Table 2. Summary for changes in METH-induced behavior and dopamine release in MMP-2-(–/–) and MMP-9-(–/–) mice

	Wild-type	MMP-2-(–/–)	MMP-9-(–/–)
Conditioned place preference	↑	±	±
+TIMP-2-AS	N.D.		
+MMP inhibitor	↓		
Hyperlocomotion (single)	↑	±	±
+TIMP-2-AS	↑		
+MMP inhibitor	↑		
Locomotor sensitization (repeated)	↑	±	±
+TIMP-2-AS	↑↑		
+MMP inhibitor	↑		
Dopamine release	↑	±	±
+TIMP-2-AS	↑↑		
+MMP inhibitor	↓		

±, No change; ↑, Significant increase;↓, Significant increase; N.D., Not determined.

binding in wild-type mice, but such changes were significantly attenuated in MMP-2-(–/–) and MMP-9-(–/–) mice (19). These results suggest that the MMP/TIMP system is involved in the METH-induced dysregulation of dopamine release and receptor signaling. As dopamine D_2 receptors function in the feedback inhibition of dopamine release (40, 41), the downregulation may contribute to an enhancement of the METH-induced increase in extracellular dopamine levels.

Recently, Kim et al. (42) have demonstrated that MMP-3 has a specific role in dopamine neuronal degeneration. They suggested that the active MMP-3 released from stressed dopamine neurons is a candidate molecule that activates microglia, leads to production of superoxide, and plays a pivotal role in dopamine neuronal death; and they proposed that abrogation of MMP-3 or inhibition of MMP-3 activity in early neuronal degeneration may be an effective means of preventing progressive degeneration of dopamine neurons. This study strongly suggests that MMPs play a crucial role in the regulation of dopaminergic neurons in various diseases.

Conclusion

As reviewed in this article, MMP-2, MMP-9, and TIMP-2 are involved in the rearrangement of the neural network in the mesocorticolimbic dopamine system, which plays a crucial role in the development of behavioral sensitization to METH (Table 2). It is likely that the MMP/TIMP system plays a role in METH-induced behavioral sensitization through modulation of the function of plasma membrane proteins such as dopamine receptors and transporters. These results, together with the fact that MMP acts to degrade components of the ECM such as laminin and collagen IV, suggest that repeated METH-induced overexpression of MMP-2, MMP-9, and TIMP-2 is associated with the structural and functional changes in the mesocorticolimbic dopamine system, leading to METH-induced behavioral sensitization and reward following repeated drug treatment. We have proposed that some cytokines and neurotrophic factors such as basic fibroblast growth factor and brain-derived neurotrophic factor act as pro-addictive cytokines, whereas glia-derived neurotrophic factor and TNF-α act as anti-addictive cytokines, which reduce the rewarding effects of drugs of abuse (11). It appears that MMP-2 and MMP-9 can be classified as pro-addictive, whereas TIMP-2 may be anti-addictive. We propose that the dynamic changes to, and balance of, levels of pro-addictive and anti-addictive factors in the brain are determinants of susceptibility to drug dependence. Furthermore, our findings suggest that inhibitions of pro-addictive factors such as MMP-2 and MMP-9 may be effective in the treatment of drug dependence.

Acknowledgments

This study was supported in part by Grants-in-Aid for Scientific Research (No. 18790052 and 19390062); the 21st Century COE Program from the Ministry of Education, Culture, Sports, Science, and Technology of Japan; and a grant from the Smoking Research Foundation, Japan. This work was also supported by Japan Society for the Promotion of Science (JSPS) and Korea Science and Engineering Foundation (KOSEF) under the Japan-Korea Basic Scientific Cooperation Program.

References

1 Shippenberg TS, Heidbreder Ch. Sensitization to the conditioned rewarding effects of cocaine: pharmacological and temporal characteristics. J Pharmacol Exp Ther. 1995;273:808–815.

2 Berke J, Hyman ST. Addiction, dopamine, and the molecular mechanisms of memory. Neuron. 2000;25:515–532.

3 Ishikawa K, Nitta A, Mizoguchi H, Mouri A, Murai R, Miyamoto Y, et al. Effects of single and repeated administration of methamphetamine or morphine on neuroglycan C gene expression in the rat brain. Int J Neuropsychopharmacol. 2006;9: 407–415.

4 Nakajima A, Yamada K, He J, Zeng N, Nitta A, Nabeshima T. Anatomical substrates for the discriminative stimulus effects of methamphetamine in rats. J Neurochem. 2004;91:308–317.

5 Kamei H, Nagai T, Nakano H, Togan Y, Takayanagi M, Takahashi K, et al. Repeated methamphetamine treatment impairs recognition memory through a failure of novelty-induced ERK1/2 activation in the prefrontal cortex of mice. Biol Psychiatry. 2006;59:75–84.

6 Yan Y, Yamada K, Niwa M, Nagai T, Nitta A, Nabeshima T. Enduring vulnerability to reinstatement of methamphetamine-seeking behavior in glial cell line-derived neurotrophic factor mutant mice. FASEB J. 2007;21:1994–2004.

7 Nakajima A, Yamada K, Nagai T, Uchiyama T, Miyamoto Y, Mamiya T, et al. Role of tumor necrosis factor-alpha in methamphetamine-induced drug dependence and neurotoxicity. J Neurosci. 2004;24:2212–2225.

8 Mizoguchi H, Yamada K, Mizuno M, Mizuno T, Nitta A, Noda Y, et al. Regulations of methamphetamine reward by extracellular signal-regulated kinase 1/2/ets-like gene-1 signaling pathway via the activation of dopamine receptors. Mol Pharmacol. 2004;65:1293–1301.

9 Robinson TE, Kolb B. Persistent structural modifications in nucleus accumbens and prefrontal cortex neurons produced by previous experience with amphetamine. J Neurosci. 1997;17: 8491–8497.

10 Nestler EJ. Molecular basis of long-term plasticity underlying addiction. Nat Rev Neurosci. 2001;2:119–128.

11 Yamada K, Nabeshima T. Pro- and anti-addictive neurotrophic factors and cytokines in psychostimulant addiction. Ann N Y Acad Sci. 2004;1025:198–204.

12 Yamada K, Nagai T, Nabeshima T. Drug dependence, synaptic plasticity, and tissue plasminogen activator. J Pharmacol Sci. 2005;97:157–161.

13 Niwa M, Nitta A, Mizoguchi H, Ito Y, Noda Y, Nagai T, et al. A novel molecule "shati" is involved in methamphetamine-induced hyperlocomotion, sensitization, and conditioned place preference. J Neurosci. 2007;27:7604–7615.

14 Nagai T, Yamada K, Yoshimura M, Ishikawa K, Miyamoto Y, Hashimoto K, et al. The tissue plasminogen activator-plasmin system participates in the rewarding effect of morphine by regulating dopamine release. Proc Natl Acod Sci U S A. 2004; 101:3650–3655.

15 Nagai T, Noda Y, Ishikawa K, Miyamoto Y, Yoshimura M, Ito M, et al. The role of tissue plasminogen activator in methamphetamine-related reward and sensitization. J Neurochem. 2005;92:660–667.

16 Nagai T, Ito M, Nakamichi N, Mizoguchi H, Kamei H, Fukakusa A, et al. The rewards of nicotine: regulation by tissue plasminogen activator-plasmin system through protease activated receptor-1. J Neurosci. 2006;26:12374–12383.

17 Ishikawa K, Nitta A, Mizoguchi H, Mouri A, Murai R, Miyamoto Y, et al. Effects of single and repeated administration of methamphetamine or morphine on neuroglycan C gene expression in the rat brain. Int J Neuropsychopharmacol. 2006;9:407–415.

18 Mizoguchi H, Yamada K, Niwa M, Mouri A, Mizuno T, Noda Y, et al. Reduction of methamphetamine-induced sensitization and reward in matrix metalloproteinase-2 and -9 deficient mice. J Neurochem. 2007;100:1579–1588.

19 Mizoguchi H, Yamada K, Mouri A, Niwa M, Mizuno T, Noda Y, et al. Role of matrix metalloproteinase (MMP) and tissue inhibitor of MMP (TIMP) in methamphetamine-induced behavioral sensitization and reward: implications for dopamine receptor downregulation and dopamine release. J Neurochem. 2007;102:1548–1560.

20 Yong VW, Power C, Forsyth P, Edwards DR. Metalloproteinases in biology and pathology of the nervous system. Nat Rev Neurosci. 2001;2:502–511.

21 Ethell IM, Ethell DW. Matrix metalloproteinases in brain development and remodeling: Synaptic functions and targets. J Neurosci Res. 2007. In press.

22 Wright JW, Reichert JR, Davis CJ, Harding JW. Neural plasticity and the brain renin-angiotensin system. Neurosci Biovehav Rev. 2002;26:529–552.

23 Baricos WH, Cortez SL, el-Dahr SS, Schnaper HW. ECM degradation by cultured human mesangial cells is mediated by a PA/plasmin/MMP-2 cascade. Kidney Int. 1995;47:1039–1047.

24 Mannello F, Gazzanelli G. Tissue inhibitors of metalloproteinases and programmed cell death: conundrums, controversies and potential implications. Apoptosis. 2001;6:479–482.

25 Sternlicht MD, Werb Z. How matrix metalloproteinases regulate cell behavior. Annu Rev Cell Dev Biol. 2001;17:463–516.

26 Rivera S, Tremblay E, Timsit S, Canals O, Ben-Ari Y, Khrestchatisky M. Tissue inhibitor of metalloproteinases-1 (TIMP-1) is differentially induced in neurons and astrocytes after seizures: evidence for developmental, immediate early gene, and lesion response. J Neurosci. 1997;17:4223–4235.

27 Szklarczyk A, Lapinska J, Rylski M, Mckay RD, Kaczmarek L. Matrixmetalloproteinase-9 undergoes expression and activation during dendritic remodeling in adult hippocampus. J Neurosci. 2002;22:920–930.

28 Vaillant C, Didier-Bazes M, Hurter A, Bein MF, Thomasset N. Spatiotemporal expression patterns of metalloproteinases and their inhibitors in the postnatal developing rat cerebellum. J Neurosci. 1999;19:4994–5004.

29 Lo EH, Wang X, Cuzner ML. Extracellular proteolysis in brain injury and inflammation: role for plasminogen activations and matrix metalloproteinases. J Neurosci Res. 2002;69:1–9.

30 Nagy V, Bozdagi O, Matynia A, Balcerzyk M, Okulski P, Dzwonek J, et al. Matrix metalloproteinase-9 is required for hippocampal late-phase long-term potentiation and memory. J Neurosci. 2006;15:1923–1934.

31 Krekoski CA, Neubauer D, Graham JB, Muir DL. Metalloproteinase-dependent predegeneration in vitro enhances axonal regeneration within a cellular peripheral nerve grafts. J Neurosci. 2002;22:10408–10415.

32 Monard D. Cell-derived proteases and protease inhibitors as regulators of neurite outgrowth. Trends Neurosci. 1988;11:541–

544.
33 Uhm JH, Dolley NP, Oh LY, Yong VW. Oligodendrocytes utilize a matrix metalloproteinases, MMP-9, to extend processes along an astrocyte extracellular matrix. Glia. 1998;22:53–63.
34 Reeves T, Prins ML, Zhu JP, Povlishock JT, Phillips LL. Matrix metalloproteinases inhibition alters functional and structural correlates of deafferentation-induced sprouting in the dentate gyrus. J Neurosci. 2003;12:10182–10189.
35 Brown TE, Forquer MR, Cocking DL, Jansen HT, Harding JW, Sorg BA. Role of matrix metalloproteinases in the acquisition and reconsolidation of cocaine-induced conditioned place preference. Learn Mem. 2007;14:214–223.
36 Sulzer D, Chen TK, Lau YY, Kristensen H, Rayport S, Ewing A. Amphetamine redistributes dopamine from synaptic vesicles to the cytosol and promotes reverse transport. J Neurosci. 1995;15:4102–4108.
37 Khoshbouei H, Sen N, Guptaroy B, Johnson L, Lund D, Gnegy ME, et al. N-terminalphosphorylation of the dopamine transporter is required for amphetamine-induced efflux. PLoS Biol. 2004;2:387–393.
38 Goldstein RZ, Volkow ND. Drug addiction and its underlying neurobiological basis: neuroimaging evidence for the involvement of the frontal cortex. Am J Psychiatry. 2002;159:1642–1652.
39 Bowers MS, McFarland K, Lake RW, Peterson YK, Lapish CC, Gregory ML, et al. Activator of G protein signaling 3: a gatekeeper of cocaine sensitization and drug seeking. Neuron. 2004;42:269–281.
40 Bowyer JF, Weiner N. Modulation of the Ca^{2+}-evoked release of [3H]-dopamine from striatal synaptosomes by dopamine (D2) agonists and antagonists. J Pharmacol Exp Ther. 1987;241:27–33.
41 Lindgren N, Xu Z-QiD, Herrera-M M, Haycock J, Hokfelt T, Fisone G. Dopamine D2 receptors regulate tyrosine hydroxylase activity and phosphorylation at Ser40 in rat striatum. Eur J Neurosci. 2001;13:773–780.
42 Kim YS, Choi DH, Block ML, Lorenzl S, Yang L, Kim YJ, et al. A pivotal role of matrix metalloproteinase-3 activity in dopaminergic neuronal degeneration via microglial activation. FASEB J. 2007;21:179–187.

厚生労働科学研究費補助金（医薬品・医療機器等レギュラトリーサイエンス総合研究推進事業）
分担研究報告書

乱用薬物への渇望（薬物探索行動）の再燃の脳内機序解明およびその治療薬開発に関する研究

分担研究者：山本経之[1]
研究協力者：縄田陽子[1]、平仁田尊人[2]
（[1]長崎国際大学薬学部薬理学研究室、[2]米国国立衛生研究所薬物乱用研究所）

[研究要旨]

ストレスは薬物依存症患者における薬物への渇望再燃の誘発因子の１つと考えられている。本研究では、①覚せい剤methamphetamine（MAP）探索行動は、ストレス負荷によって誘発されるか、またMAP探索行動の発現に②ストレス関連分子である副腎皮質刺激ホルモン放出因子（corticotropin-releasing factor; CRF）受容体ならびに③副腎皮質刺激ホルモンの遊離を亢進させるプロスタグランディンE（EP）受容体がいかなる役割を演じているかを追究した。実験は、ラットがレバーを１回押せばMAPが薬物関連刺激（cue）と共に微量注入される薬物自己投与実験法を用いて行った。10日間のMAP自己投与実験後、MAPを生理食塩液に置換し自己投与実験（cue呈示なし）を続けた（消去過程；MAP退薬）。レバー押し行動が減弱した時点で、cue呈示またはMAPの少量投与（MAP-priming）によりレバー押し行動が再発する。この反応をMAP探索行動（生理食塩液自己投与下）の指標とした。コミュニケーションボックスを用いての身体的ストレス負荷（foot shock；0.4-0.8 mA）および精神的ストレス負荷後では、レバー押し行動はいずれも認められなかった。一方、オペラントボックス内の床面の金属性グリッドを通してfoot shock（0.8 mA）を15分間負荷すると、その期間に限ってレバー押し行動は増加し、MAP探索行動が誘発された。しかし、ストレス負荷を中止すると、直ちにレバー押し行動は減弱した。一方、cueまたはMAP-primingによるMAP探索行動は、非選択的CRF受容体拮抗薬であるα-Helical CRF_{9-41}によって有意に抑制された。このMAP探索行動は、選択的CRF_1受容体拮抗薬NBI27914の脳室内投与またはONO-SF-353-02の経口投与によっても抑制された。選択的CRF_2受容体拮抗薬Astressin 2Bはcue 呈示によるMAP探索行動を抑制したが、MAP-primingによるMAP探索行動は抑制しなかった。cue呈示またはMAP-primingによるMAP探索行動は、EP1ならびにEP3受容体拮抗薬の脳室内投与によって有意に抑制されたが、EP4受容体拮抗薬ではいずれも抑制されなかった。以上より、MAP探索行動はfoot shock暴露中にのみ惹起される事が分かった。また、cue呈示によるMAP探索行動の発現にはCRF_1ならびにCRF_2受容体の活性化が、MAP-primingによるMAP探索行動の発現にはCRF_1受容体の活性化がそれぞれ関与している事が明らかとなり、MAP探索行動の動因によってCRF受容体の関与の仕方が異なる事も分かった。さらには、MAP探索行動の発現にEP1およびEP3受容体が促進的に関与する可能性が示唆され、CRFとの関連性を今後追究する。

A. 研究目的

薬物依存症患者では、長期断薬後においても薬物への渇望が容易に再燃する事が知られている。この渇望再燃を誘発する因子として、①薬物の再使用、②薬物を使用していた環境への暴露および、③ストレス負荷、の３種類が挙げられる。薬物依存症患者では健常者と比較し血中のコルチゾール量が増加している事が分かっている[8]。さらに、ストレス負荷または薬物を使用していた環境への暴露により渇望が再燃し、この時、血清中のACTHならびにコルチゾール量が増加する事も報告されている[7]。これらの知見から、薬物依存症患者の渇望再燃には視床下部－下垂体－副腎系（hypothalamic-pituitary-adrenal system; HPA 系）が関与している可能性が強く示唆されるが、その詳細なメカニズムは明らかではない。一方、HPA 系を構成し、ストレス関連分子である副腎皮質刺激ホルモン放出因子（corticotropin-releasing factor; CRF）には CRF_1 ならびに CRF_2 の２つの受容体サブタイプが知られており、これらの受容体は不安作用や鬱作用に対し相反する機能を有する事が知られている[2]。しかし、渇望再燃における機能は明らかではない。一方、プロスタグランディン E（EP）受容体は脳内で CRF 受容体と共発現しており、さらに、副腎皮質刺激ホルモン（adrenocorticotropic hormone; ACTH）の遊離を促進的に制御している事が知られている[5]。ストレスによる MAP 探索行動の発現に、CRF と EP 受容体の相互的な関与が示唆されるが、この点を明らかにした報告はない。

そこで、本年度は、覚せい剤 methamphetamine（MAP）自己投与実験法を用いて、①ストレスによって MAP 探索行動がどのような形で発現するか、ならびに MAP 探索行動発現における CRF 受容体サブタイプの役割を追究した。さらに、EP 受容体の MAP 探索行動の発現における役割を追究した。

B. 研究方法

１．実験動物

薬物自己投与実験には Wistar 系雄性ラット［㈱日本エスエルシー、300-350g］を使用した。

２．使用薬物

methamphetamine [MAP：（株）大日本製薬]ならびにmetyrapone（Sigma-Aldrich, Inc.）は生理食塩液［㈱大塚製薬］に溶解した。また、Corticotropin Releasing Factor human, rat （Sigma-Aldrich, Inc.）、α-helical CRF_{9-41} (Sigma- Aldrich, Inc.）ならびに Astressin 2B（Sigma-Aldrich, Inc.）は蒸留水に溶解した。NBI27914（Sigma- Aldrich, Inc.）は蒸留水、ethanolおよびcremophor ELの混合溶液（18:1:1）に溶解した。ONO-SF-353-02は蒸留水とメチルセルロース混合溶液に、ONO- 8713、ONO-AE3-240ならびにONO-AE3-208は、蒸留水とDMSOの混合溶液（1:1）に溶解した。

３．薬物自己投与実験法

MAP 自己投与訓練に先立ち、あらかじめ餌ペレットを正強化子としたレバー押し行動をラットに獲得させた。その後自己投与用のカテーテルの静脈内留置手術を行った。カテーテルはシラスティックカテーテル［外径 1.0 mm、内径 0.5 mm；㈱カネカメディクス］を使用し、ラットの右頚静脈から挿入し、先端を心耳の入り口直前に留置した。実験には、レバー押しに伴い薬物が注入されるアクティブレバーと、薬物が得られないインアクティブレバーの２つのレバーが装着されたオペラント箱［29 cm×23 cm×33 cm ；㈱ニューロサイエンス］を使用した。ラットがアクティブレバーを１回押せば（FR1 条件下）、MAP（0.02 mg/100 μL/infusion）が薬物関連刺激（drug-associated cue：音; 85 dB/2.9 kHz, 光; 200 lux）と共に微量注入される。10 日間の MAP 自己投与実験後、MAP を生理食塩液に置換した自己投与実験（cue 呈示

なし）を続けた（消去過程）。レバー押し行動が減弱した時点で、薬物関連刺激の呈示、MAP-priming 投与ならびにストレス負荷を行い“渇望”の指標としての MAP 探索行動（生理食塩液自己投与下でのレバー押し反応）の発現の有無を調べた。

4．エサを正の強化子としたオペラント課題

薬物自己投与実験直後に food pellet (45 mg; Holton Industries Co. LTD.) を正強化子としてオペラント行動実験 (fixed ratio 1) を行い、ラットが 30 個の food pellet を獲得するまでの時間（experimental time）を測定した。1200 秒を cut off time とした。

5．ストレス負荷

オペラントボックスの床面の金属性グリッドを通して、foot shock（0.8 mA）を 15 分間負荷した。一方、コミュニケーションボックスを用いての実験では、床面グリッドから foot shock (0.4-0.8 mA; 15 分間)を受ける群（身体的ストレス負荷群）と foot shock を受けないが身体的ストレス負荷ラットの鳴き声や行動異常等の表出下に暴露される群（精神的ストレス負荷群）に分けて行った。

6．統計学的処理

値は全て平均±標準誤差で表した。二群間の有意差検定には、Bonferroni / Dunn test を用いた。

C. 研究結果

1．ストレス負荷による MAP 探索行動の発現

MAP 自己投与最終日のラットのレバー押し回数は 35.8±5.2 回であった（FR-1）。その後 MAP を生理食塩液に置換するとそのレバー押しは徐々に低下し、5 日目のレバー押し回数は 3.6±0.7 回であった。退薬 6 日目に（生理食塩液自己投与下）、MAP 関連刺激または MAP (1.0 mg/kg, i.p.) priming 投与を行うと、有意なレバー押し回数の増加が認められ、MAP 探索行動が発現した。一方、FR5 条件下では MAP 自己投与最終日のレバー押し回数は、133±4.7 回であった。MAP を生理食塩液に置換すると 17±1.8 回 (6.5±2.5 回/15 分間）と低下した。この時、オペラントボックス床面のグリッドから foot shock を負荷すると、レバー押し行動は有意に増加し、MAP 探索行動が誘発された (Fig. 1a)。しかし、このレバー押し行動は、ストレス負荷を中止するとほとんど認められなかった。一方、コミュニケーションボックスを用いての身体的ストレス負荷および精神的ストレス負荷群のレバー押し行動の増加はいずれも認められなかった (Fig. 1b)。

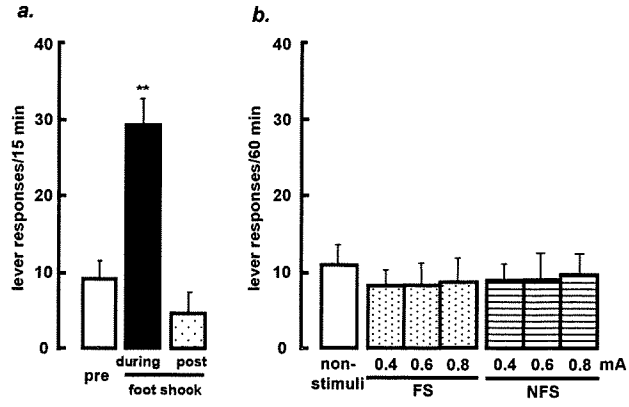

Figure 1. Effect of stress on the lever pressing behavior in MAP self-administered rats under FR5
a. Rats were exposed to electrical foot shock (FS) (0.8 mA, 15 min) in the operant box. *b.* Rats were exposed to FS or non foot shock (NFS) stress in a communication box. The rats in the FS group (physical stress; closed circle) received the electrical shock directly, and the rats in the NFS group (psychological stress; open circle) could escape from the electrical shock, but received various emotional stimuli from other foot shocked rats in the communication box. **P<0.01 versus pre group

2．MAP 探索行動の発現における CRF の関与

MAP 自己投与後退薬 6 日目に CRF (1.0, 3.2 μg/side)を脳室内微量注入すると、レバー押し行動は有意に増加し、MAP 探索行動が誘発された (Fig. 2)。逆に、MAP 関連刺激ならびに、MAP-priming 投与による MAP 探索行動は、非選択的 CRF 受容体拮抗薬であるα-helical CRF_{9-41} (3.2, 10 μg/side) の脳室内微量注入により有意に抑制された（Fig.

3)。しかしながら、CRF、MAP 関連刺激ならびに MAP-priming 投与による MAP 探索行動は、コルチコステロン合成阻害薬メチラポン（100 mg/kg, i.p.）の投与ではいずれも抑制されなかった（Fig. 4)。

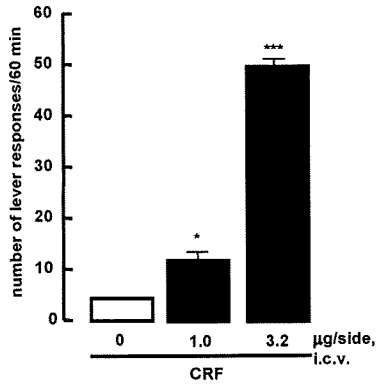

Figure 2. The reinstatement of MAP seeking behavior induced by intraventricular administration of CRF under FR1. *p<0.05, and ***p < 0.001, compared with the vehicle treated group.

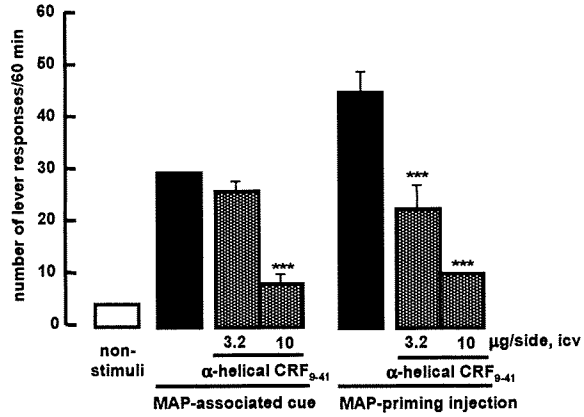

Figure 3. Effects of intraventricular administration of non-specific CRF receptor antagonist, α-Helical CRF_{9-41} on the reinstatement of MAP seeking behavior. ***p < 0.001, compared with the cue and MAP-priming injection alone.

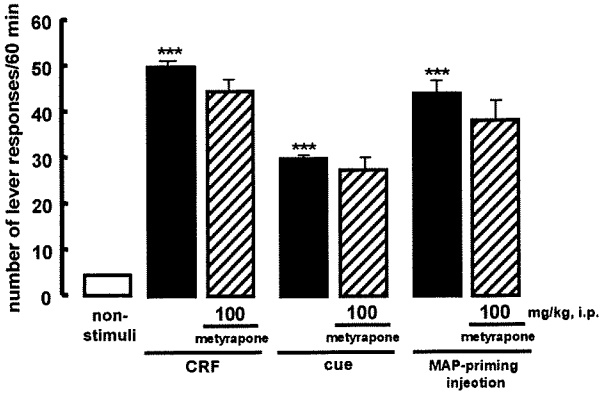

Figure 4. Effects of a corticosterone synthetic inhibitor, metyrapone on the reinstatement of MAP seeking behavior induced by CRF, MAP associated cue or MAP-priming injection. ***P < 0.001 vs. non-stimuli groups.

MAP 関連刺激ならびに MAP-priming 投与による MAP 探索行動は、いずれも選択的 CRF_1 受容体拮抗薬 NBI27914（32 μg/side）の脳室内微量注入により有意に抑制された（Fig. 5a)。さらに、経口投与可能な選択的 CRF_1 受容体拮抗薬 ONO-SF-353-02（1.0 -10 mg/kg, p.o.）もまた、両刺激による MAP 探索行動を抑制した（Fig. 6)。一方、選択的 CRF_2 受容体拮抗薬 Astressin 2B（32, 100 μg/side）の脳室内投与は、MAP 関連刺激による MAP 探索行動を抑制したが、MAP-priming による MAP 探索行動を抑制しなかった（Fig. 5b)。

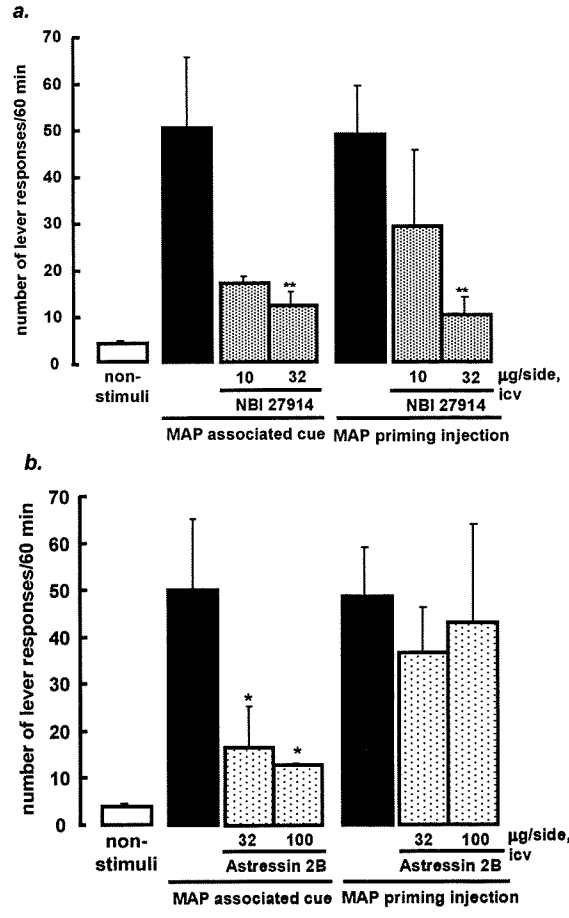

Figure 5. Effects of subtypes of CRF receptor antagonist on relapse to MAP-seeking behavior in MAP self-administered rats
a, Effects of a selective CRF_1 receptor antagonist, NBI 27914 (10, 32 μg/side; icv) on relapse to MAP-seeking behavior. b, Effects of a selective CRF_2 receptor antagonist, Astressin 2B (32, 100 μg/side; icv) on relapse to MAP-seeking behavior. *P < 0.05, **P < 0.01 vs. non-stimuli groups.

3．MAP 探索行動発現におけるプロスタグランディン E（EP）受容体の関与

MAP 関連刺激ならびに MAP-priming 投与による MAP 探索行動は、EP1 受容体拮抗薬 ONO-8713 ならびに EP3 受容体拮抗薬 ONO-AE3-240 の脳室

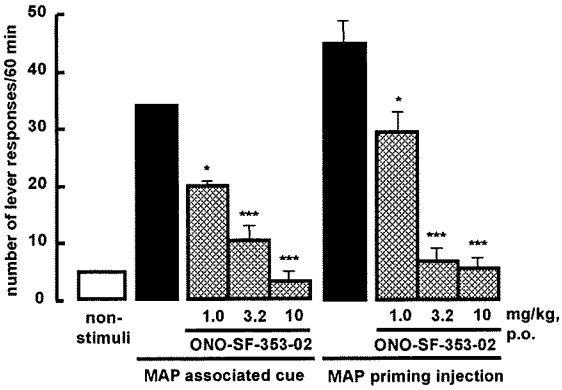

Figure 6. Effects of a selective CRF_1 receptor antagonist, ONO-SF-353-02 (p.o.) on relapse to MAP-seeking behavior in MAP self-administered rats. *P < 0.05, **P < 0.001 vs. non-stimuli group

内投与（100 μg/side）によって有意に抑制された。しかしながら、EP4 受容体拮抗薬 ONO-AE3-208 の脳室内投与（32 μg/side）ではいずれの MAP 探索行動も抑制されなかった（Fig. 7）。

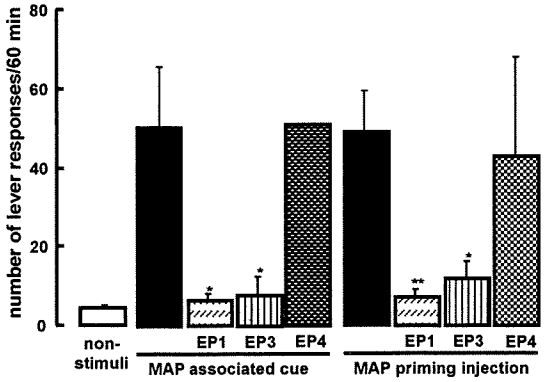

Figure 7. Effects of a prostaglandin E (EP) receptor antagonist on relapse to MAP-seeking behavior in MAP self-administered rats. *P < 0.05, *P < 0.01 vs. MAP assiciated cue or MAP priming alone, EP1: EP1 receptor antagonist (ONO-8713; 100 mg/side), EP3: EP3 receptor antagonist (ONO-AE3-240; 100 mg/side), EP4: EP4 receptor antagonist (ONO-AE3-208; 32 mg/side)

4．MAP 摂取行動における CRF 受容体の関与

MAP 自己投与実験 10 日目における MAP 摂取行動は、α-helical CRF_{9-41} の脳室内投与によって全く影響を受けなかった（Fig. 8）。

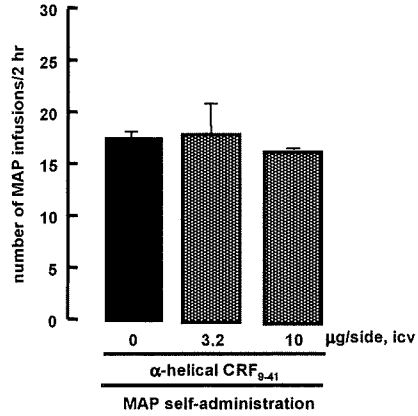

Figure 8. Effects of intraventricular administration of α-Helical CRF_{9-41} on MAP taking behavior.

D. 考察

オペラントボックス内での foot shock 負荷中のレバー押し行動は増加し、MAP 探索行動が誘発された。同様にコカインやヘロインの自己投与実験においても、オペラントボックス内での foot shock 負荷により薬物探索行動が惹起される事が報告されている[4), 6)]。しかし、コミュニケーションボックスを用いての foot shock 負荷後、レバー押し行動実験を開始しても MAP 探索行動は誘発されなかった。これらの事から、薬物探索行動は foot shock の暴露期間のみ誘発される事が分かった。

一方、MAP 関連刺激ならびに MAP priming 投与によって惹起される MAP 探索行動は、いずれも非選択的 CRF 受容体拮抗薬α-helical CRF_{9-41} の脳室内投与によって抑制された。逆に、CRF の脳室内投与により MAP 探索行動が誘発された。この結果から、MAP 探索行動の発現は CRF 受容体の活性化を介して起こっている事が示唆される。CRF はストレスに呼応して HPA 系を活性化し、ACTH やグルココルチコイド（コルチゾール／コルチコステロン）の遊離を増加させる事が知られている。しかし、本実験では CRF による MAP 探索行動はメチランポンで抑制されなかった。この事から、MAP 探索行動は、コルチコステロンを介した反応ではない事が示唆される。この事は、CRF が“ニューロモデュレーター”として働いている

可能性を示唆するものである。事実、CRF はニューロモデュレーターとして種々の中枢作用を発現する事が知られている[2]。

MAP 関連刺激による MAP 探索行動の発現には CRF_1 ならびに CRF_2 の両受容体が、また MAP priming 投与による MAP 探索行動の発現には CRF_1 受容体が促進的に関与する事を明らかにした。我々はこれまで MAP 関連刺激による MAP 探索行動の責任部位が扁桃体及び前頭前皮質であり[3]、また MAP priming 投与によるそれが前頭前皮質である事を指摘した[3]。一方、CRF_1 受容体の脳内分布は扁桃体ならびに前頭前皮質の両部位に、また CRF_2 受容体のそれは扁桃体に多く発現している事が知られている[1]。MAP 探索行動の発現における CRF 受容体サブタイプの関与の相違は、この CRF 受容体の脳内分布の違いに基因する事が推察される。

EP1 ならびに EP3 受容体拮抗薬によって MAP 探索行動の発現は抑制されたが、EP4 受容体拮抗薬では影響がなかった。このように、MAP 探索行動が EP1、EP3 受容体の活性化を介して起こる事が分かったが、本実験では EP 受容体と CRF 受容体の能動的関連性まで明らかにする事は出来なかった。

E. 結論

MAP 探索行動はストレス暴露によって誘発されるが、ストレスを中止すると消失する事が分かった。また、MAP 探索行動は CRF 受容体の活性化を介して起こり、その機構に EP 受容体の関与が示唆される。

【参考文献】

1) Chalmers DT., Lovenberg TW., De Souza EB.: Localization of novel corticotropin-releasing factor receptor (CRF2) mRNA expression to specific subcortical nuclei in rat brain: comparison with CRF1 receptor mRNA expression. J. Neurosci., **15**: 6340-6350, 1995.

2) Dautzenberg FM. and Hauger RL.: The CRF peptide family and their receptors: yet more partners discovered. *Trends Pharmacol. Sci.*, **23**: 71-77, 2002.

3) Hiranita T., Nawata Y., Sakimura K., et al.: The possibility of nicotinic activators as craving killers in methamphetamine dependence. *Proc. Natl. Acad. Sci. U S A*, **103**: 8523-8527, 2006.

4) Mantsch JR., Baker DA., Francis DM., et al.: Stressor- and corticotropin releasing factor-induced reinstatement and active stress-related behavioral responses are augmented following long-access cocaine self-administration by rats. *Psychopharmacology*, **195**: 591-603, 2008.

5) Matsuoka Y., Furuyashiki T., Bito H., et al.: Impaired adrenocorticotropic hormone response to bacterial endotoxin in mice deficient in prostaglandin E receptor EP1 and EP3 subtypes. Proc. Natl. Acad. Sci. U S A, **100**: 4132-4137, 2003.

6) Shalev U., Morales M., Hope B., et al.: Time-dependent changes in extinction behavior and stress-induced reinstatement of drug seeking following withdrawal from heroin in rats. *Psychopharmacology* , **156**: 98-107, 2001.

7) Sinha R., Talih M., Malison R., et al.: Hypothalamic-pituitary-adrenal axis and sympatho-adreno-medullary responses during stress-induced and drug cue-induced cocaine craving states. *Psychopharmacology*. **170**: 62-72, 2003.

8) Wisniewski AB., Brown TT., John M., et al.: Cortisol levels and depression in men and women using heroin and cocaine. *Psychoneuroendocrinology*, **31**: 250-255, 2006.

F. 研究発表

１．論文発表

1) Hiranita T., Nawata Y., Sakimura K., Yamamoto T.: Methamphetamine-seeking behavior is due to inhibition of nicotinic cholinergic transmission by activation of cannabinoid CB1 receptors, *Neuropharmacology*, **55**: 1300-1306, 2008

2) Sakae N., Yamasaki N., Kitaichi K., Fukuda T., Yamada M., Yoshikawa H., Hiranita T., Tatsumi Y., Kira J., Yamamoto T., Miyakawa T., Nakayama K.I.: Mice lacking the schizophrenia-associated protein FEZ1 manifest hyperactivity and enhanced responsiveness to psychostimulants. *Hum Mol Genet.*, **17**: 3191-203, 2008

3) Shigemats N., Fukuda T., Yamamoto T., Nishioku T., Yamaguchi T., Himeno M., Nakayama K., Tsukuba T., Kadowaki T., Okamoto K., Higuchi S., Yamamoto K.: Association of Catepsin E deficiency with the increase territorial aggresive response in mice. *J Neurochemistry*, **105**: 1394-1404, 2008

4) 山本経之, 縄田陽子: 大脳基底核と脳内報酬；ドパミン伝達の観点から. *分子精神医学*, **8**: 314-320, 2008

5) 縄田陽子, 山本経之: 薬物自己投与実験法を用いての薬物依存研究. *日本アルコール・薬物医学会雑誌*, **43**: 158-165, 2008

２．学会発表

1) Yamamoto T., Hiranita T., Nawata Y., Kagamiishi Y.: The role of corticotrophin-releasing factor in cue- and methamphetamine-induced reinstatement of methamphetamine-seeking in rats, CINP 50th Annual Meeting (Munich, Germany, 2008, July)

2) 北市清幸, 縄田陽子, 濱崎直孝, 山本経之: アニオン交感輸送タンパクAE3欠損マウスの行動薬理学的特性. 第18回日本臨床精神神経薬理学会・第38回日本神経精神薬理学会 合同年会（品川、2008年10月）

3) 縄田陽子, 北市清幸, 山本経之: 合成麻薬MDMA退薬時に認められる認知機能障害における脂質過酸化とカンナビノイドシステムの関与. 第18回日本臨床精神神経薬理学会・第38回日本神経精神薬理学会 合同年会（品川、2008年10月）

4) 山本経之, 縄田陽子, 平仁田尊人: シンポジウム「薬物療法による渇望感制御の可能性：基礎と臨床の接点」、薬物自己投与実験法を用いてのメタンフェタミン探索行動の発現におけるCB1受容体ならびにニコチン性ACh受容体の関与. アルコール・薬物依存関連合同学術総会（横浜、2008年9月）

5) 縄田陽子, 平仁田尊人, 山本経之: 選択的CRF_1受容体拮抗薬による覚せい剤メタンフェタミン探索行動の抑制. アルコール・薬物依存関連合同学術総会（横浜、2008年9月）

G. 知的財産権の出願・登録状況

なし

Neuropharmacology 55 (2008) 1300–1306

Contents lists available at ScienceDirect

Neuropharmacology

journal homepage: www.elsevier.com/locate/neuropharm

Methamphetamine-seeking behavior is due to inhibition of nicotinic cholinergic transmission by activation of cannabinoid CB1 receptors

Takato Hiranita [a,b,c], Yoko Nawata [a], Katsuya Sakimura [b], Tsuneyuki Yamamoto [a,*]

[a] Department of Pharmacology, Faculty of Pharmaceutical Sciences, Nagasaki International University, 2825-7 Huis Ten Bosch, Sasebo, Nagasaki 859-3298, Japan
[b] Department of Pharmacology, Graduate School of Pharmaceutical Sciences, Kyushu University, 3-1-1 Maidashi, Higashi-ku, Fukuoka 812-8582, Japan
[c] Psychobiology Section, Medications Discovery Research Branch, Intramural Research Program, Department of Health and Human Services, National Institute on Drug Abuse, National Institutes of Health, Baltimore, MD 21224, United States

ARTICLE INFO

Article history:
Received 15 September 2007
Received in revised form 12 August 2008
Accepted 14 August 2008

Keywords:
Cannabinoid CB1 receptors
Craving
Drug-seeking behavior
Methamphetamine
Nicotinic ACh receptors
Relapse

ABSTRACT

We previously reported the involvement of cannabinoid CB1 receptors (CB1Rs) and nicotinic acetylcholine receptors (nAChRs) in the reinstatement of methamphetamine (MAP)-seeking behavior (lever-pressing response for MAP reinforcement under saline infusion). The present study examined whether the reinstatement involves interactions between these receptors. Rats were trained to self-administer MAP with a light and tone (MAP-associated cues). Then, extinction sessions under saline infusion without cues were conducted. After that, a reinstatement tests were conducted by either presenting the cues or a MAP-priming injection. Systemic and intracranial administration of HU210, a cannabinoid CB1R agonist, into the nucleus accumbens core (NAC) and prelimbic cortex (PrC) reinstated MAP-seeking behavior. The reinstatement caused by the systemic HU210 treatment was attenuated by intracranial administration of AM251, a cannabinoid CB1R antagonist, into each region mentioned above. Meanwhile, reinstatement induced by the MAP-associated cues and MAP-priming injection was also attenuated by intracranial administration of AM251 in each region. In these regions, the attenuating effects of AM251 on the reinstatement induced by each stimulus were blocked by the intracranial administration of mecamylamine, a non-selective nAChR antagonist, but not by scopolamine, a muscarinic ACh receptor (mAChR) antagonist. Furthermore, the intracranial administration of DHβE, an α4β2 nAChR antagonist, but not MLA, an α7 nAChR antagonist, into each region blocked the AM251-induced attenuation of the reinstatement. These findings suggest that relapses to MAP-seeking behavior may be due to two steps, first inhibition of ACh transmission by the activation of cannabinoid CB1Rs and then the inactivation of α4β2 nAChRs.

1. Introduction

Relapse to drug-seeking behavior is a hallmark of drug dependence, but an effective treatment has yet to be developed. In human addicts and animal models of relapse, three different kinds of stimuli are capable of eliciting drug-seeking behavior: stress, cues predicting drug availability, and re-exposure to a previously self-administered drug (Shalev et al., 2002). Understanding the neural mechanisms by which these stimuli elicit relapse is a prerequisite to creating adequate pharmacotherapies for drug dependence.

We have previously demonstrated that the systemic administration of a cannabinoid CB1R antagonist SR141716A (rimonabant) (Anggadiredja et al., 2004a) and nicotine (Hiranita et al., 2004, 2006) attenuates the reinstatement of MAP-seeking behavior induced by re-exposure to MAP-associated cues, previously paired with MAP-taking, as well as a MAP-priming injection (1.0 mg/kg, i.p.); however, the site of action of the antagonist responsible for attenuating the reinstatement is unknown. Therefore, the first purpose of this study is to identify this region. We reported that the nucleus accumbens core (NAC) and prelimbic cortex (PrC) were involved in the reinstatement of MAP-seeking behavior (Hiranita et al., 2006). Recently it was reported that endocannabinoid was important for the neural plasticity of glutamatergic neurons between these regions (Robbe et al., 2003). Therefore, we focused on these two regions. Meanwhile, it has been shown that SR141716A, a cannabinoid CB1R antagonist, stimulates the release of ACh in the medial prefrontal cortex (mPFC) (Gessa et al., 1998; Tzavara et al., 2003a). Electrophysiological studies reported that endocannabinoids functioned as antagonists at nAChRs. Thus, anandamide and 2-arachidonoylglycerol (2-AG), endogenous cannabinoid CB1R agonists, inhibited the function of nAChRs (Oz, 2006; Spivak et al., 2007). Furthermore, a cannabinoid CB1R antagonist AM251 attenuated

* Corresponding author. Tel.: +81 956 20 5629; fax: +81 956 39 3111.
E-mail address: tyamamot@niu.ac.jp (T. Yamamoto).

0028-3908/$ – see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.neuropharm.2008.08.012

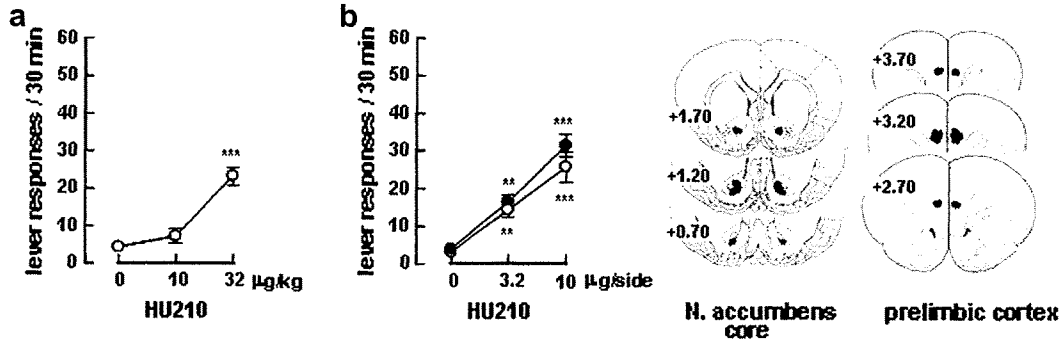

Fig. 1. Effect of HU210, a cannabinoid CB1 receptor agonist, in MAP self-administered rats. (a) Effect of systemic treatment with HU210 in MAP self-administered rats ($n = 6$). ***, $P < 0.001$ compared with vehicle. (b) Effect of the intracranial administration of HU210 into the nucleus accumbens core (open circles) and prelimbic cortex (closed circles) in MAP self-administered rats ($n = 7$, each). **, $P < 0.01$ and ***, $P < 0.001$ compared with vehicle. Coronal brain maps show a schematic representation of where the annuluses were placed. The numbers indicate the distance from the bregma in the anteroposterior plane. Mapping also includes the location of the tips of the cannulas used in all experiments.

nicotine self-administration and nicotine-seeking behavior in rats (Shoaib, 2008). Such findings indicate that the cannabinoid system modulates AChrgic transmission. Consequently, it is possible that a cannabinoid CB1R antagonist SR141716A (Anggadiredja et al., 2004a) attenuates reinstatement of MAP-seeking behavior by mediating AChrgic transmission. However, little is known about the interaction between the cannabinoid system and nAChRs during relapse to drug-seeking behavior. The second purpose of this study is to clarify the interrelation between cannabinoid CB1Rs and nAChRs.

Neuronal nAChRs are comprised of combinations of α (2–9) and β (2–4) subunits arranged to form a pentameric receptor (Grottick et al., 2000). The principal subtypes in the central nervous system are believed to be α4β2 and homomeric α7 nAChRs (Grottick et al., 2000). Their distribution in the PFC and striatum, including the PrC and NAC, respectively, is known to be similar (Gotti et al., 2006). Although it is well established that nicotine has a rewarding effect, the nAChR subtypes involved in the reinstatement of drug-seeking behavior are unknown. Therefore, the third purpose of this study was to identify the nAChR subtypes responsible for the CB1R–nAChR interaction.

2. Materials and methods

2.1. Subjects

One hundred thirty-three subjects were used. Male Wistar/ST (Nippon SLC Co., Hamamatsu, Japan) rats (250–350 g, 10 weeks old) were individually housed in a temperature- and humidity-controlled environment under a 12-h light/dark cycle (lights on at 7:00 a.m.). Food and water were available ad libitum in the home cage except when daily food intake was limited to 15–20 g after the implantation of catheters to fix the distance between the proximal position of a catheter in the vein and the surface of the atrial auricle. Rats were trained and tested between 9:00 a.m. and 5:00 p.m. Procedures for animal treatments were conducted in accordance with the Guide for the Care and Use of Laboratory Animals as adopted and promulgated by the Declaration of Helsinki and Faculty of Pharmaceutical Sciences, Kyushu University Publication, enacted 1988. In all studies, within-subject designs are used so that each animal served as its own control, and the overall number of subjects was minimal.

2.2. Surgery

Silascon catheters (inner and outer diameter: 0.5 and 1.0 mm, respectively; Kaneka Medix Co., Japan) were surgically implanted into the jugular vein under sodium pentobarbital (40 mg/kg, intraperitoneal (i.p.), Kyoritsu Seiyaku Co., Japan) anesthesia as described previously (Hiranita et al., 2006). After the surgery, catheter patency was maintained by daily infusion of 0.15 ml of a saline solution containing heparin (30 U/ml) after each session. After catheterization in the jugular vein, rats were fixed in a stereotaxic apparatus. Two guide cannulas (inner and outer diameter: 0.4 and 0.7 mm, respectively; stainless steel pipe) were bilaterally implanted 1 mm above the NAC (coordinates: anteroposterior, mediolateral, and dorsoventral, +1.2, ±1.6, and −7.8 mm relative to the bregma, midline, and skull surface, respectively), and PrC (+3.2, ±0.75, and −4.7). Two stainless steel screws were implanted in the skull for support. The cannulas and screws were held in place with dental cement. An obturator (stainless steel) was inserted into each guide cannula to prevent blockage.

2.3. Drugs

MAP HCl (Dainippon Pharmaceutical, Osaka), (Nattick, MA), mecamylamine (a non-specific nAChR antagonist, Sigma–Aldrich), (−)-scopolamine (a muscarinic AChR (mAChR) antagonist, Sigma–Aldrich), dihydro-β-erythroidine HBr (DHβE, an α4β2 nAChR antagonist, Sigma–Aldrich) and methyllycaconitine citrate (MLA, an α7 nAChR antagonist, Sigma–Aldrich) were dissolved in saline, while AM251 and HU210, a cannabinoid CB1R antagonist and agonist, respectively, were dissolved in dimethyl sulfoxide. MAP was delivered intravenous (i.v.) for self-administration (0.02 mg/0.1 ml/infusion) and i.p. for priming injections (1.0 mg/kg) 30 min before tests. Systemic administration of HU210 (10–32 µg/kg) was done subcutaneously (s.c.) 15 min before the sessions. Drugs administered intracranially (0.5 µl/side) were microinjected into the brain 5 min before sessions through an injection cannula (inner and outer diameters were 0.1 and 0.35 mm, respectively; a stainless steel tube was used) that extended 1 mm below the guide cannula (stainless steel) using a microsyringe (Hamilton).

2.4. Apparatus

The injector system contained a fluid swivel (Instech Lab., Inc. PA) mounted on the top of each operant chamber (Neuroscience, Inc. Japan). One end of the swivel was connected via polyethylene tubing (Kaneka Medix Co., Japan) encased in a protective stainless steel spring tether (Instech Laboratories, Inc. PA) to the animal's catheter while the other end of the swivel was connected via polyethylene tubing to the infusion pump. The operant chambers were enclosed in ventilated, sound-attenuating cubicles and controlled by computer software (Med Associates Inc., VT). The chamber's light was switched on throughout the session. Lever-pressing responses resulted in the infusion of MAP (0.02 mg/infusion over 6 s) accompanied by light (mounted 4 cm above the lever, 200 lux) and tone (85 dB/2.9 kHz) for 26 s (MAP-associated cues). The subsequent 20 s was a 'time out' period during which lever presses were still recorded but not accompanied with infusions.

2.5. MAP self-administration, extinction, and reinstatement

Two days after surgery, rats were trained to self-administer MAP under a fixed ratio (FR-1) schedule of reinforcement (each lever-press is reinforced) in a 2-h daily session for 10 days (MAP-taking). Each injection was accompanied by light and tone (MAP-associated cues). During this time, inactive lever responses had no programmed consequences, but were recorded. After MAP 10 days of self-administration, 5 daily extinction sessions (1-h), were conducted during which active lever responding resulted in an infusion of saline instead of MAP without presentation of the MAP-associated cues (or until the rats achieved the extinction criterion of less than 10 responses per session on the previously active lever). Reinstatement (drug-seeking behavior) tests under saline infusions were carried out for 30 min from day

Table 1
Effect of intracranial injection of HU210 on food-taking responses (responses/min)

Nucleus accumbens core			Prelimbic cortex		
HU210 (µg/side)			HU210 (µg/side)		
0	3.2	10	0	3.2	10
25.0 ± 0.9	26.3 ± 1.1	25.2 ± 1.1	25.2 ± 1.1	24.7 ± 1.1	25.4 ± 0.7

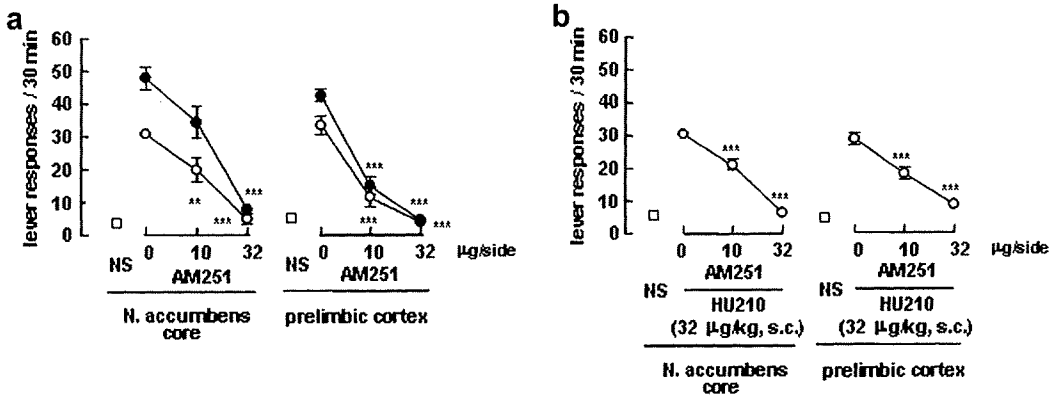

Fig. 2. Effects of the intracranial administration of AM251, a cannabinoid CB1 receptor antagonist, into the nucleus accumbens core and prelimbic cortex on the reinstatement of MAP-seeking behavior induced by MAP-associated cues, MAP-priming injections, and systemic administration of HU210. (a) Effects of the intracranial administration of AM251 into the nucleus accumbens core and prelimbic cortex on the reinstatement induced by MAP-associated cues and MAP-priming injections ($n = 7$, each). Open squares, and open and closed circles represent groups given non-stimuli (NS), MAP-associated cues, and MAP-priming injections. **, $P < 0.01$, and ***, $P < 0.001$ compared with the cue presentation and MAP-priming injection alone. (b) Effects of the intracranial administration of AM251 into the nucleus accumbens core and prelimbic cortex on the reinstatement induced by systemic treatment with HU210 (32 μg/kg, s.c.) ($n = 7$, each). Open squares and circles represent groups given non-stimuli (NS) and HU210, respectively. ***, $P < 0.001$ compared with HU210 alone. NS means lever responses under the extinction condition.

6 of extinction (or the day after rats achieved the extinction criterion) every 6 days under an FR-1 schedule. In the cue-induced test, immediately after the onset of the session, rats were re-exposed to the MAP-associated cues, and each press on the active lever resulted in presentation of the cues. In the MAP primed reinstatement test, MAP (1.0 mg/kg i.p.) was injected 30 min before the test. Each response during the test session resulted in an infusion of saline but not the MAP-associated cues. Drugs were preadministered in a counterbalanced order. Each rat was evaluated by both cue- and MAP-induced reinstatement. In our pilot study, levels of active lever responses induced by MAP-associated cues and MAP-priming injections did not change during at least the third time. Therefore, each rat was given either the cue-stimulus or MAP-priming injection alternately and had 6 reinstatement tests in total, that is, 3 tests per stimulus.

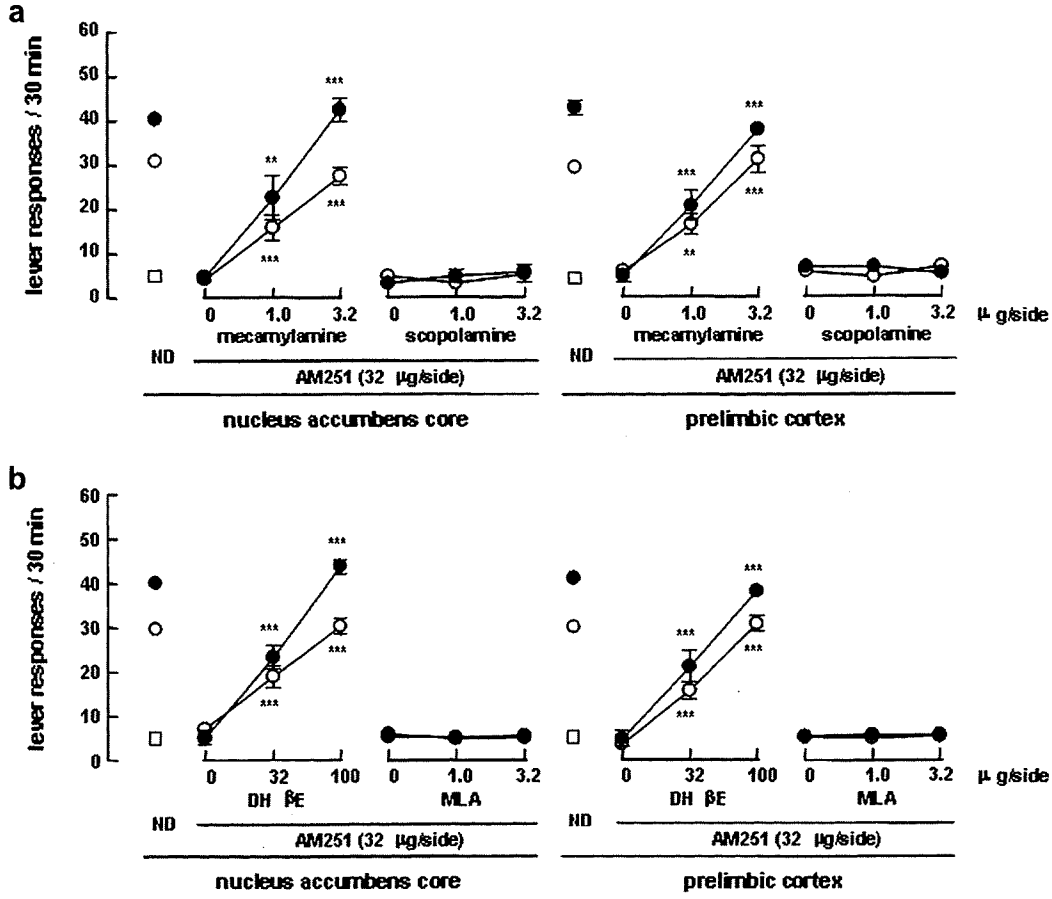

Fig. 3. Effects of the intracranial administration of cholinergic antagonists into the nucleus accumbens core and the prelimbic cortex on the AM251-induced attenuation of the reinstatement induced by the cues and MAP-priming injections. Open squares, and open and closed circles represent groups given non-stimuli (NS), MAP-associated cues, and MAP-priming injections, respectively. ND: non-drugs. **, $P < 0.01$, and ***, $P < 0.001$ compared with AM251-pretreated groups given the cues and MAP-priming injections ($n = 7$, each). (a) Effects of a non-selective nicotinic and muscarinic acetylcholine receptor antagonist (mecamylamine and scopolamine, respectively) on the reinstatement. (b) Effects of a selective α4β2 and α7 nicotinic acetylcholine receptor antagonist (DHβE and MLA, respectively) on the reinstatement. NS means lever responses under the extinction condition, whereas ND means no pretreatment with test drugs.

Table 2
Effect of intracranial injection of AM251 on food-taking responses in rats given the inductive stimuli (responses/min)

Inductive stimuli of the reinstatement	Nucleus accumbens core			Prelimbic cortex		
	AM251 (μg/side)			AM251 (μg/side)		
	0	10	32	0	10	32
MAP-associated cues	22.4 ± 1.9	21.1 ± 1.8	20.1 ± 2.1	24.1 ± 1.4	24.9 ± 0.7	25.9 ± 1.2
MAP-priming injection	22.3 ± 1.1	20.5 ± 2.0	22.5 ± 1.5	24.9 ± 1.1	24.6 ± 1.4	24.2 ± 1.2
HU210 (32 μg/kg, s.c.)	22.4 ± 1.9	21.1 ± 1.8	20.1 ± 2.1	24.1 ± 1.4	24.9 ± 0.7	25.9 ± 1.2

Table 3
Effect of coadministration of AM251 with cholinergic antagonists on food-taking responses in rats given the inductive stimuli (responses/min)

Inductive stimuli of the reinstatement	Nucleus accumbens core			Prelimbic cortex		
	AM251 (32 μg/side)			AM251 (32 μg/side)		
	Mecamylamine (μg/side)			Mecamylamine (μg/side)		
	0	1	3.2	0	1	3.2
MAP-associated cues	25.4 ± 1.2	24.0 ± 1.5	21.2 ± 1.8	25.6 ± 1.1	24.3 ± 0.8	25.1 ± 1.1
MAP-priming injection	21.2 ± 1.6	23.5 ± 2.0	23.3 ± 1.2	25.5 ± 0.9	26.1 ± 1.4	25.6 ± 1.0
	DHβE (μg/side)			DHβE (μg/side)		
	0	32	100	0	32	100
MAP-associated cues	25.6 ± 0.8	21.1 ± 0.8	22.1 ± 1.8	22.8 ± 1.6	24.3 ± 1.3	24.7 ± 1.1
MAP-priming injection	24.3 ± 1.3	24.7 ± 1.1	25.6 ± 0.8	23.9 ± 1.5	22.6 ± 1.1	22.0 ± 1.4
	MLA (μg/side)			MLA (μg/side)		
	0	1	3.2	0	1	3.2
MAP-associated cues	25.1 ± 0.8	25.0 ± 1.0	22.2 ± 1.0	25.6 ± 0.8	22.6 ± 1.3	21.7 ± 1.5
MAP-priming injection	22.9 ± 1.0	22.1 ± 1.6	25.1 ± 0.8	22.9 ± 1.1	22.5 ± 1.0	22.9 ± 1.0
	Scopolamine (μg/side)			Scopolamine (μg/side)		
	0	1	3.2	0	1	3.2
MAP-associated cues	22.7 ± 1.9	24.4 ± 1.9	24.3 ± 1.5	24.9 ± 0.8	25.3 ± 1.1	25.0 ± 1.1
MAP-priming injection	21.2 ± 1.6	23.5 ± 2.0	22.5 ± 1.6	24.6 ± 1.0	25.9 ± 1.1	25.0 ± 1.2

2.6. Operant task performance for food pellets

All subjects pressed a lever for food-pellet reinforcement under the FR-1 schedule 5 min after the self-administration session. Each test ended when rats had received 30 pellets. The time limit was 1200 s.

Table 4
Effect of AM251 or cholinergic antagonists alone on food-taking responses (responses/min)

Brain regions	AM251 (μg/side)		
	0	10	32
Nucleus accumbens core	25.3 ± 1.0	24.4 ± 1.1	26.0 ± 1.1
Prelimbic cortex	24.8 ± 0.9	23.8 ± 1.2	24.0 ± 0.9
	Mecamylamine (μg/side)		
	0	1	3.2
Nucleus accumbens core	23.8 ± 0.8	24.3 ± 1.0	25.2 ± 1.2
Prelimbic cortex	24.6 ± 1.3	24.1 ± 0.9	23.4 ± 0.8
	DHβE (μg/side)		
	0	32	100
Nucleus accumbens core	24.2 ± 1.0	25.1 ± 0.9	24.4 ± 1.3
Prelimbic cortex	24.3 ± 1.3	24.0 ± 1.3	23.5 ± 1.0
	MLA (μg/side)		
	0	1	3.2
Nucleus accumbens core	24.6 ± 101	24.6 ± 0.7	24.4 ± 0.9
Prelimbic cortex	24.4 ± 1.1	25.1 ± 1.1	25.1 ± 0.8
	Scopolamine (μg/side)		
	0	1	3.2
Nucleus accumbens core	22.5 ± 0.9	24.3 ± 1.9	24.7 ± 0.9
Prelimbic cortex	24.0 ± 1.2	25.1 ± 1.2	24.8 ± 1.0

2.7. Data analysis

Data represent the mean ± SEM number of lever responses. Response totals were analyzed by ANOVA (a within-subjects design). A one-way ANOVA was used to compare means, and Bonferroni–Dunn tests were used for post hoc analyses. Differences were considered significant at $P < 0.05$. All statistical analyses were performed by using the Stat View software program (v. 5.0; SAS Institute Inc., Cary, NC).

2.8. Histology

After the experiments, all rats were deeply anesthetized with pentobarbital (52 mg/kg, i.p.) and transcardially perfused with phosphate-buffered saline followed by 4% PLP (periodate lysine paraformaldehyde). The brains were removed, soaked in PLP for at least 24 h, sliced at a thickness of 80 μm, mounted on MAS-coated slides, and stained with cresyl violet. The positions of the injection cannulas were inspected under a light microscope.

3. Results

The total amount of MAP-intake was 3.3 ± 0.7 mg, i.v. for 10 days. Systemic administration of HU210 dose-dependently increased number of the lever responses in the MAP self-administration paradigm [MAP-seeking behavior, from 4.3 ± 0.7 to 23.2 ± 2.4, $F(2, 15) = 34.247$, $P < 0.001$] (Fig. 1a). Intracranial administration of HU210 into the NAC and PrC also increased lever responses [$F(2, 18) = 18.620$, $P < 0.001$, and $F(2, 18) = 40.443$, $P < 0.001$, respectively, Fig. 1b]. On the other hand, intracranial administration of HU210 into the NAC and PrC did not affect responses for food-reinforcement [food-taking responses] (Table 1, $P > 0.1$).

Re-exposure to the MAP-associated cues increased lever responses from 3.4 ± 0.7 to 30.7 ± 0.8 [$F(1, 16) = 515.165$, $P < 0.001$ compared with the NS group, Fig. 2a]. This increase was dose-dependently attenuated by the intracranial administration of

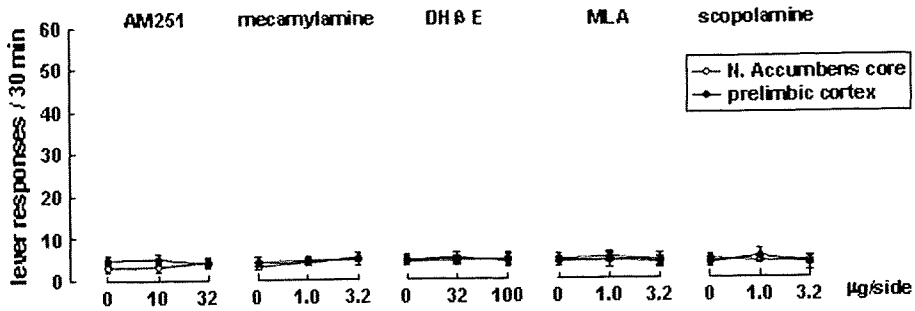

Fig. 4. Effects of intracranial priming-administration of AM251 and cholinergic antagonists alone into the nucleus accumbens core and the prelimbic cortex in MAP self-administered rats. Open and closed circles represent drug-priming injections into the nucleus accumbens core and prelimbic cortex, respectively ($n = 6$, each).

AM251 into the NAC [$F(2, 15) = 30.973$, $P < 0.001$, Fig. 2a]. MAP-priming injections also increased lever responses from 3.4 ± 0.7 to 47.8 ± 3.4 [$F(1, 16) = 308.145$, $P < 0.001$, Fig. 2a]. Similar to the cue-induced increase in lever responses, the MAP-priming injection-induced increase was also attenuated by the intracranial administration of AM251 into the NAC [$F(2, 15) = 34.475$, $P < 0.001$, Fig. 2a]. Correlation coefficients between the total amount of MAP-intake and number of active lever responses induced by MAP-associated cues ($r = 0.343$, $P < 0.01$) and MAP-priming injections ($r = -0.481$, $P < 0.001$) were revealed. As to the PrC, increased lever responses induced by MAP-associated cues and MAP-priming injections were also attenuated by the intracranial administration of AM251 [$F(2, 15) = 41.524$, $P < 0.001$ and $F(2, 15) = 95.203$, $P < 0.001$, respectively, Fig. 2a]. The intracranial administration of AM251 into the NAC and PrC also attenuated the increase in lever responses induced by systemic HU210 treatment [$F(2, 15) = 69.847$, $P < 0.001$, and $F(2, 15) = 40.745$, $P < 0.001$, respectively, Fig. 2b]. In contrast, intracranial administration of AM251 into the NAC and PrC did not affect food-taking responses in rats given the MAP-associated cues, and MAP- and HU210 (32 μg/kg, s.c.)-priming injections (Table 2, $P > 0.1$).

Mecamylamine (1.0–3.2 μg/side, intra-NAC) dose-dependently antagonized the attenuation of lever responding induced by intra-NAC AM251 [$F(2, 15) = 35.002$ (cues) and 31.138 (MAP-priming injection), respectively, $P < 0.01$, Fig. 3a]. With regard to the PrC, intracranial administration of mecamylamine also blocked the attenuating effect of the intracranial administration of AM251 on lever pressing [$F(2, 15) = 28.078$ (cues) and 51.028 (MAP-priming injection), respectively, $P < 0.001$ Fig. 3a]. However, scopolamine (1.0–3.2 μg/side, intra-NAC and intra-PrC, respectively) did not block the intra-NAC and intra-PrC AM251-induced attenuation of the lever responses induced by each stimulus ($P > 0.3$, Fig. 3a). On the other hand, intra-NAC DHβE blocked the attenuation of lever pressing induced by intra-NAC AM251 [$F(2, 15) = 38.504$ (cues) and 94.037 (MAP-priming injection), $P < 0.001$, Fig. 3b]. In the PrC, intracranial administration of DHβE also antagonized the attenuation induced by the intracranial administration of AM215 [$F(2, 15) = 67.111$ (cues) and 48.347 (MAP-priming injection), $P < 0.001$, Fig. 3b]. However, MLA (1.0–3.2 μg/side, intra-NAC and intra-PrC, respectively) did not block the intra-NAC and intra-PrC AM251-induced attenuation ($P > 0.7$, Fig. 3b). Meanwhile, the coadministration of AM251 with cholinergic antagonists did not affect food-taking responses (Table 3, $P > 0.1$).

Microinjection of neither AM251 nor cholinergic antagonists alone into the NAC and PrC reinstated MAP-seeking behavior (Fig. 4). In addition, these treatments did not alter food-taking responses (Table 4, $P > 0.1$).

4. Discussion

MAP-associated cues and MAP-priming injections reinstated MAP-seeking behavior. Systemic administration of HU210, a cannabinoid CB1R agonist, also reinstated this behavior. Reinstatement produced by these three stimuli was attenuated by the intracranial administration of AM251, a CB1R antagonist, into the NAC and PrC. The treatments did not affect food-taking behavior, indicating these effects of AM251 to be due to specific behavioral effect on MAP. These findings suggest that CB1Rs in these two regions have an important role in reinstatement of MAP-seeking behavior. Regarding the cannabinoid system, this is the first identification of the regions responsible for MAP cravings. In each region, the attenuating effect of AM251 was blocked by mecamylamine, a non-selective nAChR antagonist, but not by scopolamine, an mAChR antagonist. The effects of mecamylamine were mimicked by DHβE, but not by MLA, an α4β2 and α7 nAChR antagonist, respectively. These findings suggest an important role of interaction between CB1Rs and α4β2 nAChRs in reinstatement of MAP-seeking behavior. Recently, CB1R agonists, such as Δ^9-tetrahydrocannabinol (THC) and WIN55,212-2, inhibited ACh release in the mPFC and hippocampus (Gessa et al., 1998; Tzavara et al., 2003b). These inhibitory effects were suppressed by SR141716A, a CB1R antagonist. SR141716A alone promoted ACh release in both regions (Gessa et al., 1998; Tzavara et al., 2003b). These findings suggest the cannabinoid system to be an inhibitory modulator of AChrgic transmission. Considering this inhibitory regulation of CB1Rs, our findings suggest that relapses to MAP-seeking behavior may be due to two steps, first inhibition of ACh transmission by the activation of CB1Rs and then the inactivation of α4β2 nAChRs. Additionally, we previously reported that SR141716A, nicotine, and donepezil, an acetylcholinesterase inhibitor, attenuated reinstatement of MAP-seeking behavior (Anggadiredja et al., 2004a; Hiranita et al., 2004, 2006). Nicotine and ACh have greater affinity for α4β2 (K_i values: 0.79 and 44 nM) than for α7 nAChRs (5000 and 14,300 nM) (Decker et al., 1995; Gotti et al., 2006). Considering this preference of nicotine and ACh for α4β2 nAChRs, our previous and present findings support an important role for α4β2 nAChRs in the reinstatement of drug-seeking behavior.

In our previous study, systemic administration of mecamylamine alone neither reinstated MAP-seeking behavior nor potentiated the reinstatement induced by cues and MAP-priming injections (Hiranita et al., 2006). The present study demonstrates that intracranial injection of neither mecamylamine nor DHβE alone reinstated MAP-seeking behavior. Therefore, these results suggest the functional normalization of α4β2 nAChRs to be important for the blockade of reinstatement of MAP-seeking behavior. However, ACh and nicotine have less affinity for α7 than for α4β2 nAChRs (Decker et al., 1995; Gotti et al., 2006). Therefore, these findings suggest that activation of α7 nAChRs through 'endogenous' ACh might not be enough to produce a behavioral effect. Meanwhile, CB1Rs in the NAC and PrC have an inhibitory role in glutamatergic transmission (Mackie, 2005; Robbe et al., 2001), whereas endocannabinoid is involved in the neural plasticity of glutamatergic neurons between these two regions (Robbe et al., 2003). Recently, the activation of glutamatergic neurons was

reported to reinstate cocaine-seeking behavior (Kalivas and McFarland, 2003). Although the present results did not demonstrate a role for α7 nAChRs in reinstatement, α7 nAChRs are expressed in glutamatergic terminals from the cortex to the striatum and ventral tegmental area (VTA) (Mansvelder et al., 2002). Therefore, the distribution of α7 nAChRs has a very similar distribution to that of CB1Rs. This finding suggests the activation of glutamatergic transmission via α7 nAChRs to contribute to the reinstatement of drug-seeking behavior. Therefore, α4β2 and α7 nAChRs might have opposite roles in the reinstatement of drug-seeking behavior, inhibitory and facilitatory, respectively.

This report is the first indication that α4β2, but not α7, nAChRs have an inhibitory role in relapse to drug-seeking behavior. To attenuate the relapse, it may be important to target the rewarding property of α4β2 nAChRs. Indeed, regarding nicotine, DHβE reduced self-administration (Corrigall et al., 1994; Grottick et al., 2000) and conditioned place preference (Walters et al., 2006), whereas MLA affected neither self-administration (Grottick et al., 2000) nor the threshold elevation of electric brain stimulation during nicotine withdrawal (Markou and Paterson, 2001). α4β2 nAChR knockout mice showed decreases in nicotine place preference (Cincotta et al., 2008). Mutant mice lacking the β2 subunit showed decreased nicotine self-administration relative to the wild-type mice (Picciotto et al., 1998). The mice did not exhibit nicotine place preference, while α7 nAChR knockout mice did (Cincotta et al., 2008; Walters et al., 2006). Considering the rewarding property of α4β2 nAChRs, activation of this receptor may be important in preventing cravings for not only MAP but also other abusive drugs.

There is evidence demonstrating an involvement of α7 nAChRs in the effects of CB1R agonists. In rats, systemic administration of MLA, but not DHβE, antagonized the discriminative effects of Δ^9-THC and reduced self-administration of WIN55,212-2 (Solinas et al., 2007). Meanwhile, we previously showed that effect of a cannabinoid agonist altered the MAP withdrawal/extinction state. Repeated administration of the cannabinoid agonist, Δ^8-THC, during the extinction phase, suppressed reinstatement of MAP-seeking behavior induced by cues and a MAP-priming injection (Anggadiredja et al., 2004a). However, after extinction training, although Δ^8-THC had no effect by itself, coadministration of the agonist and MAP at small doses reinstated MAP-seeking behavior (Anggadiredja et al., 2004a). These findings suggest that the interaction between nAChR subtypes and the cannabinoid system may switch from α7 to α4β2 nAChRs before and after MAP withdrawal/extinction. In contrast to the cannabinoid system, α4β2, but not α7, nAChR, contributed to the effect of nicotine effects before and after withdrawal. As shown above, α4β2, but not α7, nAChRs contribute to nicotine reinforcement before nicotine withdrawal (Corrigall et al., 1994; Grottick et al., 2000). During the nicotine withdrawal phase, the administration of DHβE precipitated the signs of withdrawal (Malin et al., 1998), whereas MLA did not affect the threshold elevation of electric brain stimulation during nicotine withdrawal (Markou and Paterson, 2001).

The present study showed an interaction between CB1Rs and α4β2 nAChRs in the NAC and PrC for the reinstatement of MAP-seeking behavior. Other regions might also be involved. We previously demonstrated the involvement of the amygdala and hippocampus in reinstatement of MAP-seeking behavior (Hiranita et al., 2006). The involvement of information processing from these two regions to the PrC or NAC in reinstatement is reported (Di Ciano and Everitt, 2004; Fuchs et al., 2007; Miller and Marshall, 2005). Additionally, intra-amygdala SR141716A failed to affect cue-induced reinstatement of heroin-seeking behavior (Alvarez-Jaimes et al., 2007). Therefore, it is unlikely that there is interaction between CB1Rs and nAChRs in the amygdala as there is in the NAC and PrC. Whether such interaction occurs in the hippocampus, however, remains to be elucidated. Meanwhile, several studies reported the involvement of nAChRs in the VTA in the behavioral effects of nicotine. Microinjection of DHβE into the VTA reduced nicotine self-administration (Corrigall et al., 1994). In a place conditioning procedure, DHβE was found to block both the rewarding and the aversive properties of intra-VTA nicotine (Laviolette and van der Kooy, 2003). However, MLA blocked nicotine reward and switched the motivational valence from rewarding to aversive (Laviolette and van der Kooy, 2003). Nicotine produced a reduction in intracranial self-stimulation threshold, while intra-VTA MLA attenuated the effect of nicotine (Panagis et al., 2000). On the other hand, rats self-administered Δ^9-THC into the VTA (Zangen et al., 2006). As to the VTA, these findings may suggest the involvement of not only α4β2 but also α7 nAChRs in the cannabinoid system. Therefore, the manner in which CB1R and nAChR subtypes interact might not be the same in each region of the brain.

Despite differences in brain regions and incentive stimuli, AM251 and DHβE on reinstatement of MAP-seeking behavior were equipotent in their effect. In our previous study, nicotine equipotently attenuated reinstatement induced by cues or MAP-priming injections (Hiranita et al., 2006). The effects of nicotine were attenuated by mecamylamine at the same dose range (Hiranita et al., 2006). In other laboratories, the microinjection of SR141716A into different brain regions equipotently attenuated heroin- (Alvarez-Jaimes et al., 2007) and nicotine-seeking behavior (Kodas et al., 2007) induced by cues. Additionally, systemic administration of SR141716A equipotently attenuated the reinstatement of heroin-seeking behavior induced by cues or heroin-priming injections (De Vries et al., 2003). However, cannabinoid CB1Rs are expressed more densely in the cortex than in the nucleus accumbens (Mackie, 2005), whereas both α4β2 and α7 nAChRs are expressed densely in the PFC and striatum (Gotti et al., 2006). We previously reported that an opioid receptor antagonist, naltrexone, attenuated the reinstatement of MAP-seeking behavior induced by cues, but not MAP-priming injections (Anggadiredja et al., 2004b). A corticotrophin-releasing factor receptor antagonist, CP-154,526, attenuated reinstatement induced by cues more effectively than that induced by MAP-priming injections (Moffett and Goeders, 2007). Considering these differences, it is very surprising that singular mechanisms regulate reinstatement.

In summary, the present study demonstrated that CB1Rs and α4β2 nAChRs play a key role in the relapse to MAP-seeking behavior. These findings further provide support for considering substances that inactivate cannabinoid CB1Rs and α4β2 nAChR agonists for relieving drug cravings.

Acknowledgements

We thank Dr. Paul L. Soto, National Institute on Drug Abuse, for checking grammar and spelling of this manuscript. This study was supported by Grants-in-Aid for scientific research from the Ministry of Education, Culture, Sports, Science and Technology, the Ministry of Health, Labor, and Welfare and Japan and Smoking Research Foundation, Japan.

References

Alvarez-Jaimes, L., Polis, I., Parsons, L.H., 2007. Attenuation of cue-induced heroin-seeking behavior by cannabinoid CB(1) antagonist infusions into the nucleus accumbens core and prefrontal cortex, but not basolateral amygdala. Neuropsychopharmacology.

Anggadiredja, K., Nakamichi, M., Hiranita, T., Tanaka, H., Shoyama, Y., Watanabe, S., Yamamoto, T., 2004a. Endocannabinoid system modulates relapse to methamphetamine seeking: possible mediation by the arachidonic acid cascade. Neuropsychopharmacology 29, 1470–1478.

Anggadiredja, K., Sakimura, K., Hiranita, T., Yamamoto, T., 2004b. Naltrexone attenuates cue- but not drug-induced methamphetamine seeking: a possible

mechanism for the dissociation of primary and secondary reward. Brain Res. 1021, 272–276.
Cincotta, S.L., Yorek, M.S., Moschak, T.M., Lewis, S.R., Rodefer, J.S., 2008. Selective nicotinic acetylcholine receptor agonists: potential therapies for neuropsychiatric disorders with cognitive dysfunction. Curr. Opin. Investig. Drugs 9, 47–56.
Corrigall, W.A., Coen, K.M., Adamson, K.L., 1994. Self-administered nicotine activates the mesolimbic dopamine system through the ventral tegmental area. Brain Res. 653, 278–284.
De Vries, T.J., Homberg, J.R., Binnekade, R., Raaso, H., Schoffelmeer, A.N., 2003. Cannabinoid modulation of the reinforcing and motivational properties of heroin and heroin-associated cues in rats. Psychopharmacology (Berl.) 168, 164–169.
Decker, M.W., Brioni, J.D., Bannon, A.W., Arneric, S.P., 1995. Diversity of neuronal nicotinic acetylcholine receptors: lessons from behavior and implications for CNS therapeutics. Life Sci. 56, 545–570.
Di Ciano, P., Everitt, B.J., 2004. Direct interactions between the basolateral amygdala and nucleus accumbens core underlie cocaine-seeking behavior by rats. J. Neurosci. 24, 7167–7173.
Fuchs, R.A., Eaddy, J.L., Su, Z.I., Bell, G.H., 2007. Interactions of the basolateral amygdala with the dorsal hippocampus and dorsomedial prefrontal cortex regulate drug context-induced reinstatement of cocaine-seeking in rats. Eur. J. Neurosci. 26, 487–498.
Gessa, G.L., Casu, M.A., Carta, G., Mascia, M.S., 1998. Cannabinoids decrease acetylcholine release in the medial–prefrontal cortex and hippocampus, reversal by SR 141716A. Eur. J. Pharmacol. 355, 119–124.
Gotti, C., Zoli, M., Clementi, F., 2006. Brain nicotinic acetylcholine receptors: native subtypes and their relevance. Trends Pharmacol. Sci. 27, 482–491.
Grottick, A.J., Wyler, R., Higgins, G.A., 2000. The alpha4beta2 agonist SIB 1765F, but not the alpha7 agonist AR-R 17779, cross-sensitises to the psychostimulant effects of nicotine. Psychopharmacology (Berl.) 150, 233–236.
Hiranita, T., Anggadiredja, K., Fujisaki, C., Watanabe, S., Yamamoto, T., 2004. Nicotine attenuates relapse to methamphetamine-seeking behavior (craving) in rats. Ann. N.Y. Acad. Sci. 1025, 504–507.
Hiranita, T., Nawata, Y., Sakimura, K., Anggadiredja, K., Yamamoto, T., 2006. Suppression of methamphetamine-seeking behavior by nicotinic agonists. Proc. Natl. Acad. Sci. U.S.A. 103, 8523–8527.
Kalivas, P.W., McFarland, K., 2003. Brain circuitry and the reinstatement of cocaine-seeking behavior. Psychopharmacology (Berl.) 168, 44–56.
Kodas, E., Cohen, C., Louis, C., Griebel, G., 2007. Cortico-limbic circuitry for conditioned nicotine-seeking behavior in rats involves endocannabinoid signaling. Psychopharmacology (Berl.) 194, 161–171.
Laviolette, S.R., van der Kooy, D., 2003. The motivational valence of nicotine in the rat ventral tegmental area is switched from rewarding to aversive following blockade of the alpha7-subunit-containing nicotinic acetylcholine receptor. Psychopharmacology (Berl.) 166, 306–313.
Mackie, K., 2005. Distribution of cannabinoid receptors in the central and peripheral nervous system. Handb. Exp. Pharmacol., 299–325.
Malin, D.H., Lake, J.R., Upchurch, T.P., Shenoi, M., Rajan, N., Schweinle, W.E., 1998. Nicotine abstinence syndrome precipitated by the competitive nicotinic antagonist dihydro-beta-erythroidine. Pharmacol. Biochem. Behav. 60, 609–613.
Mansvelder, H.D., Keath, J.R., McGehee, D.S., 2002. Synaptic mechanisms underlie nicotine-induced excitability of brain reward areas. Neuron 33, 905–919.
Markou, A., Paterson, N.E., 2001. The nicotinic antagonist methyllycaconitine has differential effects on nicotine self-administration and nicotine withdrawal in the rat. Nicotine Tob. Res. 3, 361–373.
Miller, C.A., Marshall, J.F., 2005. Altered Fos expression in neural pathways underlying cue-elicited drug seeking in the rat. Eur. J. Neurosci. 21, 1385–1393.
Moffett, M.C., Goeders, N.E., 2007. CP-154,526, a CRF type-1 receptor antagonist, attenuates the cue-and methamphetamine-induced reinstatement of extinguished methamphetamine-seeking behavior in rats. Psychopharmacology (Berl.) 190, 171–180.
Oz, M., 2006. Receptor-independent actions of cannabinoids on cell membranes: focus on endocannabinoids. Pharmacol. Ther. 111, 114–144.
Panagis, G., Kastellakis, A., Spyraki, C., Nomikos, G., 2000. Effects of methyllycaconitine (MLA), an alpha 7 nicotinic receptor antagonist, on nicotine- and cocaine-induced potentiation of brain stimulation reward. Psychopharmacology (Berl.) 149, 388–396.
Picciotto, M.R., Zoli, M., Rimondini, R., Lena, C., Marubio, L.M., Pich, E.M., Fuxe, K., Changeux, J.P., 1998. Acetylcholine receptors containing the beta2 subunit are involved in the reinforcing properties of nicotine. Nature 391, 173–177.
Robbe, D., Alonso, G., Duchamp, F., Bockaert, J., Manzoni, O.J., 2001. Localization and mechanisms of action of cannabinoid receptors at the glutamatergic synapses of the mouse nucleus accumbens. J. Neurosci. 21, 109–116.
Robbe, D., Alonso, G., Manzoni, O.J., 2003. Exogenous and endogenous cannabinoids control synaptic transmission in mice nucleus accumbens. Ann. N.Y. Acad. Sci. 1003, 212–225.
Shalev, U., Grimm, J.W., Shaham, Y., 2002. Neurobiology of relapse to heroin and cocaine seeking: a review. Pharmacol. Rev. 54, 1–42.
Shoaib, M., 2008. The cannabinoid antagonist AM251 attenuates nicotine self-administration and nicotine-seeking behaviour in rats. Neuropharmacology 54, 438–444.
Solinas, M., Scherma, M., Fattore, L., Stroik, J., Wertheim, C., Tanda, G., Fratta, W., Goldberg, S.R., 2007. Nicotinic alpha 7 receptors as a new target for treatment of cannabis abuse. J. Neurosci. 27, 5615–5620.
Spivak, C.E., Lupica, C.R., Oz, M., 2007. The endocannabinoid anandamide inhibits the function of alpha4beta2 nicotinic acetylcholine receptors. Mol. Pharmacol. 72, 1024–1032.
Tzavara, E.T., Davis, R.J., Perry, K.W., Li, X., Salhoff, C., Bymaster, F.P., Witkin, J.M., Nomikos, G.G., 2003a. The CB1 receptor antagonist SR141716A selectively increases monoaminergic neurotransmission in the medial prefrontal cortex: implications for therapeutic actions. Br. J. Pharmacol. 138, 544–553.
Tzavara, E.T., Wade, M., Nomikos, G.G., 2003b. Biphasic effects of cannabinoids on acetylcholine release in the hippocampus: site and mechanism of action. J. Neurosci. 23, 9374–9384.
Walters, C.L., Brown, S., Changeux, J.P., Martin, B., Damaj, M.I., 2006. The beta2 but not alpha7 subunit of the nicotinic acetylcholine receptor is required for nicotine-conditioned place preference in mice. Psychopharmacology (Berl.) 184, 339–344.
Zangen, A., Solinas, M., Ikemoto, S., Goldberg, S.R., Wise, R.A., 2006. Two brain sites for cannabinoid reward. J. Neurosci. 26, 4901–4907.

[Jpn. J. Alcohol & Drug Dependence
43 (3), 158～165, 2008]

Mini Review

薬物自己投与実験法を用いての薬物依存研究

縄田陽子，山本経之

長崎国際大学薬学部薬理学研究室

（受理：平成20年4月21日）

Drug self-administration methods to the study of drug craving (drug seeking behavior)

Yoko NAWATA and Tsuneyuki YAMAMOTO

Department of Pharmacology, Faculty of Pharmaceutical Science, Nagasaki International University, 2825-7, Huis Ten Bosch, Sasebo, Nagasaki 859-3298, Japan

(Accepted : April 21, 2008)

Summary

The drug self-administration procedure is necessary for the study of addiction in terms of two similarities with human. One similarity is drug taking pattern, and the other is reinstatement of drug-seeking responses after the withdrawal. This procedure consists of two phase, called as the acquisition of drug taking behavior and the extinction. The drug taking behavior is formed on the base of reinforcing effects of drug under a fixed ratio schedule. On the other hands, a progressive ratio schedule is a useful procedure to estimate the potency of reinforcing/rewarding effect. Clinically, drug craving can be triggered by taking the other drug which has the effects similar to abused drug, by stimuli previously associated with drug-taking, or by exposure to stressors. In preclinical study, these three initiating factors also reinstate drug-seeking. From the studies that clarify the responsible brain regions, the importance of the nucleus accumbens, prefrontal cortex and hippocampus is clarified. The brain regions associated with drug craving are identified using a PET and fMRI technologies. In the future study, it is expected to integrate/reconstruct between the identified brain regions that provoke drug craving in humans and responsible brain regions that induce reinstatement of drug-seeking in animal model of drug dependence.

※教育委員会からの推薦論文：啓蒙的な実験技術や実験方法

Key words: drug taking behavior, drug seeking behavior, drug priming injection, drug associated cue, stress
薬物自己投与行動，薬物探索行動，薬物プライミング投与，薬物関連刺激，ストレス

はじめに

薬物依存症は，薬物を摂取したいという強い欲望／強迫感にかられ，薬物摂取行動を制御する事が困難となり，薬物への渇望の再燃と薬物の反復使用を繰り返す進行性疾患と考えられている[1]．渇望再燃の危険性を予測する事は臨床上大切な事であるが，現在ではCocaine Rapid Evaluation Screening Trial（CREST）法などにより患者の渇望程度をスコア化（Craving Scale）し推察している．さらに，Craving Scaleだけに頼るのではなく，心拍数や血圧などの自律神経反応および血中コルチゾール等のストレスマーカーも調べ，総体的に渇望の再燃を捉える試みもなされている[2]．一方，動物実験における薬物依存に関する研究は，これまで薬物の報酬効果を中心になされてきた．しかし，臨床上の問題点は薬物への渇望の再燃であり，この点に重きが置かれている．

本稿では，ヒトの薬物摂取行動様式と類似する薬物自己投与実験法を取り上げ，薬物探索行動の発現に焦点を当て概説した．

1. 薬物の“自己投与”と“他己投与”に基づく相違

大部分の実験系での薬物投与様式は，実験者が投与する“他己”投与で行われている．薬物依存に関する研究では，ヒトでの薬物摂取様式を念頭におけば，おのずと実験動物が自発的に薬物を摂取する自己投与実験法に，より妥当性があると考えられる．一方，自己投与と他己投与では，薬物の作用発現に差異がある事が“yoked”実験法から明らかにされた．“yoked”実験法は，連動した2つの実験系で行われる．動物は自らレバーを押す事によって報酬としての薬物が自動的に注入される（自己投与群）．この時，自らレバーを押して薬物を注入する事が出来ない別の装置の動物に，自己投与群のレバー押しに呼応して同時期に同容量の薬物が注入される（yoked群）．自らの意思でレバーを押すか否かの相違はあるが，薬物注入に関して両者間には差がなく，全く同じである．それにも関わらず，脳内報酬系を構成する側坐核ならびに扁桃体におけるドパミン遊離量は，コカインやアンフェタミン自己投与群およびyoked群の両群で有意な増加は認められるものの，その増加は自己投与群で有意に高い事が明らかとなった[3-5]．さらに，コカイン退薬時に薬物探索行動を誘発する自己投与群では，側坐核でのグルタミン酸の遊離が著しく増加するが，yoked群ではそのような変化が認められていない[6]．このように，薬物投与形態が受動的／能動的であるかによって，薬物の作用態度は薬物の投与量・投与パターンが同一であっても，異なる事が分かる．この事は薬物摂取に対する“意欲”がもたらす薬理学的変容と考えると興味深い．

2. 薬物摂取行動と薬物探索行動の発現

薬物自己投与実験法は，①薬物摂取行動の獲得過程，②薬物を生理食塩液に切り替えての消

表1 アカゲザルでの薬物静脈内自己投与実験における比率累進試験法を用いてのレバー押し反応の最終比率（final ratios; breaking point）

Drug	Uni Dose (mg/kg/inj)	Pretreatment (2～4weeks)	Final Ratios (high range)
Morphine	0.25	−	1,350～ 1,600
		+	1,260～ 6,400
Dihydrocodeine	1.0	−	950～ 1,900
		+	4,530～10,760
Pentazocine	1.0	−	1,350～ 3,810
		+	2,260～ 3,810
Alcohol	800	−	1,600～ 6,400
		+	3,200～ 6,400
Diazepam	1.0	−	950～ 3,200
		+	670～ 1,900
Cocaine	0.11	−	1,600～ 6,400
		+	800～ 3,200
Nicotine	0.25	−	1,350～ 2,690
		+	670～ 1,900

比率累進試験に先立ち，2～4週間の被験薬の反復強制投与（+），または生理食塩液の反復強制投与（−）を行っている．文献7）より引用

去（退薬）過程の2つの要素から成る．薬物依存研究の中では，前者においては薬物の持つ強化効果／報酬効果の評価系として，また後者においては薬物への渇望（薬物探索行動）発現の評価系として利用されている．

薬物摂取行動

薬物自己投与行動実験には，定率（Fixed-Ratio；FR）実験法と比率累進（Progressive-Ratio；PR）実験法が用いられている．FR実験法では，動物は特定した回数の反応によって報酬としての薬物を得る事が出来る．一方，PR実験法は1回の摂取に必要なレバー押し回数を累進増加させる方法で，薬物摂取を断念する直前のレバー押し回数（ブレーキングポイント）で評価される．PR実験法は，強化効果／報酬効果の強さを定量的に評価する上で有用な方法である．表1は，サルを用いた代表的な薬物におけるブレーキングポイントを示している[7]．薬物自己投与行動が確立された後，さらに被験薬を2～4週間反復強制投与すると，モルヒネおよびジヒドロコデインでは身体依存が形成される．このような状況下では，ブレーキングポイントは生理食塩液反復強制投与群に比べて著しく増加する．これらの行動パターンは，退薬症候の苦痛から反復投与を繰り返し，精神依存がさらに増強される臨床像を彷彿させる．一方，反復強制投与しても身体依存を起こさないジアゼパム，コカインならびにニコチンでは，ブレーキングポイントは，生理食塩液反復強制投与群に比べて逆に減少する傾向にあった．

薬物探索行動

臨床上，最も重要な事は薬物への渇望の再燃メカニズムを明らかにする事である．渇望を誘