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chains located in the central region of the molecule, and contributes two α-helices in the formation of the exocytotic fusion complex together with syntaxin-1 and synaptobrevin, which is required for vesicle fusion (Jahn et al., 2003; Jahn and Scheller, 2006). Generation of SNAP-25 null mutant mice revealed that SNAP-25 is not required for stimulus-independent neurotransmitter release, but is essential for evoked synaptic transmission (Washbourne et al., 2002). Furthermore, clostridial toxins, which specifically cleave selected components of the SNARE complex, have unequivocally demonstrated the requirement of SNAP-25, syntaxin and synaptobrevin in vesicle exocytosis (Jahn et al., 2003; Schiavo et al., 2000). Besides its cognate SNARE proteins, SNAP-25 also interacts with the synaptic vesicle protein synaptotagmin I (Schiavo et al., 1997), providing an essential mechanism for triggering the Ca^{2+}-dependent membrane fusion (Zhang et al., 2002) and for controlling fusion pore dynamics during the final steps of exocytosis (Bai et al., 2004). Increasing evidence supports that SNAP-25 also modulates various voltage-gated ion channels (reviewed in Catterall, 1999; Zamponi, 2003). SNAP-25 therefore represents a multifunctional protein involved in the control of neurotransmitter secretion via several interactions. The concept that SNAP-25 plays additional functions besides formation of the pore complex is in line with the evidence that the protein, which is probably the most abundant protein in the brain, making up 1% of brain protein (Walch-Solimena et al., 1995), is not exclusively localized at synaptic sites but is also present all along axons and dendrites (Galli et al., 1995).

In 2004, we reported that SNAP-25 is virtually absent from mature GABAergic inhibitory synapses (Verderio et al., 2004). Given the central role of SNAP-25 in the formation of the SNARE complex for synaptic vesicle exocytosis, the unexpected absence of SNAP-25 from mature inhibitory synapses raised a debate among neuroscientists: is there a SNARE substituting SNAP-25 at GABAergic synapses? Do low levels of SNAP-25, undetectable by immunocytochemistry, still control exocytosis at inhibitory synapses? In the past few years, several papers have been published reporting apparently contradicting evidence. The aim of this review is to summarize the available literature, to define universal concepts, and to highlight the crucial questions which remain unanswered.

SNAP-25 EXPRESSION IN EXCITATORY AND INHIBITORY NEURONS

The first evidence showing that SNAP-25 is heterogeneously expressed in distinct populations of neurons derives from the pioneering study of Oyler et al. (1989) who described the isolation and characterization of cDNA clones encoding a novel 25-kDa synaptosomal protein, SNAP-25. This report was subsequently followed up by other studies indicating that SNAP-25 is expressed in specific subtypes of conventional synapses, but not ribbon synapses (Catsicas et al., 1992), that variable SNAP-25 expression occurs in autonomic vasoconstrictor and vasodilator neurons in central and peripheral inputs to sympathetic neurons (Gibbins et al., 2003; Morris et al., 2000), and that heterogeneous levels of the protein are present in VAChT-containing varicosities of the ventral horn of the spinal cord (Hellstrom et al., 1999). Duc and Catsicas (1995) in particular reported that only a subset of CNS synapses displays SNAP-25 immunostaining at the electron microscopy level.

Verderio et al. (2004) reported the lack of SNAP-25 immunoreactivity at inhibitory synapses of mature rat hippocampal cultures and adult rat hippocampus. This observation was subsequently extended to adult mouse hippocampus (Frassoni et al., 2005) and human brain (Garbelli et al., 2008). Notably, a partial colocalization between SNAP-25 and GABAergic markers was detected in the prenatal mouse hippocampus and in developing neurons at early stages in culture (Frassoni et al., 2005), indicating a developmentally-regulated disappearance of SNAP-25 immunoreactivity during interneuron differentiation (Fig. 1A). It was also reported that the progressive reduction of SNAP-25 immunoreactivity in interneurons occurs at a greater speed in neuronal cultures obtained from rat compared with mice (Frassoni et al., 2005). The absence of SNAP-25 immunoreactivity in adult GABAergic synapses (Fig. 1D) was more recently confirmed by Bragina et al. (2007), who extended the analysis to the cerebral cortex, and by Garbelli et al. (2008) who combined pre-embedding immunolabeling for SNAP-25 with post-embedding immunogold localization of the neurotransmitter GABA. In contrast with these data, Tafoya et al. (2006) reported the presence of SNAP-25 immunoreactivity in developing cultured neurons from mouse (Fig. 1B), and also showed the presence of high SNAP-25 levels at perisomatic basket cell terminals on CA1 pyramidal cells of the adult mouse hippocampus and in adult mouse thalamus. As these results have been obtained using the same source of commercial antibodies (SMI 81, Tafoya et al., 2006; Garbelli et al., 2008), the reasons for these discrepancies are not clear, although they could be attributed to either methodological differences (Bragina et al., 2007) or to alterations in tissue preservation (Garbelli et al., 2008).

Differences in the levels of the protein at inhibitory and excitatory hippocampal synapses may be consequences of mechanisms operating at the post-transcriptional level. Indeed, SNAP-25 immunoreactivity was occasionally detected in the Golgi area of mature interneurons in culture (Frassoni et al., 2005) and mRNA for SNAP-25 was present in adult hippocampal inhibitory neurons (Boschert et al., 1996; Tafoya et al., 2006), thus excluding the possibility that GABAergic neurons do not synthesize this protein. The differential accumulation of SNAP-25 in specific nerve terminals may be due instead to differences in mRNA translation, to the rapid turnover of the SNAP-25 polypeptide at specific nerve terminals, or could reflect differential axonal processing or transport (see also Oyler et al., 1989) that is restricted to mature interneurons. Notably, a robust reduction of SNAP-25 expression in fast-spiking GABAergic interneurons (Berghuis et al., 2004) is associated with their progressive morphogenesis and func-