Role of Dopamine D2 Receptor/Fatty Acid-Binding Protein 3 Signaling in Nicotine-Induced Addiction

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（ニコチン依存症におけるドパミン D2 受容体と脂肪酸結合タンパク質 3 シグナルの役割）

[Background]

Smoking was reported as the second leading reason for disability and early death in 2015. Smoking causes blood vessels to become thicker and narrower, which could increase cardiac beat and blood pressure. Moreover, smoking leads to 90% of all lung cancer deaths, and causes approximately 80% of all deaths from chronic obstructive pulmonary disease. Nicotine is a predominant addictive compound present in tobacco and exerts physical and psychological dependence through activating nicotinic acetylcholine receptors in the central nervous system (CNS). Nicotine exerts its physiological function by interacting with nicotinic acetylcholine receptors (nAChRs) in the ventral tegmental area (VTA), through binding with pentameric ligand-gated ion channels. Numerous investigations have reported that dopamine D1 receptors (D1Rs) are fully involved in nicotine-induced addiction, and participate in signaling pathways related with addiction. Recently, Emerging evidence demonstrated that not only D1Rs, dopamine D2 receptors (D2Rs) are also critical for the acquisition of nicotine-induced behaviors. Our previous data suggested that D2R knockout ($D2R^{-/-}$) mice failed to exhibit conditioned place preference (CPP) scores following 28 days of consecutive nicotine administration.

Long-chain polyunsaturated fatty acids (LCPUFAs) are fatty acids with 18-20 carbons or more, and are abundantly expressed in the brain and retina. It is necessary for fatty acid-binding proteins (FABPs) to function as cellular shuttles to transport LCPUFAs to proper intracellular compartments since LCPUFAs are not soluble in water. Among FABPs, FABP3 is the particular isoform expressed in neurons in the mature brain. FABP3 is associated with D2R, and especially correlated with D2LR and binds with D2LR at the insert region of 29 amino acid sequences (G242-V270) in the third cytoplasmic loop. Moreover, previous investigations defined that fatty acid-binding protein 3 (FABP3) is critical for dopamine D2 receptor (D2R) function in the mouse striatum, suggesting that D2R and FABP3 signaling mediates the extrapyramidal behaviors in mice. In this context, we hypothesize that FABP3 mediates the nicotine-induced CPP in mice. To define how FABP3 elicits the D2R-mediated psychomotor behaviors, nicotine-induced CPP behaviors were investigated in FABP3 null ($FABP3^{-/-}$) mice.

[Methods]

Nicotine-induced CPP behaviors were evaluated with preference scores using CPP apparatus. The acclimatization phase persisted for 4 days. In this period, each mouse was placed into the neutral compartment and was allowed to freely explore all three compartments for a total of 10 min. On day 5, the preconditioning test was performed. Each mouse was placed in the neutral compartment for 5 min with two guillotine doors closed. Afterwards, two guillotine doors were removed, and each mouse was allowed to access all three compartments for 15 min. The formula for documenting CPP preference score was as follows: Preference score = Retention time in related conditioning compartment/Total time in all conditioning compartments × 100%. Subsequently, in the conditioning phase, mice were administered

nicotine or saline and were confined in the related compartments with guillotine doors closed for 30 min daily for 14 consecutive days. CPP preference scores were calculated using the same method as described above. In the withdrawal phase, mice received saline administration prior to placement in the related compartments for 5 days. In the nicotine relapse phase, mice were administered saline or nicotine once. Thirty minutes later, each mouse was placed in the neutral compartment in turn, and allowed to freely explore for the whole three compartments for 15 min.

Afterwards, the responsiveness of Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) autophosphorylation and extracellular signal-regulated kinase (ERK) phosphorylation levels were examined by immunoblotting assay using the nucleus accumbens (NAc) and the hippocampal CA1 brain region extracts. The neuronal activities of NAc neurons with D1Rs and D2Rs were evaluated by calculating the number of immunoreactive double-positive cells with c-Fos and autophosphorylated CaMKII immunoreactivities.

[Results]

In CPP behavioral tests, we observed significantly increased CPP scores in 14 consecutive days of nicotine-treated WT mice in the comparison of base level of precondition in the CPP conditioning phase. However, the failure of CPP induction in FABP3$^{-/-}$ mice following 14 consecutive days of nicotine exposure was observed in the nicotine conditioning phase. Identical to the results in the conditioning phase, the complete inhibition of nicotine-induced CPP scores were also observed in the withdrawal and nicotine relapse phases.

Consistent with failed induction of CPP behaviors, FABP3$^{-/-}$ mice showed lack of responsiveness of CaMKII autophosphorylation and ERK phosphorylation levels in the context of nicotine exposure in the NAc and the hippocampal CA1 region. Interestingly, we observed a significantly increased basal level of CaMKII autophosphorylation in saline-treated FABP3$^{-/-}$ mice NAc relative to that in WT mice. Although we did not observe significantly increased basal levels of ERK phosphorylation in FABP3$^{-/-}$ NAc, the trend toward enhancement of ERK phosphorylation levels was observed.

In the purpose of elucidating mechanism underlying significantly increased basal level of CaMKII autophosphorylation in FABP3$^{-/-}$ mice NAc, and insufficient responsiveness of CaMKII activities to chronic nicotine exposure, we next carried out experiments in evaluating CaMKII autophosphorylation levels in D1R- and D2R- positive cells in the NAc by using immunofluorescence staining, since the equilibrium of D1Rs and D2Rs are reported to involved in neuropsychiatric disorders including addiction, and CaMKII activities are important in D1R- and D2R-mediated signaling. Intriguingly, CaMKII autophosphorylation level in FABP3$^{-/-}$ mice D1R in the NAc, which evaluated by calculating the number of D1R/pCaMKII double-positive cells, was significantly decreased relative to the basal level in similarly treated WT mice. Importantly, a lack of responsiveness in terms of CaMKII autophosphorylation level to nicotine treatment in D1R-positive cells, was also observed in FABP3$^{-/-}$ mice NAc. However, conversely, a significantly increased basal number of D2R/pCaMKII double-positive cells in the NAc were observed

in FABP3$^{-/-}$ mice administered saline. Moreover, a lack of responsiveness of CaMKII autophosphorylation level to nicotine treatment in D2R-positive cells in the NAc, was observed in FABP3$^{-/-}$ mice.

Subsequently, we observed that c-Fos levels were closely correlated with CaMKII autophosphorylation levels in D2R-positive cells in the NAc. We found a significantly increased number of D2R-positive cells and lack of responsiveness of nicotine-induced elevation in the number of D2R-positive cells in FABP3$^{-/-}$ mice. We then analyzed c-Fos activities in D2R-positive cells. As a result, a significantly increased number of D2R/c-Fos double-positive cells and lack of responsiveness of c-Fos activities to chronic nicotine treatment were observed in FABP3$^{-/-}$ mice NAc

[Conclusion]

In summary, we demonstrate for the first time that FABP3$^{-/-}$ mice failed to establish nicotine-induced CPP behaviors in conditioning, withdrawal, as well as nicotine relapse phases. These impaired acquisition of CPP behaviors were closely correlated with lack of responsiveness of CaMKII autophosphorylation and ERK phosphorylation levels in the NAc and hippocampal CA1 region. Whereas, a significantly elevated CaMKII autophosphorylation level was observed in FABP3$^{-/-}$ mice NAc. Consistent with CaMKII autophosphorylation level, we observed that c-Fos level was identically robustly reduced in D1R-positive cells, while rapidly elevated in D2R-positive cells in FABP3$^{-/-}$ mice NAc. Collectively, our results indicate that FABP3 could be a novel therapeutic target intended to treat nicotine-induced addiction, and other psychostimulant-induced addictive behaviors which affect DAergic systems in the CNS.

[Discussion]

Numerous investigations indicate that D1R and D2R are both positively involved in nicotine and other psychostimulant-induced behaviors. Although some contradictory studies exist, we speculated that aberrant D2R signaling due to lack of FABP3 through affecting D1R, causes impaired acquisition of nicotine-induced CPP behaviors. We found the significantly decreased number of D1R-positive cells and increased number of D2R-positive cells while functions are abnormal, in FABP3$^{-/-}$ mice NAc. Indeed, the balance between D1R and D2R in the NAc is crucial, whereas we did not observe that FABP3 deficiency facilitates D1R functions in nicotine-induced addiction. Further investigations in detailed correlation between D1R and D2R in drug of abuse are still required.

We provided a possible schematic representation to explain the failure of nicotine-induced CPP in FABP3$^{-/-}$ mice. The cAMP/Ca^{2+} signaling which was originally regulated by negative D2R/FABP3 signaling in WT mice NAc, was derepressed in FABP3$^{-/-}$ mice due to the aberrant D2R signaling through FABP3 deficiency, thereby causing significantly elevated basal levels of CaMKII autophosphorylation and CREB/c-Fos signaling. These constitutive elevations failed to induce nicotine-induced CPP behaviors in FABP3$^{-/-}$ mice.

However, some opposing investigations reported that an increase in intracellular levels of Ca^{2+}/CaMKII induced by D2R stimulation through agonist quinpirole was observed in NG108-15 cells overexpressing D2R. However, this study was carried out in cell lines but not in mice. D2LRs partially but not thoroughly

interact with FABP3, and some of D2LRs mediate CaMKII independent of FABP3. Moreover, the functions of FABP3 in which it was not colocalized with D2LR are still unclear. Furthermore, CaMKII autophosphorylation levels in D1R null mice should be addressed in future investigations.