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Impairment in extinction of auditory fear memory in syntenin-1 knockout mice

シンテニンー１ノックアウトマウスにおける音恐怖条件付け記憶の消去障害

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【学位論文内容の要約】

Objective

Syntenin-1 (Syndecan-binding protein, Sdcbp) is a PDZ domain-containing intracellular scaffold protein for various receptor proteins involved in exosome production, synapse formation, and synaptic plasticity. Since syntenin-1 has the ability to interact with all four ionotropic α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA)-type GluR subunits (AMPARs GluA1-4), we speculated that syntenin-1 may have the role to regulate learning and memory through effects on synaptic plasticity *in vivo*.

Materials and Methods

1) Animals: Syntenin-1 knockout (KO) and wild type (WT) mice were produced by breeding homozygous parents and housed in a temperature-controlled environment under a 12 h light/dark cycle as previously described. Animal care and experimental protocols were approved by the Animal Experiment Committee of the University of Toyama (Authorization No. A2012MED-35 and A2015MED-25).

2) Western blot and immunohistochemical analyses: The protein samples extracted from amygdala of the mouse brain were separated by SDS-PAGE and blotted on PVDF membranes. The membranes were incubated with the primary antibodies and then with HRP-conjugated secondary antibodies. Protein bands were detected using a LAS4000 mini-digital imager (GE Healthcare). The WT and syntenin-1 KO mice were deeply anesthetized and perfused transcardially with ice-cold PBS followed by 4% paraformaldehyde. Brains were removed, post-fixed, and transferred into a 30% sucrose/PBS solution. Brains were frozen and cut into 30-μm thick coronal sections using a freezing microtome (LEICA CM 1850). Free-floating brain sections were incubated with the primary antibodies, then with the secondary antibodies. Images were obtained using a confocal laser scanning microscope (Leica TCS SP5 II).

3) Behavioral tests: The open field test was performed to measure locomotor activity and basal anxiety in WT and KO mice using SCANET MV-40 (MELQUEST Co.). Auditory fear conditioning was conducted in a conditioning chamber (17 × 15 × 13 cm (CL-M3, O'Hara & Co.)). Mice were placed in the conditioning chamber for 60 s and then presented with a tone of 65 dB for 30 s.

At the end of the tone presentation, the mice were given a footshock (0.75 mA, 2 s). The conditioning protocol consists of three tone−shock pairings at 1 min intervals. Extinction training was conducted 24 h after fear conditioning. Animals received 18 tone presentations (30 s) at random intervals in the same chamber used for the auditory-cued test. Contextual fear memory and auditory-cued fear memory were evaluated as the percent freezing.

Results and Discussion

1) Syntenin-1 is expressed in amygdala, medial prefrontal cortex, hippocampus, cerebral cortex, thalamus, and hypothalamus.

2) Genetic disruption of syntenin-1 had little effect on contextual and auditory fear conditioning. In addition, no changes in locomotion and anxiety level were observed in syntenin-1 KO mice in open-field test.

3) The WT and syntenin-1 KO mice showed the similar level of freezing in fear conditioning and in extinction training. However, the syntenin-1 KO mice showed the higher levels of freezing than the WT mice during the auditory cued test after the extinction, which suggest the selective impairment in extinction of auditory fear memory in syntenin-1 KO mice.

4) This extinction deficit was associated with reduced c-Fos-positive neuron number in the BLA and IL after the extinction training and increased c-Fos-positive neuron number in BLA after the cued test in syntenin-1 KO mice compared with WT mice.

5) The expression levels of phospho-GluA1 at serine-831, phospho-GluA1 at serine-845 and total GluA1 in the amygdala were comparable between WT and syntenin-1 KO mice at basal state, after fear conditioning, and after extinction training.

Conclusion

Selective impairment of cued fear memory extinction in syntenin-1 KO mice is associated with decreased activation of c-Fos neurons of BLA and IL after extinction training.