Synaptic Plasticity in Angelman Syndrome

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ABSTRACT : Angelman syndrome (AS) is a neurodevelopmental disorder characterized by intellectual disability and autism. The genetic cause is the absence of UBE3A, an E3 ubiquitin ligase, from the maternal chromosome which can arise from multiple origins. Recently discovered targets of Ube3a are important for activity dependent changes in synaptic transmission and spine morphology. Plasticity studies in an AS mouse model is important for basic plasticity research with regard to understanding protein homeostasis as well as the search for therapeutic targets for the patients. The progress on synaptic plasticity from this unique disorder is reviewed.

Key words : Angelman syndrome, Plasticity

INTRODUCTION

One goal of animal models for neurodevelopmental disorders is to find the mechanism underlying human symptoms and clues for therapy targets. Transgenic animal models are validated through comparison of the animal phenotypes and human symptoms. The next step is to explain the pathophysiological process mediating the molecular defect to the animal's behavior. Synaptic dysfunction is common in neurodevelopmental disorders (Zoghbi and Bear, 2012). Synaptic plasticity is sensitive to abnormal changes in the synapse. Long-term potentiation (LTP) and long-term depression (LTD) are robust phenomena with easy induction. From the study of these phenomena, discovery of therapeutic targets is possible. An example is the enhancement of metabotropic glutamate receptor (mGluR) dependent LTD in Fragile X syndrome, where LTD investigation led to the discovery of signaling pathway altered in this disease and now a target of clinical trials (Bhakar et al., 2012).

Angelman syndrome (AS) is characterized by profound intellectual disability, movement disorders, absence of speech, epilepsy, and autistic behaviors (Buntinx et al., 1995). The molecular defects for AS include maternally derived micro-deletions of 15q11-q13, point mutations in the maternal copy of UBE3A gene, imprinting center defects, and paternal uniparental disomy (Dan, 2009). Despite the different molecular defects, deficiency of brain-specific maternally expressed UBE3A gene is responsible for most clinical features in AS (Moncal et al., 1999). To model AS in mice, the first Ube3a knock-out mouse targeting exon 2 of Ube3a was reported in 1998 that recapitulated the major features of AS in maternal deficiency mice (Ube3a m-/p+) (Jiang et al., 1998). Another Ube3a mutant mouse with a mutation in exon 10 encoding the ubiquitin ligase domain was also reported (Miura et al., 2002). However, the Ube3a exon 2 deletion mutant mice have been used more widely in the literature.

The expression of Ube3a is activity dependent, which predicts that activity dependent plasticity is impaired in the AS mouse (Greer et al., 2010). Plasticity in AS mouse is a clinical example in that the cause is the malfunction in the protein degradation. Protein degradation plays a role in plasticity and memory (Kaang and Choi, 2012). For example, activity dependent spine growth was decreased by proteasome inhibition (Hamilton et al., 2012). Defect in this process may cause structural and functional plasticity

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impairments, which in turn contribute to behavioral symptoms. The understanding of the plasticity process may help identify therapeutic targets for AS, but it also provide a chance for better understanding plasticity itself.

PLASTICITY

Plasticity as well as basal neurotransmission in hippocampus have been tested because maternal copy of Ube3a was absent in hippocampal neurons and hippocampus dependent memory was impaired in behavioral studies. Consistent with these data, LTP in CA1 was decreased in Ube3a m-/p+ compared to m+/p+. However baseline synaptic responses were not different (Jiang et al., 1998). In later studies, the difference in LTP was in induction threshold, not in the induction machinery itself. When a strong LTP induction protocol (3 times more trains) was used in CA1, Ube3a m-/p+ showed LTP comparable to that of wild type (WT) mice (Weeber et al., 2003; Table 1). In the same study, NMDA receptor-independent LTP was also impaired (three sets of 200 Hz trains with 4 min intervals).

In regard to the LTP threshold issue, a similar phenomenon was reported in other models such as Fmr1 knock-out (KO) (Fragile X syndrome model) mice and aged rats. In Fmr1 KO, initial reports did not find impaired LTP, but with a weaker stimulation protocol, reduced LTP was exposed (Lauterborn et al., 2007). This deficit could be reversed by brain-derived neurotrophic factor (BDNF) treatment. A similar phenomenon was reported from cognitively impaired aged rats (Tombaugh et al., 2002). In this case, a selective muscarinic M2 antagonist enhanced the impaired LTP to the level of cognitively unimpaired aged rats. It will be interesting to test if treatment with BDNF or M2 antagonists can also reverse the LTP deficit in Ube3a m-/p+ mice. Still, it is not clear what factors are responsible for the stimulation protocol effect on the maintenance of plasticity. For LTP maintenance, cytoskeletal stabilization is an important factor. Many other molecules including calcium are involved in this process, too. The importance of the intact induction machinery is in the possibility that activity modulation or chemical agents (BDNF, M2 antagonist) can alleviate learning and memory problem *in vivo*.

The other brain area subjected to plasticity studies was neocortex (Yashiro et al., 2009). In visual cortex, LTP was reduced in Ube3a m-/p+. In a similar fashion as in CA1, the difference disappeared if LTP was induced with a relatively stronger protocol (Table 1). Note that this

Table 1. Synaptic plasticity in Ube3a m-/p+

Brain areas	Plasticity types	Protocols	Effects	References
CA1	LTP	2 HFS, interval 20 sec	↓	Jiang et al., 1998
CA1	LTP	2 HFS, interval 20 sec	↓	Weeber et al., 2003
CA1	LTP	3 set of trains (10 min interval), one set (2 HFS, 20 sec interval)	NS	Weeber et al., 2003
CA1	LTP	200 Hz (100 pulse), 3 trains, interval 2 min	↓	Weeber et al., 2003
CA1	LTP	2 HFS, interval 20 sec	↓, rescue	van Woerden et al., 2007
visual cortex	LTP	40 Hz (40 pulse) trains, 3times (interval 10 sec)	↓	Yashiro et al., 2009
visual cortex	LTP	2 HFS, interval 15 sec	NS	Yashiro et al., 2009
visual cortex	LTD	1 Hz, 900 pulse	↓	Yashiro et al., 2009
CA1	LTP	2 HFS, interval 20 sec	↓, partial rescue	Daily et al., 2012

HFS (high frequency stimulation, 100Hz, 100 pulses), LTP (long-term potentiation), LTD (long-term depression), LFS (low frequency stimulation, 1Hz, 900 pulse), NS (no significant difference), CA1 (stimulation at Schaffer collateral, recording from striatum radiatum), visual cortex (stimulation at layer 4, recording from layer 2/3), ↓ (decrease), ↑ (increase)

stimulation was a modest stimulation protocol in CA1 hippocampus (Weeber et al., 2003). In neocortex as well as CA1, the impaired process is the maintenance. At least in the two brain regions, similar LTP mechanisms seem to be at work in the mutants. It is a question how far this can be generalized to other brain areas. Because cerebellum is another Ube3a imprinted region, plasticity data from the cerebellum is worth pursuing.

Long term depression (LTD) is a long-lasting decrease in synaptic transmission (Collingridge et al., 2010). Two major types of LTD are *N*-Methyl-D-aspartate (NMDA) dependent and mGluR dependent ones. In Ube3a m-/p+ neocortex, NMDA dependent LTD was reduced (Yashiro et al., 2010). LTD in hippocampus was not reported yet, but it is expected that NMDA dependent LTD in CA1 changes in a similar fashion as in neocortex. mGluR LTD in CA1 should also be examined. This type of LTD is impaired in other neurodevelopmental disorders. Interesting examples are the models for Tuberous Sclerosis Complex (TSC) and Fragile X syndrome. DHPG induced group I mGluR LTD (DHPG LTD) is increased in Fmr1 KO, but decreased in Tsc2+/- (Auerbach et al., 2011). In both disorders, gene transcription has increased, so the different subset of genes altered in each disorder may determine the direction of LTD change.

There are interesting new findings from the visual cortex. For plasticity deficits to occur, visual experience is required (Yashiro et al., 2010). Without visual experience as in dark rearing, plasticity was restored in both LTP and LTD experiments on m-/p+ mice. This is probably because activity dependent proteins were not expressed in the dark and thus not subject to Ube3a dysregulation. In another report from visual cortex, the ocular dominance plasticity after monocular deprivation was present, but at a slower speed and with a smaller magnitude in m-/p+ (Sato and Stryker, 2010). At least in CA1 and visual cortex, absence of the maternal copy of Ube3a impaired synaptic plasticity. This may affect normal synapse development during critical periods.

MOLECULAR PATHWAY

CaMKII is important in intracellular signaling related to plasticity. In m-/p+ CA1, increased inhibitory autophophorylation of CaMKII was found (Weeber et al., 2003). This reduction of CaMKII activity in the postsynaptic compartment could reduce plasticity. In a rescue experiment, mutation at the inhibitory autophosphorylation site of CaMKII (CaMKII-T305/T306A) reversed the LTP deficit (van Woerden et al., 2007). This manipulation also reduced audiogenic seizures by 75% in the mutant compared with m-/p+ mice lacking CaMKII mutations. It is an interesting correlation of LTP rescue and seizure rescue by enhanced CaMKII activity. This suggests that the reduction of CaMKII activity is a common cause for LTP impairment and seizure.

The increase or decrease of 2-amino-3-(5-methyl-3-oxo-1,2-oxazol-4-yl) propanoic acid (AMPA) receptors on postsynaptic spines in CA1 is correlated with the expression of LTP or LTD respectively. In cultured neurons of Ube3a knock-down by shRNA, the GluR1 subunit of the AMPA receptor is reduced in the plasma membrane while NR1 subunit of NMDA receptor is unchanged (Greer et al., 2010). This pattern of receptor subunit changes was consistent with AMPA and NMDA receptor mediated current changes. In CA1, AMPA current evoked by electrical stimulation is decreased in m-/p+, while NMDA current is unchanged (Greer et al., 2010). In spontaneous activity, miniature excitatory postsynaptic current (mEPSC) frequency, not amplitude, was decreased in visual cortex and CA1 (Yashiro et al., 2009; Greer et al., 2010). In contrast to the excitatory neurotransmission, miniature inhibitory postsynaptic current (mIPSC) was not changed (Greer et al., 2010). These data support the idea that excitatory neurotransmission through AMPA receptors is decreased, which mediates the plasticity impairment in m-/p+.

Spine morphology in parallel with neurotransmission changes in an activity dependent way. Ube3a is expressed in the spine as well as in the nucleus (Dindot et al.,

2008). Spine number was decreased in CA1 and cortex of m-/p+ mice, and spine length was decreased in CA1 (Dindot et al., 2008). Interestingly, spine density was dependent on visual experience because the density difference disappeared in dark reared m-/p+ mice (Yashiro et al., 2009). This indicates that Ube3a has an important role in translating neural activity into spine morphology.

The current data strongly suggest that Ube3a binding proteins are related to plasticity or excitatory neurotransmission. Because the main function of Ube3a is to promote degradation of proteins, the absence of Ube3a in m-/p+ is expected to increase the target proteins above the normal level which in turn cause the cellular defects. The search for the binding proteins is critical for understanding the molecular pathways to behavioral deficits in m-/p+ mice. In addition to p53, two more targets were discovered recently, Arc and ephexin 5 (Jiang et al., 1998; Greer et al., 2010; Margolis et al., 2010). Arc and ephexin 5 are especially relevant to synaptic plasticity. Arc is important for both LTP and LTD (Bramham et al., 2008; Shepherd and Bear, 2011). The primary function of Arc is mediating AMPA receptor endocytosis. Increased Arc protein may explain the change in both LTP and LTD in Ube3a m-/p+ mice. Arc is a target protein common to Fragile X syndrome (FXS) and AS (Shepherd and Bear, 2011). Arc mRNA is regulated by fragile X mental retardation protein (FMRP) and Arc protein degradation is mediated by Ube3a. This gives motivation to compare plasticity and signaling pathways between the two disorders. In mGluR LTD, FMRP inhibits Arc translation while phosphorylated elongation factor 2 (P-EF2) stimulates the process (Park et al., 2008). In Fmr1 KO mice, P-EF2 increase upon DHPG stimulation is higher than that of WT (Ronesi et al., 2012). The dysregulation of this pathway should be tested in Ube3a m-/p+.

Ephexin regulates spine morphology (Margolis et al., 2010). In a general scheme, ephrin activates its receptor, Eph, to phosphorylate ephexin acting as guanine exchange factor (GEF) for RhoA. Activated RhoA eventually inhibits cytoskeleton. Ephexin 5 binds to EphB2 which increases spine density if stimulated by Ephrin-B1/B2 (Margolis et al., 2010). Ube3a absence leads to abundant ephexin 5 which in turn inhibits EphB2 and spine growth. The morphological phenotype in Ube3a m-/p+ may be due to the uncontrolled ephexin 5 at least in some degree. Furthermore, ephexin 5 may affect activity dependent plasticity by modulating the signaling pathway downstream of ephrin and Eph. EphB and ephrin B both are implicated in LTP and LTD (Grunwald et al., 2001; Grunwald et al., 2004). Ephrin activation facilitates DHPG LTD (Piccinin et al., 2010). If this facilitatory effect was decreased or absent in AS, DHPG LTD may decrease in Ube3a m-/p+ contrary to the prediction from increased Arc and AMPA receptor endocytosis. Ephexin 5 may be another critical molecule for activity dependent plasticity in addition to Arc.

FUTURE DIRECTION AND CONCLUSIONS

The understanding of neural plasticity in Ube3a mutant mice underwent great progress recent years. However, much work is in need to catch up with the mechanistic understanding we have of the synaptic defects of Fragile X syndrome. There is also a question of whether activation of the paternal copy of Ube3a will rescue the plasticity and seizure abnormalities (Huang et al., 2011; Daily e al., 2011). LTD in hippocampus has not been studied in detail yet. In particular, mGluR LTD is an important experiment to undertake, the results of which will allow us to compare Ube3a m-/p+ with other models of neurodevelopmental disorders (Auberbach et al., 2011).

1. Plasticity in Inhibitory Transmission and Presynaptic Plasticity

Plasticity in Ube3a m-/p+ focused on glutamatergic neurotransmission and excitatory synapses through studies of dendritic spines. This was reasonable considering decreased AMPA current, mEPSC frequency decrease and

no change in mIPSC. At the same time, reduced excitation and increased seizure activity is a puzzle. However, decreased inhibitory transmission was found in young adult Ube3a m-/p+ mice (Wallace et al., 2012). After an electrical stimulus train (10 Hz, 800 stimuli), the recovery of inhibitory transmission was much slower in Ube3a m-/p+ slices. The reduced inhibition during this period may contribute to hyperexcitability. One candidate mechanism for the slow recovery is the decreased number of synaptic vesicles at the presynaptic terminal of GABAergic interneurons. This also points to the importance of the presynaptic site of plasticity which needs to be further studied. More work on the plasticity of inhibitory transmission can give hints to seizure generation mechanism in Ube3a m-/p+.

2. Epilepsy and Plasticity

Epilepsy is a very common comorbidity with neurodevelopmental disorders (Leung and Ring, 2011). The cellular process of plasticity and epileptogenesis may be related in this group of patients. Hyperexcitability and plasticity may share signaling pathways in the spine (McNamara et al., 2006). CaMKII and calcineurin are key molecules to LTP and LTD, but they are also important in epileptogenesis. Reduction of CaMKII can induce epileptogenesis, while inhibition of calcineurin impairs epileptogenesis (McNamara et al., 2006). According to this idea, the net dephosphorylation is positively correlated with epileptogenesis. The finding of the key substrates regulated by phosphorylation is critical. Plasticity is a good tool to find the substrate. If a certain molecule's phosphorylation state is found to be altered from the plasticity work, the molecule can be tested for its role in epileptogenesis.

The Ube3a m-/p+ model mouse was the first example in mammals to show that protein degradation had a role in synaptic plasticity. This is an important animal model to study the role of protein homeostasis in plasticity. Research in this direction can also help to find therapeutic targets for AS patients.

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