Role of dopamine D2 receptor in the generation of nicotine dependence(ニコチン依存性形成におけるドパミンD2受容体の役割に関する研究)

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Role of dopamine D2 receptor in the generation of nicotine dependence

(ニコチン依存性形成におけるドパミン D2 受容体の役割に関する研究)

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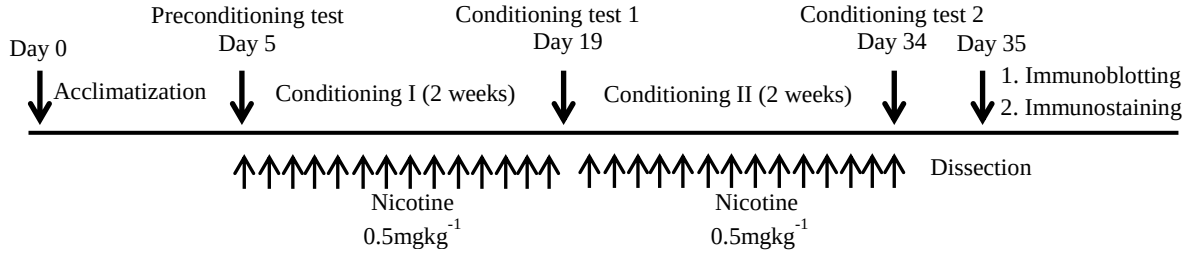
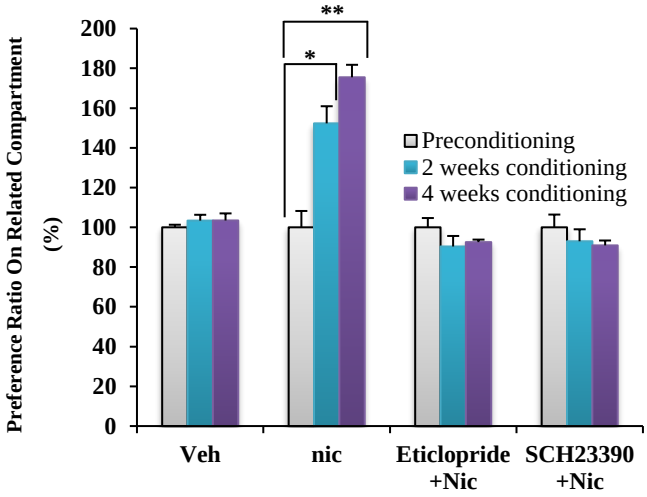
Abstract

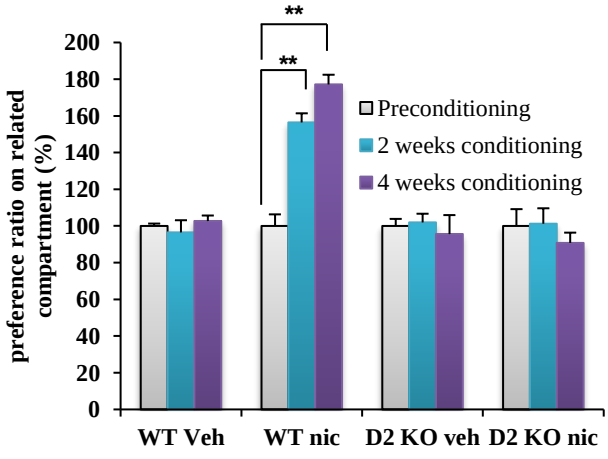
Nicotine is a stimulatory component in tobacco and induces psychological dependence through its rewarding effect in the central nervous system (CNS), thereby leading to the nicotine addiction. The therapeutics of nicotine dependence to improve nicotine addiction and quality of life have not been established. We first found that curcuminoid, anti-inflammatory agent prevents both nicotine dependence and relapse when mice were assessed by the conditioned placed preference (CPP) test. Curcuminoid (1, 3.2, and 10 mg·kg^{-1}, oral) dose-dependently inhibited development of nicotine dependence when it was administrated 30 minutes prior to nicotine administration (0.5 mg·kg^{-1}, i.p.) for consecutive 7 days. In addition, curcuminoid significantly suppressed the priming effects of nicotine, in which inhibition of acetylcholinesterase activity is associated. However, further extensive studies are required to define the inhibitory mechanism of acetylcholinesterase activity by curcuminoid.

We performed CPP test for 28 days administration of nicotine to establish the nicotine-induced CPP as representative of nicotine reward and reinforcement. Since dopamine mediates nicotine dependence through the nucleus accumbens and hippocampus, we assessed Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) and extracellular signal-regulated kinase (ERK) signals.



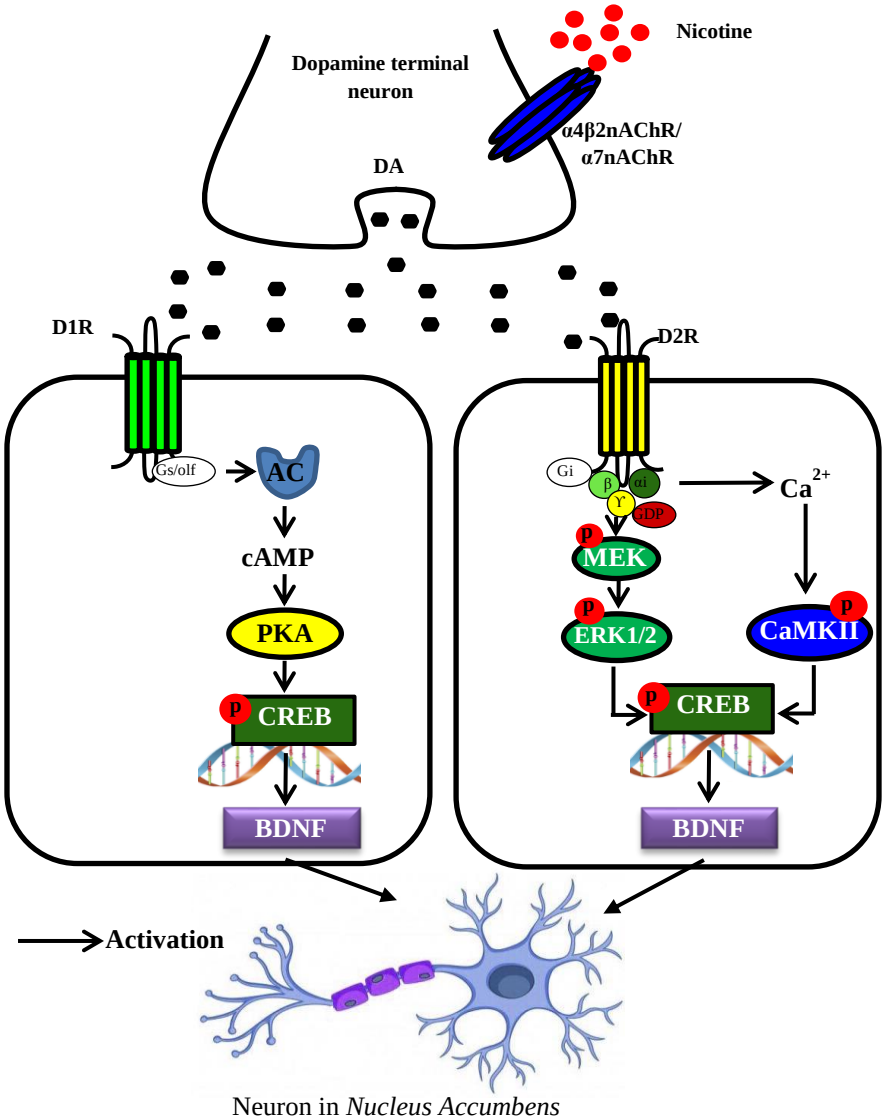


We first found that, in addition to D1R antagonist (SCH23390), D2R antagonist (eticlopride) administration with 30 minutes prior to nicotine administration completely abolishes the nicotine dependence in both 2 and 4 weeks nicotine conditioning mice as shown in figure. Although the involvement of dopamine D1 receptor (D1R) has been well documented in the nicotine dependence, however, the role of D2R remains unclear. Thus, we investigated whether D2R is critical for nicotine dependence behaviors using D2R knock out (D2RKO) mice. We first assessed the nicotine-induced CPP behaviors in wild type (WT) and D2RKO mice. D2RKO mice were then subjected to neurochemical analyses after nicotine-conditioning with CPP. Interestingly, D2RKO mice failed to develop the nicotine-induced CPP behaviors after continuous nicotine administration with 0.5 mg/kg for 4 weeks as shown in figure.



Since both nucleus accumbens and hippocamus have central role in the nicotine-induced CPP behaviors, we investigated both CaMKII and ERK signaling both in the nucleus accumbens and hippocampal CA1 region after 4 weeks administration of nicotine. Notably, both CaMKII and ERK phosphorylation in both regions were elevated by nicotine administration in WT mice. However, these kinase pathways were unchanged in D2RKO mice. Moreover, the basal levels of CaMKII and ERK phosphorylation were significantly reduced in D2RKO mice. Consistent with immunoblotting results, immunostaining analyses showed the number of phosphorylated CaMKII and ERK positive cells in nucleus accumbens elevated on WT mice but not in D2KO mice after nicotine treatment for 4 weeks administration.

The nicotine-induced CPP behaviors were associated with elevation of Pro-BDNF and BDNF protein levels in WT mice. On the other hand, Pro-BDNF and BDNF protein levels were unchanged after chronic nicotine administration for 28 consecitive days in D2RKO mice. Taken together we propose the possible mechanism how the dopamine D2R pathway generates nicotine-induced CPP behaviors. Nicotine administration activates α4β2nAChRs and α7nAChRs in the dopaminergic terminals as shown in figure. Nicotine administration enhances DA release by stimulation of both nAChRs. The released DA stimulates D2R thereby enhancing MEK and ERK1/2 pathway through enhancement of cytosolic tyrosine kinase and in turn causes CREB phosphorylation in nucleus accumbens. Additionally, D2R activation elevates CaMKII signaling by elevation of intracellular calcium. The CaMKII activation also mediates CREB phosphorylation followed by pro-BDNF production. The pro-BDNF is degraded by proteolytic enzyme to produce mature BDNF. BDNF may be essential for synaptic rearrangement accounting for nicotine-induced CPP behavior. As shown previously, D1R stimulation also mediates BDNF expression through cyclic AMP-dependent protein kinase (PKA) pathway in the different neurons from D2R expressing neurons.



Neuron in *Nucleus Accumbens*

In conclusion, activation of CaMKII and ERK1/2 through D2R are associated with nicotine-induced CPP behavior. Like D1R, D2R is critical for nicotine-induced CPP behavior. We also propose curcuminoid as therapeutic candidate to ameliorate nicotine dependence and relapse.

別紙様式（Ａ４版）

論文審査結果の要旨

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論 文 題 目:Role of dopamine D2 receptor in the generation of nicotine dependence(ニコチン依存性形成におけるドパミン D2 受容体の役割に関する研究)

タバコの成分であるニコチンは中枢神経系を活性化する物質であり、ドパミン系などの報酬系を活性化してニコチン依存性を生じる。慢性喫煙は高血圧等の循環器疾患を起こすことから、禁煙によるニコチン依存症を改善することは生活習慣病の治療にも繋がる。本研究では天然に存在するクルクミノイド（クルクミン類）がニコチン依存形成を抑制することを証明した。その機序の１つにはアセチルコリン分解酵素の阻害作用が関与することを証明した。その効果は代表的アセチルコリン分解酵素阻害薬ドネペジルと同等であり、重要な知見である。クルクミン類は抗炎症、抗酸化作用、抗がん作用が報告されており、食品にも使われることから安全性の高いサプリメントとして期待できる。次に、ニコチン依存形成にドパミン D2 受容体が関与することを D2 受容体欠損マウスで証明した。これまでにも D1 受容体が cAMP 系を介してニコチン依存形成に関わることが報告されているが、D2 受容体の役割については不明である。本研究では D2 受容体欠損マウスでニコチン依存が形成されないこと、ニコチン依存形成には脳内でのカルシウム依存性プロテインキナーゼの活性化とその活性化による神経栄養因子（BNDF）の誘導が重要であることを明らかにした。さらに、ニコチン依存形成に重要な側座核において D2 受容体欠損マウスでは D1 受容体の機能も低下することを明らかにした。以上のことから、ニコチン依存形成を抑制する治療薬開発には D2 受容体を標的にすることが重要であること示した。

以上、本研究ではニコチン依存性形成におけるドパミン D2 受容体の重要な役割を明らかにした。さらに、食品にも使われるクルクミン類がニコチン依存形成を抑制することを明らかにした。今後は、クルクミン類の効能についてヒトでの検討が必要である。Wilar 氏の母国であるインドネシアでは少年期からのタバコ汚染が社会問題となっている。タバコ喫煙による依存症、生活習慣病を予防するためにも本研究が貢献することが期待できる。

よって、本論文は博士（薬科学）の学位論文として合格と認める。