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Mini-Review

Role of drebrin A in dendritic spine plasticity and synaptic function

Implications in neurological disorders

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Drebrin A is one of the most abundant neuron-specific binding proteins of F-actin and its expression is increased in parallel with synapse formation. Drebrin A is particularly concentrated in dendritic spines, postsynaptic sides of excitatory glutamatergic synapses. More recently, Ferhat and colleagues reported the functional role of drebrin A in regulating synaptic transmission. Indeed, our study showed that overexpression of drebrin A induced an increase of glutamatergic but not GABAergic synapses and resulted in the alteration of the normal excitatory-inhibitory ratio in favor of excitation in mature hippocampal neurons. Downregulation of drebrin A expression by antisense oligonucleotides resulted in the decrease of both miniature- glutamatergic and GABAergic synaptic activities without affecting the excitatory-inhibitory ratio. Studies performed in heterologous cells revealed that drebrin A reorganized the actin filaments and stabilized them and that these effects are depend upon its actin-binding domain. These results suggest that drebrin A regulates dendritic spine morphology, size and density, presumably via regulation of actin cytoskeleton remodeling and dynamics. These data demonstrate for the first time that an actin-binding protein such as drebrin A regulates both glutamatergic and GABAergic synaptic transmissions, probably through an increase of active synaptic site density for glutamatergic transmission and through homeostatic mechanisms for the GABAergic one.

It is appealing to suggest that abnormalities in the expression of drebrin A may result in aberrant synapse development and/or loss of synapses leading to synaptic dysfunction, which underlies cognitive impairment accompanying neurological disorders such as Alzheimer's disease, Down syndrome as well as normal aging.

The majority of excitatory glutamatergic synapses in the CNS are found on dendritic spines.[1] These small protrusions emerging from dendritic shafts constitute sites for the development of neuronal networks and it is believed to provide a cellular substrate for synaptic plasticity.[2] Several studies have shown that spines are very dynamic structures, and their shape, size and number change during development and adulthood. During development, dendritic protrusions initiate out as filopodia, which mature into spines.[3] In adults, these changes are modulated by synaptic activity and plasticity,[4-6] and also associated with learning,[7] aging,[8] as well as diseases such as mental retardation.[9]

Actin filaments are the major cytoskeletal components of dendritic spines.[10,11] These actin filaments appeared to be the key target of molecular mechanisms modulating spine plasticity because drugs that inhibit actin dynamics caused a loss of spines and inhibition of their motility.[12] Time-lapse studies of neurons expressing actin tagged with green fluorescent protein (GFP-actin) revealed that actin-based plasticity in dendritic spines is activity dependent.[12] Consistent with this observation, it has been shown that long-term potentiation (LTP), a known form of experimental synaptic plasticity, is accompanied by enhanced F-actin content in dendritic spines in vivo[13] and in vitro.[14] Thus, the identification of the molecular basis involved in the spine plasticity are essential to understand the mechanisms of synaptic plasticity as well as some neurological disorders such as mental retardation.

The properties of actin cytoskeleton are determined by several proteins that bind to actin filaments. The adult isoform of drebrin, drebrin A (DA), a major neuron specific binding protein of F-actin, emerges as a candidate protein that confers particular characteristics for actin cytoskeleton of dendritic spines.[15] This is because DA is specifically localized at dendritic spines of mature neurons,[16,17] and is shown to inhibit the actin-binding activity of tropomyosin, fascin and α-actinin.[18,19] In vitro, DA also inhibits the interaction between actin and myosin,[16,20] suggesting that it possibly regulates actin filament contractility. In fibroblast cells, the overexpression of DA induces reorganization of actin filaments, leading to alteration in their cell shape.[21] In mature cortical neurons, the overexpression of DA causes elongation of their dendritic spines.[21] Furthermore, the reduction of DA expression by antisense oligonucleotide treatment in developing hippocampal neurons significantly decreases the width and density of filopodia-spines.[22,23]

Beside its role in cell shape and dendritic spine plasticity, DA may play a role in synaptic function. Indeed, it has been shown that DA

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induces spinous clustering of the post-synaptic density (PSD) scaffold protein, PSD-95,[22] as well as activity-dependent synaptic targeting of NMDA receptors.[23] Upon induction of LTP in the hippocampus, drebrin expression is enhanced within dendritic spines.[13] Furthermore, the reduction of DA mediated by antisense oligonucleotides causes cognitive deficits.[24] These observations also correlate well with a major loss of DA in dendritic spines of patients with Alzheimer's disease and Down's syndrome.[25-27] Studies using animal model of Alzheimer's disease revealed also an alteration in DA levels.[28] The level of reduction of DA within dendritic spines seems to correlate well with the severity of cognitive impairment.[28] Significant decreases in DA levels were also reported in normal aging.[26] Altogether, these observations support strongly that the content of DA in dendritic spines is strongly associated with synaptic function.

To evaluate this hypothesis, Ivanov et al.[29] studied the effects of DA on the regulation of dendritic spine plasticity and analyzed its electrophysiological consequences of this regulation in mature cultured hippocampal neurons. The results showed that postsynaptic expression of GFP-tagged DA increases dendritic spine length, size and density and that these effects require the actin-binding domain of DA. Studies in heterologous cells revealed that DA reorganized the actin filaments and stabilized them and that these effects were also mediated by its actin-binding domain. Taken together, these findings show that DA regulates dendritic spine plasticity, presumably via regulation of actin cytoskeleton reorganization and dynamics. The close apposition of the presynaptic marker such as synaptophysin or vGlut1 to the long spines-induced by DA suggested the existence of functional excitatory synaptic contacts. Furthermore, we showed that DA increases the density of glutamatergic synapses. Electrophysiological data showed that overexpression of DA increased the glutamatergic synaptic transmission, probably through an increase of active synaptic site density. Surprisingly, enhanced expression of DA also affected the frequency, amplitude and kinetics of miniature inhibitory postsynaptic currents, despite the fact that GABAergic synapse density and transmission efficacy were not modified. Thus, DA increases the glutamatergic to GABAergic synapse ratio and leads to alteration of the normal excitatory-inhibitory balance in favor of excitation. These observations are fundamental because the excitatory to inhibitory synapse ratio is believed to be critical for normal computation of neuronal excitation and is generally kept constant by a homeostatic feedback mechanism.[30-34]

The identification and characterization of some factors that control the overall change in the ratio of excitatory/inhibitory synapses number and activity have only recently been discovered. Indeed, several studies have involved the synaptic cell adhesion molecules called neuroligin proteins (NLG) and the scaffolding postsynaptic density protein, PSD-95.[35,36] The importance of our findings is emphasized by the recent discovery that drebrin level is increased in the superior frontal cortex in neurological disorders accompanied by mild cognitive impairment (MCI), where the synaptic function is known to be reduced.[28] Thus, these newly discovered mechanisms could provide an important implication in neurological disorders. An alteration in the ratio excitation/inhibition synaptic activities was also suggested in several neurodevelopmental psychiatric disorders, including autism and some forms of mental retardation.[35-37] This notion is emphasized by the numerous studies showing that chromosomal reorganizations in areas that harbor the *NLG1*, *NLG2* and *PSD-95* genes[38-40] and mutations of the X-linked genes encoding neuroligins NLGN3 and NLGN4,[41-44] have been associated in autism. Furthermore, an alteration in PSD-95 expression was also been implicated in fragile-X syndrome.[45] It has been shown that the overexpression of DA induces the accumulation of PSD-95 into dendritic filopodia.[46] In addition, the synaptic clustering of DA is necessary for that of PSD95 in developing neurons.[22] Taken together, it is tempting to speculate that DA might be also altered in fragile-X-syndrome leading to an alteration of PSD-95 expression. So, further analysis of the cellular, molecular and physiological defects caused by DA expression level in vitro may, therefore, provide a useful framework for understanding the physiopathological defects of fragile-X syndrome as well as other neurological disorders.

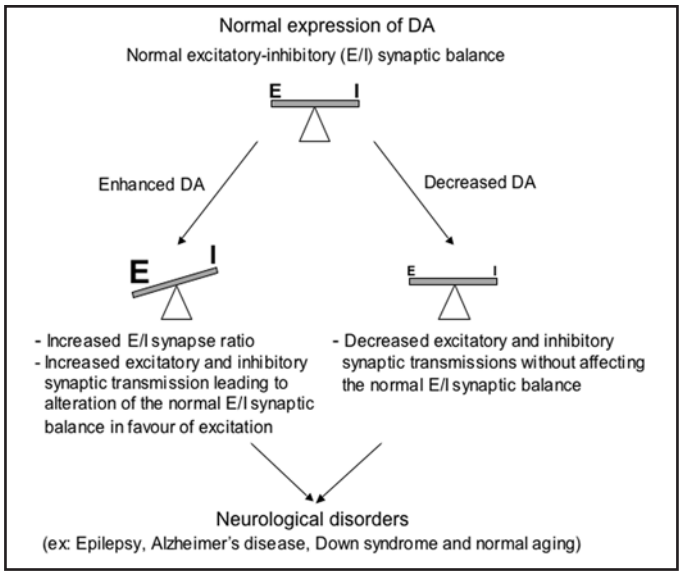

Figure 1. Relative levels of DA expression control synaptic activity leading or not to the alteration of the normal excitatory-inhibitory (E/I) synaptic activity ratio. DA alterations (overexpression or underexpresssion) might result in either strengthening or weakening of synaptic transmission, which in turn could regulate the normal excitatory-inhibitory balance. In all cases, these synaptic alterations lead in synaptic dysfunction, which according to different brain regions involved, could underlies complex psychiatric disorders such as autism and mental retardation or the cognitive impairment accompanying normal aging and neurological disorders, including Epilepsy, Alzheimer's disease and Down's syndrome.

In our study, we investigated also the functional consequences of reduced DA expression in mature cultured hippocampal neurons. Indeed, electrophysiological data showed that the downregulation of DA results into the reduction of both glutamatergic and GABAergic synaptic transmissions. Despite these synaptic transmission changes, the excitatory-inhibitory ratio was not altered by the 73% reduction of DA expression. These data indicated that 27% of residual DA was sufficient to maintain the functional balance between excitation and inhibition. Thus, the remaining DA expression can participate to the homeostatic mechanism that maintains the normal excitatory-inhibitory functional balance. The importance of our results is emphasized also by the recent discovery that a decrease level in DA content is reported in the superior temporal cortex from no cognitive impairment to MCI and to Alzheimer's disease. Furthermore, the level of DA expression has been reported to correlate well with the

severity of cognitive impairment.[28] These observations suggested that a critical amount of DA protein might be required for normal function. Therefore, the misregulation of the actin-based machinery by the loss of DA protein will lead to alteration of synaptic transmission underlying the cognitive impairment accompanying neurological disorders.[37] Thus, we propose a model (see Fig. 1) in which alterations in the amounts of DA (overexpression or underexpresssion) might result in strengthening or weakening of synaptic transmission and in turn could modulate the normal excitatory-inhibitory balance. In all cases, these synaptic alterations result in synaptic dysfunction, which according to different brain regions involved, may underlie some neurological disorders.

In summary, the work of Ivanov and colleagues[29] provides the first evidence for the involvement of DA in the regulation of excitatory and inhibitory synaptic transmission in mature hippocampal neurons. Future work on the identification and characterization of new synaptic partners of DA involved in synaptogenesis is needed to clarify the physiological mechanisms of synaptic plasticity as well as the cellular, molecular and physiological defects found in neurological disorders.

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