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Sommario

Synapses are highly dynamic structures that undergo continuous functional and structural changes in response to alterations of neuronal activity. This remodeling is critical for brain development, synaptic transmission and neuronal plasticity. At the post-synaptic site two classes of molecules play a crucial role in synaptic organization: scaffolding and cell adhesion proteins. Scaffolds ensure the accurate accumulation of neurotransmitter receptors in precise apposition to pre-synaptic release sites. In addition, they provide the physical constraints for maintaining a high concentration of receptors at synapses, and for regulating the constant flux of receptors and scaffolding elements in and out of post-synaptic sites. Scaffolds also regulate downstream signaling pathways to adjust the molecular composition of the post-synaptic devices necessary to sustain synaptic plasticity. Cell adhesion molecules bridge pre- and postsynaptic specializations through specific interactions of their extracellular domains. Such interactions do not simply provide a mechanical link between pre- and post-synaptic sites but are instrumental in activating transduction signals necessary for the recruitment of various synaptic components. In this context phosphorylation processes are critical for modulating changes in the molecular composition of the post-synaptic device. While the impact of phosphorylation of neurotransmitter receptors has been extensively characterized much less is known about the effect of

these post-translational modifications on scaffolding and cell adhesion molecules. At GABAergic synapses specific phosphorylation events targeting the scaffolding molecule gephyrin were shown to alter its oligomerization properties, thus producing concomitant changes in the numbers of receptors trapped by the scaffold and synaptic strength. Most of these phosphorylation events occur at serine or threonine residues preceding a proline, underlying a potential role of proline-directed phosphorylation as modulator of synaptic strength. At excitatory synapses, mass spectrometric analysis performed on isolated postsynaptic density proteins (PSD) has led to the identification of a number of novel serine/proline phosphorylation sites on scaffolding MAGUKs. In addition the prolyl-isomerase activity of Pin1 has been shown to regulate protein synthesis necessary to sustain the late phase of long-term potentiation. Based on these evidences, the aim of my thesis was to study the functional role of proline-directed phosphorylation in remodeling the post-synaptic devise of both inhibitory and excitatory systems by acting on pivotal constituents such as protein scaffolds and cell adhesion molecules. By combining molecular biology, immunocytochemistry and electrophysiological recording I initially investigated the impact of Pin1-dependent signaling on GABAergic transmission. I found that Neuroligin2, the cell adhesion molecule constitutively present at GABAergic synapses, undergoes post-phosphorylation, prolyl-isomerization modulation of its activity. Proline-directed phosphorylation at Serine 714 of NL2 negatively impacts on NL2 ability to complex with gephyrin. As a consequence, an enhanced accumulation of NL2, gephyrin and GABAA receptors was detected at GABAergic synapses in the hippocampus of Pin1-knockout mice (Pin1?/?), which was accompanied by a concomitant increase in amplitude of spontaneous GABAA-mediated post-synaptic currents. These results suggest that Pin1-dependent signalling represents a mechanism to modulate GABAergic transmission by regulating NL2/gephyrin interaction. Then I focused on the impact of Pin1-dependent signaling on excitatory glutamatergic transmission. In particular, I investigated whether the scaffolding molecule PSD-95, a member of the Disc-Large (DGL)-Membrane-associated guanylate kinase, and know to be phosphorylated by several proline-directed kinases, could be a target of Pin1-dependent modulation. I observed that Pin1 is recruited by PSD-95 at specific Serine-Threonine/Proline consensus motifs localized in the linker region connecting PDZ2 to PDZ3 domains and exerts a negative control on PSD-95 ability to complex with NMDARs. Indeed an enhanced PSD-95/NMDA complex formation was detected in brain extracts derived from Pin1-/- mice. In electrophysiological experiments, larger NMDA-mediated synaptic currents were detected in CA1 principal cells in hippocampal slices obtained from Pin1-/- mice as compared to controls, an effect that was associated with an enhancement in spine density and size. The correct functioning of the central nervous system relies on the rapid and efficient communication between neurons. This occurs at highly specialized functional points of contact called synapses. Synapses are extremely plastic in structure and function, strongly influenced by their own histories of impulse traffic and by signals from nearby cells. Synaptic contacts are fundamental to the development, homeostasis and remodeling of neural circuits. All these events are achieved through different mechanisms operating at both pre- and postsynaptic sites. At the level of the post synaptic density (PSD) compartment, scaffolding molecules and trans-membrane proteins are known to orchestrate proper synapses formation, maturation and

rearrangement required to sustain plasticity processes. Protein phosphorylation represents one of the most important mechanisms engaged in affecting the molecular composition of the post-synaptic device. Most studies have focused on the impact of phosphorylation on the gating properties, surface mobility and trafficking of neurotransmitter receptors while much less is known about the effect of post-translational modifications on scaffolding and cell adhesion molecules functionally linked to neurotransmitter receptors. At GABAergic synapses specific phosphorylation events of the scaffolding molecule gephyrin were shown to alter its multimerization properties, thus producing parallel changes in the number of receptors trapped by the scaffold leading to alterations of synaptic strength. Most of these phosphorylation events occur at serine or threonine residues preceding a proline, underlying a potential role of proline-directed phosphorylation as modulator of synaptic strength. The key player of such signalling cascade is represented by a small enzyme called peptidyl-prolyl isomerase Pin1 (protein interacting with NIMA 1). Pin1, upon recruitment by its substrates in a phosphorylation-dependent manner, catalyzes the cis/tran isomerization of phospho?Ser/Thr?Pro motifs leading to changes in target protein conformation and biological activity. Pin1 is highly expressed in neurons suggesting that it can exert a crucial role in synaptic transmission and plasticity processes at both inhibitory and excitatory synapses. In the first part of my PhD thesis I focused on the impact of Pin1-dependent signalling on GABAergic transmission. I found that the cell adhesion molecule of the neuroligin family enriched at GABAergic synapses, Neuroligin 2 (NL2), undergoes post-phosphorylation prolyl-isomerization modulation of its activity. Using biochemical approaches I found that the unique Pin1 consensus motif present within the cytoplasmic tail of NL2, Serine 714-proline, is indeed phosphorylated in vivo. Proline-directed phosphorylation at Serine 714 of NL2 strongly impacts on NL2 ability to complex with gephyrin. In particular, at this site, post-phosphorylation prolyl-isomerization negatively regulates the ability of NL2 to interact with gephyrin. In line with biochemical results, immunocytochemical analysis reveal that, in the absence of Pin1 expression, NL2/gephyrin complexes are enriched at GABAergic post-synaptic sites and this enrichment is accompanied by an enhanced synaptic recruitment of GABAA receptors (GABAAR). This effect was associated with a concomitant increase in the amplitude, but not in frequency, of spontaneous inhibitory post-synaptic currents (IPSCs). These findings unveil the existence of a new signalling pathway operating at inhibitory GABAergic synapses able to alter the efficacy of GABAergic transmission by modulating NL2/gephyrin interaction. Given the high abundance of Pin1 at excitatory synaptic contacts, in the second part of my PhD thesis I focused on the impact of Pin1-dependent signalling on excitatory glutamatergic transmission. In particular, I started to investigate whether the scaffolding molecule PSD-95, a member of the Disc-Large (DGL)-Membrane-associated guanylate kinase, could be a target of Pin1-dependent signalling cascade. I observed that Pin1, known to reside in post-synaptic structures, is recruited by PSD-95 at specific Serine-Threonine/Proline consensus motifs localized in the linker region connecting PDZ2 to PDZ3 domains. These sites are represented by Treonine287-Proline, Serine290-Proline and Serine295-Proline, and deletion of all of them almost completely abolished Pin1 interaction with PSD-95. Pin1 exerts a negative control on PSD-95 ability to complex with N-Methyl-D-Aspartate receptors (NMDARs). Indeed an enhanced PSD-

	95/NMDAR complex formation was detected in brain extracts derived from Pin1-/- mice. In electrophysiological experiments, larger NMDA-mediated synaptic currents were detected in CA1 principal cells in hippocampal slices obtained from Pin1-/- mice as compared to controls, an effect that was associated with an enhancement in spine density and size. These data indicate that Pin1 controls the synaptic content of NMDARs via PSD-95 prolyl-isomerization and the expression of dendritic spines, both required for the maintenance of long-term potentiation. Overall, this study highlights the crucial role of Pin1-dependent signalling in the functional organization of both inhibitory and excitatory synapses.
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