京都大学	博士（医学）	氏　名	郭　婷婷
論文題目	A combination of dietary fat intake and nicotine exposure enhances CB1 endocannabinoid receptor expression in hypothalamic nuclei in male mice （高脂肪食とニコチンの複合作用としてのマウス視床下部 CB1 カンナビノイド受容体発現の増加）		

（論文内容の要旨）

Obesity and smoking habits, top-ranked risk factors for all-cause mortality, often co-exist in the same individual. Obesity is caused by a dysfunction of the hypothalamus, which is the regulatory center of appetite and energy expenditure. Obesity and substance abuse, such as tobacco smoking, share certain central mechanisms and the endocannabinoid system may play a part. Endocannabinoids are endogenous lipids that activates CB1 receptor (CB1R) and modulates synaptic transmission in appetite and reward centers of the brain. In fact, CB1R antagonist, rimonabant, has shown both potent weight-reducing and tobacco-quitting effects in clinical studies. However, detailed localization of CB1R within the hypothalamus and its potential regulation in obese smokers has not been fully elucidated.

Hypothalamic expression of CB1R was first examined by quantitative PCR analysis of micro-dissected brain regions and by immunohistochemistry in C57BL/6 mice. Secondly, receptor expression was analyzed in murine model of high fat diet (HFD)-induced obesity treated with nicotine.

CB1R mRNA was detected in all micro-dissected brain samples, including hypothalamic nuclei; arcuate (ARC), paraventricular (PVN), ventral/dorsal medial (V/DMH), and lateral (LH) and hippocampus. CB1R mRNA levels in ARC was significantly higher than PVN or V/DMH. CB1R expression was even higher in the hippocampus. By immunohistochemistry, CB1R protein was detected as punctate signal within the hippocampus and multiple hypothalamic region including ARC, PVN and V/DMH.

Intraperitoneal injections of nicotine (12ug/g body weight per day) significantly reduced food intake both in standard diet (STD)- and HFD-fed mice by 29±5% and 53±13%, respectively. Body weight was also decreased by nicotine in both diet groups, confirming that nicotine is a potent weight-reducing agent.

Expression of appetite-regulating neuropeptides was then analyzed in hypothalamic nuclei. mRNA expression of NPY, an orexigenic peptide, was decreased by nicotine in ARC by 48% only in HFD-fed obese mice. Reduction of NPY signaling may partly be attributable for the decrease in energy intake in HFD-fed, nicotine-treated animals.

Four-week HFD alone did not alter CB1R mRNA levels in most hypothalamic nuclei except a 20% decrease in PVN. Likewise, in lean mice fed STD, treatment with nicotine alone reduced CB1R expression only in LH by 17%. In contrast, when HFD-fed obese mice were administered with nicotine, CB1R mRNA expression was significantly augmented in ARC, PVN, V/DMH, LH and hippocampus by 45%, 65%, 34%, 50% and 31%, respectively. Since either HFD or nicotine alone did not provoke such an increase, enhancement of CB1R expression in all tested brain areas by HFD and nicotine together suggests a combinatorial effect of these two insults on the endocannabinoid system.

Recent years have witnessed the co-occurrence of multiple addictive behaviors, such as dependence on the game, food, drug and tobacco. Multiple addictions partially result from the same mechanism such as the malfunction of the reward system. Termination of one addictive behavior may worsen another. For example, smoking cessation is reported with body weight gain.

This study demonstrates hypothalamic CB1R distribution and shows that hypothalamic CB1R expression is enhanced only in mice fed HFD and administered with nicotine. The finding may suggest the presence of a certain common pathology underlying cross-addiction for palatable food and nicotine.

In conclusion, tissue distribution of CB1R endocannabinoid receptor in the hypothalamic nuclei is shown and the upregulation of the hypothalamic expression of endocannabinoid receptor is demonstrated in a murine model of obese smokers.

（論文審査の結果の要旨）

肥満と喫煙習慣はしばしば同一個体に重複して認められ、いずれも重大な死亡リスクとなる。肥満とタバコへの依存には一部共通の機序の関与が示唆されているが、詳細は未解明である。エンドカナビノイド受容体（CB1R）拮抗薬リモナバンは体重減少と禁煙補助に共に有効であることから、肥満と喫煙に共通の病因としてのエンドカナビノイド・システムの重要性が示唆される。しかし、食欲中枢である視床下部での CB1R の発現分布や、肥満・喫煙状態における発現変化は明らかではない。

本研究では、第１に独自のマイクロダイセクション法で採取したマウス視床下部神経核を用いた定量 PCR 解析と免疫染色により CB1R の発現分布を解析し、食欲関連神経核群における CB1R のmRNA とタンパク質発現を明らかにした。第２に高脂肪食により肥満を呈したマウスにタバコの主成分、ニコチンを腹腔内投与すると、視床下部における食欲促進因子 NPY の減少と食欲抑制因子 CRHR1 の増加を認め、ニコチンによる摂食量・体重減少効果の一部を説明するものと考えられた。一方、ニコチンの投与は高脂肪食マウスにおいて視床下部各神経核での CB1R 発現を増加させた。これら遺伝子発現変化は高脂肪食による肥満マウスでのみ観察されたことから、肥満とニコチン作用の複合的効果の存在が示唆された。

これら研究成果は、肥満・喫煙個体の脳に生じる病態生理の解明に寄与するところが多い。

したがって、本論文は博士（医学）の学位論文として価値あるものと認める。 なお、本学位授与申請者は、令和２年　２　月　２７　日に実施された論文内容とそれに関連した試問を受け、合格と認められたものである。

要旨公開可能日：　　年　　月　　日　以降