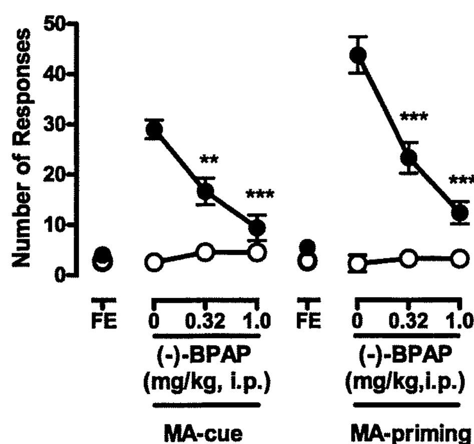

Fig. 3. Effects of acute administration of (−)-BPAP on reinstatement of methamphetamine-seeking behavior. (−)-BPAP was administered i.p. 30 min before the session. Closed and open circles indicate responding on active and inactive levers, respectively. ** $P<0.01$, and *** $P<0.001$ versus a vehicle-treated group challenged with methamphetamine-associated cues or methamphetamine-priming injections. FE; final extinction. In the test on methamphetamine-associated cues, the sample sizes of the FE session and (−)-BPAP pretreatment at the dose of 0, 0.32, and 1.0 mg/kg were 31, 10, 11, and 10, respectively. In the test on methamphetamine-priming the injection, sample sizes of the FE session and (−)-BPAP pretreatment at the dose of 0, 0.32, and 1.0 mg/kg were 23, seven, seven, and nine, respectively.

stated methamphetamine-seeking behavior (Fig. 3). One-way ANOVA indicated significant effects of methamphetamine-associated cues and methamphetamine-priming injections on active lever responses ($P<0.001$, both) but not inactive lever responses ($P=0.792$ and 0.184, respectively). *Post hoc* analysis (Dunn's method) indicated significant effects of methamphetamine-associated cues and methamphetamine-priming injections on active lever responses ($Q=5.060$, $P<0.05$, and $Q=4.208$, $P<0.05$, respectively). The total amount of methamphetamine intake was not correlated with the increase induced by either methamphetamine-associated cues or methamphetamine-priming injections ($r=0.459$ and -0.611, $P=0.182$ and 0.145, $n=10$ and 7, respectively).

Similar to the repeated administration of (−)-BPAP during the extinction phase, acute administration of (−)-BPAP (0.32 and 1.0 mg/kg i.p.) on test day also attenuated an increase in active lever responses induced by methamphetamine-associated cues and methamphetamine-priming injections (Fig. 3). Two-way measures ANOVA indicated significant effects of (−)-BPAP dose ($F(2,48)=47.455$, $P<0.001$) and reinstatement factor ($F(1,48)=14.097$, $P<0.001$), but not the interaction ($F(2,48)=2.620$, $P=0.083$) on active lever responses. The *post hoc* analysis indicated significant effect of (−)-BPAP at the dose of 0.32 and 1.0 mg/kg on active lever responses induced by methamphetamine-associated cues ($t=3.605$, $P=0.002$ and $t=5.584$, $P<0.001$) and methamphetamine-priming injections ($t=4.903$, $P<0.001$ and $t=7.969$, $P<0.001$, respectively). However, acute administration of (−)-BPAP had non-significant effects on inactive lever responses ($P\geq0.25$). Two-way measures ANOVA indicated non-significant effect of (−)-BPAP on (−)-BPAP dose, reinstatement factor, and the interaction ($F(2,48)=0.678$, $P=0.513$; $F(1,48)=0.679$, $P=0.414$; and $F(2,48)=0.074$, $P=0.929$, respectively). As indicated in Fig. 4, pre-session treatment with SCH-23390, a dopamine D_1-like receptor antagonist, (0.1, 1.0, and 10 μg/kg s.c.) did not significantly reverse the attenuating effect of pre-session treatment with (−)-BPAP on the active lever responses induced by methamphetamine-associated cues or methamphetamine-priming injections. Two-way measures ANOVA indicated non-significant effects of SCH-23390 dose, reinstatement factor, and the interaction on the (−)-BPAP-induced decrease in active lever responses ($F(3,38)=1.664$, $P=0.191$; $F(1,38)=1.845$, $P=0.182$; and $F(3,38)=2.720$, $P=0.058$, respectively). When pre-treated with (−)-BPAP, SCH-23390 did not affect inactive lever responses (Fig. 4). Two-way measures ANOVA indicated non-significant effect of the pre-session treatment with SCH-23390 on the drug dose ($F(3,38)=0.261$, $P=0.853$), reinstatement factor ($F(1,38)=2.261$, $P=0.141$), and the interaction ($F(3,38)=0.821$, $P=0.821$). Meanwhile, pretreatment with amisulpride, a dopamine D_2-like receptor antagonist, (3.2 or 10 mg/kg i.p.) failed to affect the attenuating effects of (−)-BPAP on increased active lever responses induced by methamphetamine-associated cues or methamphetamine-priming injections (Fig. 5). Two-way measures ANOVA indicated non-significant effect of amisulpride dose ($F(3,34)=2.166$, $P=0.110$) and reinstatement factor ($F(1,34)=0.576$, $P=0.453$) on the active lever responses. Pre-session treatment with amisulpride (3.2 or 10 mg/kg i.p.) failed to affect inactive lever responses (Fig. 5). Two-way measures ANOVA indicated non-significant effect of amisulpride dose ($F(3,34)=0.570$, $P=0.639$) and reinstatement factor ($F(1,34)=0.254$, $P=0.617$) on the inactive lever responses. Additionally, co-pretreatment with SCH-23390

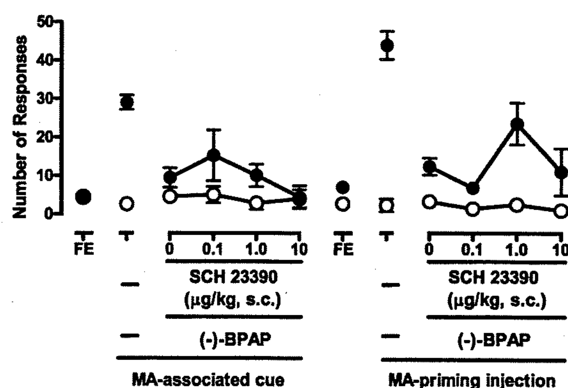

Fig. 4. Effect of acute administration of (−)-BPAP (1.0 mg/kg i.p.) with pre-session treatment with SCH-23390, a dopamine D_1-like receptor antagonist, on reinstatement of methamphetamine-seeking behavior. SCH-23390 was administered s.c. and concurrently with (−)-BPAP 30 min before the session. Closed and open circles indicate responding on active and inactive levers. FE; final extinction. In the test on methamphetamine-associated cues, the sample sizes of the FE session, vehicle alone, and (−)-BPAP co-pretreated with SCH-23390 at the dose of 0, 0.1, 1.0 and 10 mg/kg were 19, 10, five, five, five, and four, respectively. In the test on methamphetamine-priming injections, the sample sizes of the FE session, vehicle alone, and (−)-BPAP co-pretreated with SCH-23390 at the dose of 0, 0.1, 1.0 and 10 mg/kg were 10, nine, five, four, and three, respectively.

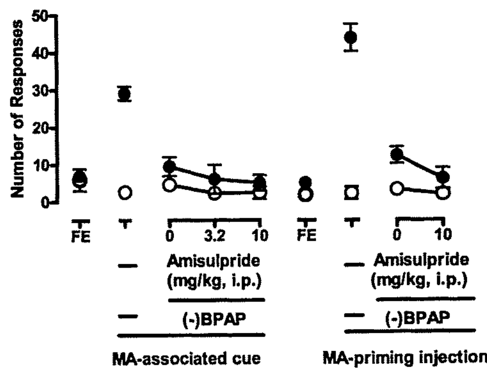

Fig. 5. Effect of acute administration of (−)-BPAP (1.0 mg/kg i.p.) with pre-session treatment of amisulpride, a dopamine D_2-like receptor antagonist, on reinstatement of methamphetamine-seeking behavior. Amisulpride and (−)-BPAP were administered i.p. 1 h and 30 min before the session, respectively. Closed and open circles indicate responding on active and inactive levers. FE; final extinction. In the test on methamphetamine-associated cues, the sample sizes of the FE session, vehicle alone, and (−)-BPAP co-pretreated with amisulpride at the dose of 0, 3.2, and 10 mg/kg were eight, 10, five, four and five, respectively. In the test on methamphetamine-priming injections, the sample sizes of the FE session, vehicle alone, and (−)-BPAP co-pretreated with amisulpride at the dose of 0, and 10 mg/kg were five, nine, five, and six, respectively.

(10 μg/kg s.c.) and amisulpride (10 mg/kg i.p.) failed to block the attenuating effect of pre-session treatment with (−)-BPAP on the increased active lever responses induced by methamphetamine-associated cues and methamphetamine-priming injections (Fig. 6). Two-way measures ANOVA indicated non-significant effect of drug treatment ($F(1,24)=3.138$, $P=0.089$), reinstatement factor ($F(1,24)=0.589$, $P=0.450$), and the interaction ($F(1,24)=0.0616$, $P=0.806$) on the active lever responses. The co-pretreatment with SCH-23390 and amisulpride also failed to alter responses on inactive levers (Fig. 6). Two-way measures ANOVA indicated non-significant effect of drug treatment ($F(1,24)=0.971$, $P=0.334$), reinstatement factor ($F(1,24)=0.0277$, $P=0.869$) and the interaction ($F(1,24)=0.324$, $P=0.574$) on the active lever responses.

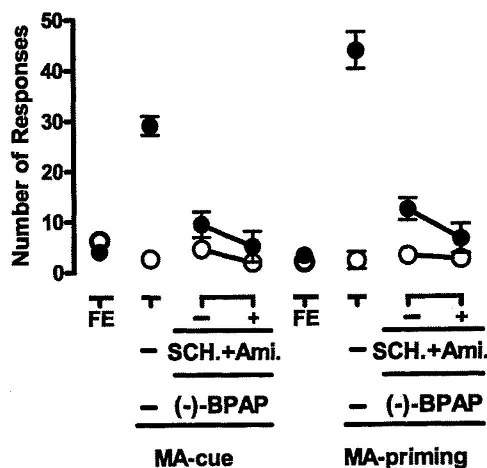

Fig. 6. Effect of pre-session treatment with (−)-BPAP (1.0 mg/kg i.p.) combined with both SCH-23390 (10 μg/kg s.c.) and amisulpride (10 mg/kg i.p.) on reinstatement of methamphetamine-seeking behavior. (−)-BPAP, and SCH-23390 were administered 30 min before the session, whereas amisulpride was administered 1 h before the session. Closed and open circles indicate responding on active and inactive levers. FE; final extinction. In the test on methamphetamine-associated cues, the sample sizes of the FE session, vehicle alone, and pre-session treatment with (−)-BPAP co-pretreated with and without combination of SCH-23390 and amisulpride were five, 10, five, and five, respectively. In the test on methamphetamine-priming injections, the sample sizes of the FE session, vehicle alone, and pre-treatment with (−)-BPAP co-pretreated with and without combination of SCH-23390 and amisulpride were four, nine, five, and four, respectively.

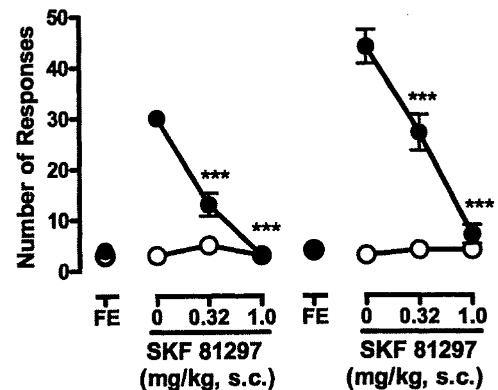

Fig. 7. Effects of pre-session treatment with SKF-81297, a dopamine D_1-like receptor agonist, on reinstatement of methamphetamine-seeking behavior. SKF-81297 was administered s.c. 15 min before the session. Closed and open circles indicate responding on active and inactive levers, respectively. *** $P<0.001$ versus groups challenged with methamphetamine-associated cues or methamphetamine-priming injections alone. FE; final extinction. In the test on methamphetamine-associated cues, the sample sizes of the FE session and SKF-81297 pretreatment at the dose of 0, 0.32, and 1.0 mg/kg were 24, eight, eight, and eight, respectively. In the test on methamphetamine-priming injections, the sample sizes of the FE session and SKF-81297 pretreatment at the dose of 0, 0.32, and 1.0 mg/kg were 24, eight, eight, and eight, respectively.

Effect of pre-session treatment with SKF-81297, a dopamine D_1-like receptor agonist, on reinstatement of methamphetamine-seeking behavior

In an experiment on controls, methamphetamine-associated cues and methamphetamine-priming injections reinstated methamphetamine-seeking behavior (Fig. 7). One-way ANOVA indicated significant effects of methamphetamine-associated cues on active lever responses ($F(1,30)=579.836$, $P<0.001$) and methamphetamine-priming injections on active and inactive lever responses ($P<0.001$ and $=0.041$). *Post hoc* analysis indicated significant effects of methamphetamine-associated cues on active lever responses ($t=24.080$, $P<0.001$, Bonferroni t-test) and methamphetamine-priming injections on active and inactive lever responses ($Q=4.178$, $P<0.05$, and $Q=2.024$, $P<0.05$, respectively, Dunn's Method). Total amount of methamphetamine intake was not correlated with the increase induced by either methamphetamine-associated cues or methamphetamine-priming injections ($r=-0.681$ and -0.401, $P=0.0628$ and 0.325, $n=8$ and 8, respectively).

Pre-session treatment with SKF-81297 (0.32–1.0 mg/kg s.c.) dose-dependently attenuated an increase in active lever responses induced by methamphetamine-associated cues and methamphetamine-priming injections (Fig. 7). Two-way measures ANOVA indicated significant effects of SKF-81297 dose ($F(2,47)=88.858$, $P<0.001$), reinstatement factor ($F(1,42)=30.898$, $P<0.001$), but not the interaction ($F(2,42)=3.163$, $P=0.053$) on increase in active lever responses induced by methamphetamine-associated cues and methamphetamine-priming injections. The *post hoc* analysis indicated significant effect of SKF-81297 at the dose of 0.32 and 1.0 mg/kg on an increase in active lever responses induced by methamphetamine-associated cues ($t=5.001$, $P<0.001$, and $t=7.911$, $P<0.001$) and methamphetamine-priming injections ($t=4.889$, $P<0.001$ and $t=10.934$, $P<0.001$, respectively). The pre-session treatment with SKF-81297 failed to affect responses on inactive levers (Fig. 7). Two-way measures ANOVA indicated non-significant effect of SKF-81297 dose ($F(2,47)=1.719$, $P=0.192$), reinstatement factor ($F(1,47)=0.0559$, $P=0.814$) and the interaction ($F(2,47)=0.753$, $P=0.477$) on the inactive lever responses. Pre-session treatment with SCH-23390 (0.1 and 1.0 μg/kg s.c.) dose-dependently blocked the attenuating effect of pre-session treatment SKF-81297 (1.0 mg/kg i.p.) on methamphetamine-associated cues as well as methamphetamine-priming injections (Fig. 8). Two-way measures ANOVA indicated significant effects of SCH-23390 dose ($F(2,30)=91.427$, $P<0.001$), reinstatement factor ($F(1,30)=8.728$, $P<0.001$), and the interaction ($F(2,30)=3.978$, $P=0.0029$) on the active lever responses induced by methamphetamine-associated cues and methamphetamine-priming injections. The *post hoc* analysis indicated significant effect of SCH-23390 at the dose of 0.1 and 1.0 μg/kg on the active lever responses induced by methamphetamine-associated cues ($t=3.049$, $P=0.014$, and $t=7.546$, $P<0.001$) and methamphetamine-priming injections ($t=5.272$, $P<0.001$, and $t=11.526$, $P<0.001$, respectively). However, SCH-23390 did not affect inactive lever responses (Fig. 8). Two-way measures ANOVA indicated significant effects of SCH-23390 dose ($F(2,30)=0.935$, $P=0.404$), reinstatement factor ($F(1,30)=1.555$, $P=0.222$), and the interaction ($F(2,30)=0.004\ 31$, $P=0.996$) on the inactive lever responses.

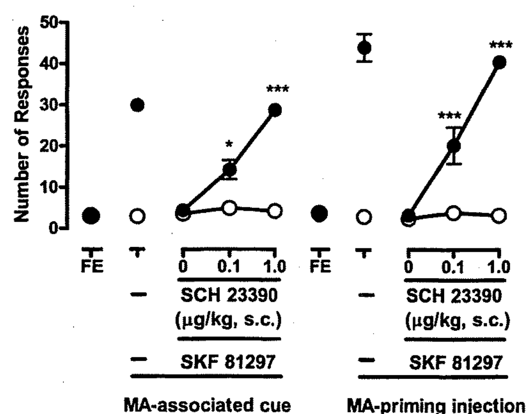

Fig. 8. Effects of pre-session treatment with SKF-81297 (1.0 mg/kg s.c.) combined with SCH-23390 (0, 0.1 and 1.0 μg/kg s.c.) on reinstatement of methamphetamine-seeking behavior. SKF-81297 and SCH-23390 were administered 15 and 30 min before the session. Closed and open circles indicate responding on active and inactive levers. * $P<0.05$ and *** $P<0.001$ versus SKF-81297-pretreated groups challenged with methamphetamine-associated cues or methamphetamine-priming injections alone. FE; final extinction. In the test on methamphetamine-associated cues, the sample sizes of the FE session, vehicle alone, and SKF-81297 co-pretreated with SCH-23390 at the dose of 0, 0.1, and 1.0 mg/kg were 26, eight, six, six, and six, respectively. In the test on methamphetamine-priming injections, the sample sizes of the FE session, vehicle alone, and (−)-BPAP co-pretreated with SKF-81297 at the dose of 0, 0.1, and 1.0 mg/kg were 26, eight, six, six, and six, respectively.

Effects of administration of (−)-BPAP, SKF-81297 or SCH-23390 alone under extinction condition in methamphetamine self-administered rats after extinction sessions

(−)-BPAP alone (1.0–3.2 mg/kg i.p.) failed to affect lever responses on active and inactive levers (Fig. 9A). At the higher dose (10 mg/kg i.p.), (−)-BPAP increased in both active and inactive lever responses (Fig. 9A). One-way ANOVA and the subsequent *post hoc* analysis with Dunn's method indicated significant effect of (−)-BPAP at the dose of 10 mg/kg on active lever responses ($Q=3.598$, $P<0.05$). In addition, one-way ANOVA indicated significant effect of (−)-BPAP at 10 mg/kg on inactive lever responses ($P=0.038$); however, the subsequent *post hoc* analysis with Dunn's method indicated non-significant effect of (−)-BPAP at 10 mg/kg on inactive lever responses ($Q=2.071$, $P\geq0.05$) compared with that at 0 mg/kg. Total amount of methamphetamine intake was correlated with responses on neither active nor inactive responses ($r=0.4598$ and 0.2169, $P=0.3589$ and 0.6797, respectively). On the other hand, neither SKF-81297 (0.032–1.0 mg/kg s.c., Fig. 9B) nor SCH-23390 (1–100 μg/kg s.c., Fig. 9C) affected responses on active and inactive levers. One-way ANOVA indicated non-significant effect of SKF-81297 and SCH-23390 on active ($F(3,32)=0.139$, $P=0.936$ and $F(3,32)=0.077$, $P=0.972$) and inactive ($F(3,32)=0.615$, $P=0.611$ and $F(3,32)=0.171$, $P=0.915$, respectively) lever responses.

Effects of pre-session treatment with (−)-BPAP on methamphetamine self-administration

On day 9 of methamphetamine self-administration, the number of methamphetamine infusions was 16.5±2.2 (Fig. 10). On day 10, pre-session treatment with (−)-BPAP (1.0 mg/kg i.p.) did not affect the number of methamphetamine infusions (Fig. 10, 16.8±1.6). Twenty four hours after the (−)-BPAP pretreatment, the number of methamphetamine infusions (Fig. 10, 16.8±2.9) was unchanged from those on the previous 2 days. Similar to the number of methamphetamine infusions, pre-session treatment with (−)-BPAP failed to affect responses on active and inactive levers during these 3 days (date not shown). One-way repeated measures ANOVA indicated non-significant effects of pre-session treatment with (−)-BPAP on total amount of methamphetamine intake ($F(5,10)=2.006$,

P=0.185), and active (F(5,10)=0.0185, P=0.982) and inactive (F(5,10)=0.200, P=0.822) lever responses.

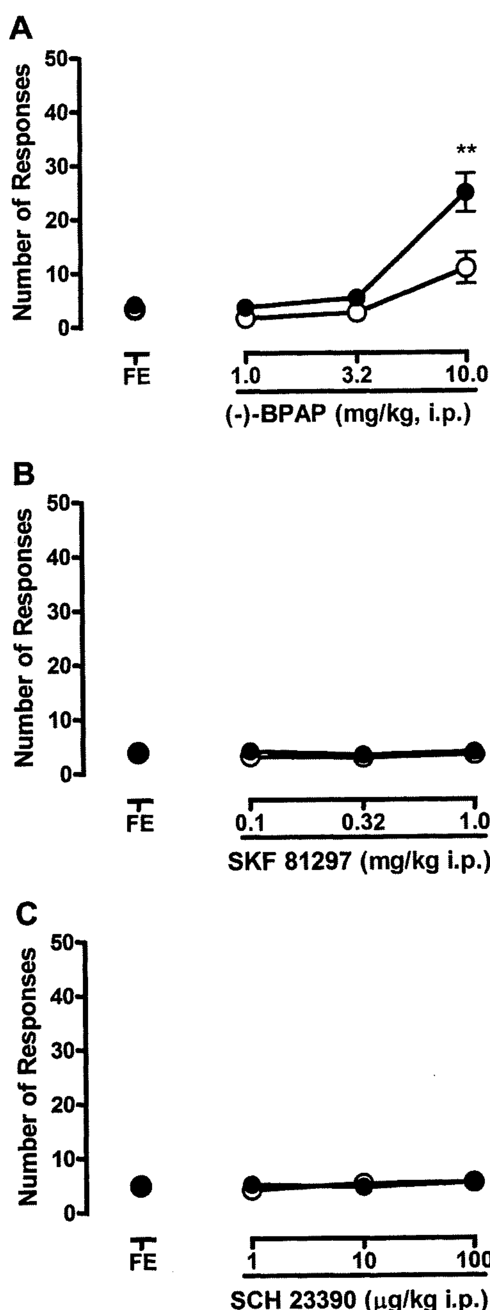

Fig. 9. Priming effects of pre-session treatment with (−)-BPAP, (A) SKF-81297, (B) and SCH-23390, (C) alone under extinction condition in methamphetamine self-administered rats after extinction sessions. (−)-BPAP was administered i.p. 30 min before the sessions, whereas SKF-81297 and SCH-23390 were administered s.c. 15 and 30 min before the session. Closed and open circles indicate responding on active and inactive levers. ** P<0.01 versus responding on active or inactive levers on final extinction (FE) day. (A) The sample sizes of the FE session and (−)-BPAP at the dose of 1.0, 3.2, and 10 mg/kg were 16, six, four, and six, respectively. (B) The sample sizes of the FE session, and SKF-81297 at the dose of 0.1, 0.32, and 1.0 mg/kg were 18, six, six, and six, respectively. (C) The sample sizes of the FE session, and SCH-23390 at the dose of 1.0, 10, and 100 μg/kg were 18, six, six, and six, respectively.

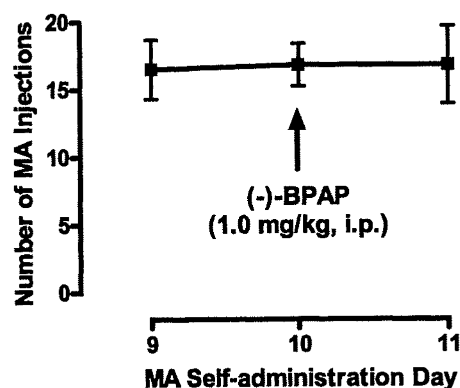

Fig. 10. Effects of pre-session treatment with (−)-BPAP in methamphetamine self-administering rats (n=6). (−)-BPAP was administered i.p. 30 min before the sessions.

Effects of (−)-BPAP and SKF-81297 on food-maintained behavior 5 min after the reinstatement sessions

In contrast to reinstatement of methamphetamine-seeking behavior, no pre-treatment with (−)-BPAP or SKF-81297 across the dose ranges tested had significant effects on food-maintained responses (Table 1). Two-way measures ANOVA indicated non-significant effect of repeated administration of (−)-BPAP on the dose (F(1,23)=2.824, P=0.106), reinstatement factor (F(1,23)=0.192, P=0.665), and the interaction (F(1,23)=0.295, P=0.592). Regarding single administration of (−)-BPAP, two-way measures ANOVA indicated a non-significant effect of (−)-BPAP on the dose (F(2,48)=0.170, P=0.844), reinstatement factor (F(1,48)=0.150, P=0.700), and the interaction (F(2,48)=0.244, P=0.784). In addition, two-way measures ANOVA indicated non-significant effect of SKF-81297 on SKF-81297 dose (F(2,42)=0.162, P=0.851), reinstatement factor (F(1,42)=0.119, P=0.732), and the interaction (F(2,42)=0.0315, P=0.969).

DISCUSSION

Repeated administration of (−)-BPAP during extinction sessions attenuated reinstatement of methamphetamine-seeking behavior induced by methamphetamine-associ-

Table 1. Effect of (−)-BPAP or SKF-81297 on food-maintained behavior 5 min after the reinstatement sessions. Regarding the sample sizes, see Figs. 2, 3 and 7

Treatment	MA-associated cue	MA-priming injection
Repeated (−)-BPAP (mg/kg/day, i.p.)		
0	10.8±1.2	12.0±1.3
1.0	9.5±0.4	9.4±1.2
Single (−)-BPAP (mg/kg, i.p.)		
0	11.4±1.3	11.0±1.6
0.32	10.7±0.9	10.9±1.5
1.0	10.9±1.4	12.4±1.7
SKF-81297 (mg/kg, s.c.)		
0	10.6±0.4	11.1±0.4
0.32	10.9±0.4	10.8±0.4
1.0	10.8±0.5	10.8±0.6

ated cues and methamphetamine-priming injections. This result may suggest a preventive role of (−)-BPAP against the development of relapse to methamphetamine craving. Moreover, even a single pre-session treatment with (−)-BPAP also attenuated the reinstatement induced by methamphetamine-associated cues and methamphetamine-priming injections in a dose-related manner. Surprisingly, neither pre-session treatment with SCH-23390, a dopamine D_1-like receptor antagonist, nor amisulpride, a dopamine D_2-like receptor antagonist, across dose ranges tested appreciably reversed the inhibitory effect of single pre-session treatment with (−)-BPAP. In addition, combined pre-session treatment with SCH-23390 and amisulpride failed to reverse the effect of a single pre-session treatment with (−)-BPAP. This finding suggests that (−)-BPAP blocks the reinstatement of methamphetamine-seeking behavior through mechanisms other than dopamine receptors. On the other hand, pre-session treatment with SKF-81297, a dopamine D_1-like receptor agonist, dose-dependently attenuated the reinstatement of methamphetamine-seeking behavior induced by either methamphetamine-associated cues or methamphetamine-priming injections similar to the result obtained from the single pre-session treatment with (−)-BPAP. In contrast to (−)-BPAP, SCH-23390 dose-dependently reversed the inhibitory effect of SKF-81297. Additionally, SCH-23390 alone failed to reinstate methamphetamine-seeking behavior. Therefore, these results suggest an inhibitory role of dopamine D_1-like receptors to reinstate methamphetamine-seeking behavior in rats. Several studies demonstrated inactivated function of dopamine D_1-like receptors. For example, clinical study demonstrated reduced activity of adenyl cyclase after striatal dopamine D_1-like receptor-stimulation in methamphetamine abusers (Tong et al., 2003). In preclinical study, rats that self-administered methamphetamine exhibited downregulation of dopamine D_1-like receptor protein in the nucleus accumbens during withdrawal (Stefanski et al., 1999). However, inability of SCH-23390 to reinstate methamphetamine-seeking behavior in the present study suggests that blockade of dopamine D_1-like receptors by itself is insufficient to reinstate methamphetamine-seeking behavior.

Decrease in lever responding might result from a general overactivation or suppression of behavioral activity. However, neither repeated nor single pretreatment of (−)-BPAP decreased in responding maintained by food reinforcement. Furthermore, pre-session treatment with SKF-81297 also failed to affect food-maintained responding. Additionally, the half-life of radio-labeled [(−)-BPAP-14C] has been reported to be 5.5 to 5.8 h, which is long enough for (−)-BPAP to continue the action during sessions on food reinforcement (Magyar et al., 2002). Therefore, blocking effects of (−)-BPAP or SKF-81297 on the reinstatement of methamphetamine-seeking behavior do not result from nonspecific behavioral effects.

Pre-session treatment with (−)-BPAP at a dose of 1.0 mg/kg selectively affected reinstatement of methamphetamine-seeking behavior, but not methamphetamine self-administration. Radio-labeled [(−)-BPAP-14C] has been reported to be well-absorbed after the i.p. and s.c. treatment and the peak concentration in the rat brain has been reached at 30 to 60 min following s.c. administration (Magyar et al., 2002). Therefore, during the session on reinstatement and self-administration in the present study, the concentration of (−)-BPAP in the brain appears to reach a peak. Considering these findings, our data suggest that methamphetamine's reinforcing effect might be less sensitive to actions of (−)-BPAP compared to the reinstatement of methamphetamine-seeking behavior. Clinical study also demonstrated that the reinforcing effect of psychostimulants is extremely robust and simply unaltered by even substantial medication effects on drug "craving" or its subjective effects. Thus, maintenance on the antidepressant desipramine in volunteers with a history of cocaine abuse resulted in a 40% decrease in ratings of "I want cocaine," yet had no effect on the amount of cocaine self-administered (Fischman et al., 1990). Therefore, (−)-BPAP may be effective as an anti-relapse therapeutic; however, (−)-BPAP may not work sufficiently as anti-methamphetamine abuse medication.

Pre-session treatment with (−)-BPAP alone at the dose 1.0 and 3.2 mg/kg did not reinstate methamphetamine-seeking behavior, whereas (−)-BPAP at only the highest dose (10.0 mg/kg) demonstrated moderate reinstatement. A tenfold higher dose was needed to reinstate methamphetamine-seeking behavior compared with the dose at which pre-session treatment with (−)-BPAP (1.0 mg/kg) attenuated the reinstatement of methamphetamine-seeking behavior induced by methamphetamine-associated cues and methamphetamine-priming injections. On the other hand, agonist/substitution therapies for opiate abuse with methadone and tobacco addiction with various formulations of nicotine have been reported to be effective (Henningfield, 1995; Kreek, 1996). In addition to the beneficial results, self-administration of methadone (Altshuler et al., 1975; Werner et al., 1976; Oei et al., 1980; Martin et al., 2007) and nicotine (Le Foll and Goldberg, 2005) in humans or experimental animals has been reported. Considering the positive and negative reports on methadone and nicotine, the potential ability of (−)-BPAP at the high dose to reinstate methamphetamine-seeking behavior in clinical situations may not discredit its clinical application as an anti-relapse agent for methamphetamine abusers.

(−)-BPAP has been reported to be an inhibitor of monoamine uptake in HEK cell (IC_{50} values: [^{3}H] dopamine, [^{3}H] noradrenaline, and [^{3}H] serotonin; 42, 52, and 640 nM, respectively) (Shimazu et al., 2003b), suggesting possible involvement of the relatively higher affinity for noradrenaline transporters in inhibitory effects of (−)-BPAP on the reinstatement of methamphetamine-seeking behavior. Moreover, methamphetamine has an at least twofold higher affinity for noradrenaline transporters than dopamine transporters but negligible affinity for serotonin transporters (Rothman and Baumann, 2003). However, dissimilar to methamphetamine (Yoneda et al., 2001) and tyramine (Shimazu et al., 2003b), (−)-BPAP alone does not release catecholamines. In addition, (−)-BPAP blocked tyramine-induced noradrenaline and dopamine

release from rat brain synaptosomes (Shimazu et al., 2003b), rather than potentiating the release. If binding of (−)-BPAP at noradrenaline transporters contributes to an inhibitory effect of (−)-BPAP on the reinstatement of methamphetamine-seeking behavior, selective noradrenaline uptake inhibitors would mimic the effect. So far, no studies in rats have reported pre-session treatment effect of selective noradrenaline uptake inhibitors on reinstatement of drug-seeking behavior, whereas a few studies in squirrel monkeys and humans have reported such effects. In squirrel monkeys, pre-session treatment with selective noradrenaline uptake inhibitors nisoxetine and talsupram both produced leftward shift of dose-effect curve of reinstatement of cocaine-seeking behavior induced by cocaine-priming injections; however, neither of the two selective noradrenaline uptake inhibitors affected the dose-effect curve of reinstatement of cocaine-seeking behavior induced by priming injections of GBR 12909, a selective dopamine uptake inhibitor (Platt et al., 2007). Furthermore, priming injections of nisoxetine and talsupram alone reinstated cocaine-seeking behavior (Platt et al., 2007). On the contrary, one clinical study has found positive results of the selective noradrenaline uptake inhibitor reboxetine to maintain cocaine abstinence (Szerman et al., 2005). Although the findings of clinical and preclinical studies seem to be inconsistent, the clinical evidence may support possible involvement of monoamine uptake inhibition in blocking effect of (−)-BPAP on the reinstatement of methamphetamine-seeking behavior, especially via noradrenaline transporters.

Alternatively, (−)-BPAP has been reported to be a highly potent enhancer (0.1 μg/kg s.c.; Yoneda et al., 2001) of electrically-stimulated monoamine release (Miklya and Knoll, 2003), whereas standard monoamine uptake inhibitors do not share this effect (Miklya and Knoll, 2003). Therefore, these findings suggest (−)-BPAP as an atypical monoamine uptake inhibitor. Meanwhile, previous studies reported "atypical" dopamine uptake inhibitors, including benztropine analogues with pharmacological profiles unlike that of cocaine (Newman et al., 1995; Katz et al., 1999; Beuming et al., 2008; Loland et al., 2008). Among benztropine analogues, several *N*-substituted benztropine analogues exhibited reduced cocaine-like effects (Katz et al., 2004), antagonized cocaine-stimulated activity (Desai et al., 2005), and failed to substitute for cocaine in rats trained to self-administration cocaine (Hiranita et al., 2009). One of the possible targets underlying the "atypical" property of *N*-substituted benztropines analogues appear to be the σ1 receptor (σ1-R), because (1) *N*-substituted benztropine analogues have a high affinity for this protein with the nanomolar order of Ki values (Katz et al., 2004), (2) rimcazole, a σ1-R antagonist with a high affinity for the dopamine transporters (Cao et al., 2003), shows reduced cocaine-like behavioral effects (Katz et al., 2003) and (3) these two analogues show different molecular interactions at the dopamine transporters from cocaine (Loland et al., 2008). Interestingly, (−)-BPAP has been reported to be a ligand at σ1-R (Hamabe et al., 2000). Furthermore, *in vitro* study demonstrated that enhancement of cellular survival activity on cortical neurons by incubation with (−)BPAP was blocked by pre-incubation with *N*-[2-(3, 4-dichlorophenyl) ethyl]-4-methylpiperazine (BD 1063), a σ1-R antagonist (Hamabe et al., 2000). A behavioral study also demonstrated that (1) methamphetamine self-administration upregulated σ1-R mRNA and protein levels in several limbic regions (Stefanski et al., 2004). Meanwhile, we have reported that donepezil, an inhibitor of choline esterase, dramatically attenuated the reinstatement of methamphetamine-seeking behavior induced by methamphetamine-associated cues and methamphetamine-priming injections (Hiranita et al., 2006). Interestingly, donepezil has been reported to have very high affinity for σ1-Rs (IC_{50} value; 14.6 nM) (Kato et al., 1999). Although the involvement of σ1-Rs in the reinstatement of methamphetamine-seeking behavior has not been well understood, the σ1-Rs may be considered as the possible target underlying blocking effect of (−)-BPAP on the reinstatement of methamphetamine-seeking behavior.

In the present study, pre-session treatment with SCH-23390 dose-dependently reversed the blocking effect of SKF-81297 on the reinstatement of methamphetamine-seeking behavior induced by methamphetamine-associated cues and methamphetamine priming injections. In contrast, at even the tenfold higher dose, pre-session treatment with SCH-23390 appreciably failed to reverse the effect of the single pre-session treatment with (−)-BPAP on the reinstatement of methamphetamine-seeking behavior. The role of dopamine D_1-like receptors on reinstatement of drug-seeking behavior seems complicated. Systemic administration of the agonist (SKF-81297) and antagonist (SCH-23390) both have been reported to attenuate cocaine-seeking behavior induced by cocaine-priming injections (15 mg/kg i.p.) or cocaine-associated cues in rats (Alleweireldt et al., 2002, 2003). On the contrary, systemic administration of SCH-23390 (up to 10 μg/kg) has been reported to fail to attenuate reinstatement of cocaine-seeking behavior induced by priming injections of cocaine (5.0, 10 and 20 mg/kg i.p.) or a selective dopamine uptake inhibitor, WIN 35,428 (Schenk and Gittings, 2003). The mechanisms underlying the inhibitory effect of both the dopamine D_1-like receptor agonist and antagonist on the reinstatement of cocaine-seeking behavior and inconsistent results of SCH-23390 on the primed-cocaine-induced reinstatement are unknown. However, considering the inhibitory effect of SCH-23390 on reinstatement of cocaine-seeking behavior in the studies by Alleweireldt et al. (2002, 2003), inability of SCH-23390 to reverse the attenuating effect of pre-session treatment with (−)-BPAP on the reinstatement of methamphetamine-seeking behavior in the present study might result from the possible inhibitory action of SCH-23390 on reinstatement of drug-seeking behavior.

On the other hand, pre-session treatment with amisulpride up to 10 mg/kg appreciably failed to reverse the effect of the single pre-session treatment with (−)-BPAP. In Chinese hamster ovary cells expressed with human dopamine D_2 and D_3 receptors, amisulpride has been reported to have very high selectivity (Ki values; 21 and 2.9 nM) (Schoemaker et al., 1997). The effective dose of amisulpride for 50% occupancy for dopamine $D_{2/3}$ recep-

tors in rat brain has been reported to be 4.68 mg/kg (s.c.) (Natesan et al., 2008). Furthermore, pretreatment with 10 mg/kg of amisulpride has been reported to reverse amphetamine (1.0 or 2.0 mg)-stimulated locomotor activity to the vehicle level (Perrault et al., 1997; Natesan et al., 2008). In addition, administration of a higher dose of amisulpride alone (20 mg/kg i.p.) has been reported to suppress 10% sucrose feeding in rats (Schneider et al., 1986). Therefore, it is unlikely that the dose of amisulpride tested (10 mg/kg) is insufficient to work as a dopamine D_2-like receptor antagonist without impairment of non-specific operant behavior in the present study. Alternatively, systemic administration of dopamine D_2-like (eticlopride (Schenk and Gittings, 2003), raclopride (Cervo et al., 2003) and haloperidol (Gal and Gyertyan, 2006)) and selective dopamine D_3 receptor antagonists (SB-277011-A (Vorel et al., 2002; Gilbert et al., 2005; Gal and Gyertyan, 2006; Cervo et al., 2007) and NGB 2904 (Gilbert et al., 2005; Xi et al., 2006; Xi and Gardner, 2007)) were found consistently to attenuate reinstatement of cocaine-seeking behavior induced by cocaine-priming injections or cocaine-associated cues in rats. Therefore, lack of amisulpride effect to reverse the attenuating effect of pre-session treatment with (−)-BPAP on the reinstatement of methamphetamine-seeking behavior in the present study might be also masked due to the possible inhibitory action of amisulpride on reinstatement of drug-seeking behavior.

CONCLUSION

In summary, activation of dopamine D_1-like receptors resulted in attenuation of the reinstatement of methamphetamine-seeking behavior in rats. Although the attenuating effect of pre-session treatment with (−)-BPAP may be unrelated to dopamine D_1-like receptors, our results suggest a specific blocking effect of pre-session treatment with (−)-BPAP without affecting the reinforcing effect of methamphetamine. Extending this conclusion to the treatment of drug dependence, (−)-BPAP and dopamine D_1-like receptor agonists may be useful as anti-relapse agents in methamphetamine dependence.

Acknowledgments—We thank Fujimoto Pharmaceutical Corporation for the gift of (−)-BPAP. We also thank Drs. Mary Pfeiffer and 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 of Japan and Smoking Research Foundation, Japan. This work was also partially supported by the Intramural Research Program of the National Institutes of Health, National Institute on Drug Abuse, and JSPS Research Fellowship for Japanese Biomedical and Behavioral Researchers at NIH.

REFERENCES

Alleweireldt AT, Kirschner KF, Blake CB, Neisewander JL (2003) D1-receptor drugs and cocaine-seeking behavior: investigation of receptor mediation and behavioral disruption in rats. Psychopharmacology 168:109–117.

Alleweireldt AT, Weber SM, Kirschner KF, Bullock BL, Neisewander JL (2002) Blockade or stimulation of D1 dopamine receptors attenuates cue reinstatement of extinguished cocaine-seeking behavior in rats. Psychopharmacology 159:284–293.

Altshuler H, Weaver S, Phillips P (1975) Intragastric self-administration of psychoactive drugs by the rhesus monkey. Life Sci 17:883–890.

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.

Beuming T, Kniazeff J, Bergmann ML, Shi L, Gracia L, Raniszewska K, Newman AH, Javitch JA, Weinstein H, Gether U, Loland CJ (2008) The binding sites for cocaine and dopamine in the dopamine transporter overlap. Nat Neurosci 11:780–789.

Cao J, Kulkarni SS, Husbands SM, Bowen WD, Williams W, Kopajtic T, Katz JL, George C, Newman AH (2003) Dual probes for the dopamine transporter and sigma1 receptors: novel piperazinyl alkyl-bis(4′-fluorophenyl)amine analogues as potential cocaine-abuse therapeutic agents. J Med Chem 46:2589–2598.

Cervo L, Carnovali F, Stark JA, Mennini T (2003) Cocaine-seeking behavior in response to drug-associated stimuli in rats: involvement of D3 and D2 dopamine receptors. Neuropsychopharmacology 28:1150–1159.

Cervo L, Cocco A, Petrella C, Heidbreder CA (2007) Selective antagonism at dopamine D3 receptors attenuates cocaine-seeking behaviour in the rat. Int J Neuropsychopharmacol 10:167–181.

Desai RI, Kopajtic TA, Koffarnus M, Newman AH, Katz JL (2005) Identification of a dopamine transporter ligand that blocks the stimulant effects of cocaine. J Neurosci 25:1889–1893.

Edwards S, Whisler KN, Fuller DC, Orsulak PJ, Self DW (2007) Addiction-related alterations in D1 and D2 dopamine receptor behavioral responses following chronic cocaine self-administration. Neuropsychopharmacology 32:354–366.

Fischman MW, Foltin RW, Nestadt G, Pearlson GD (1990) Effects of desipramine maintenance on cocaine self-administration by humans. J Pharmacol Exp Ther 253:760–770.

Gal K, Gyertyan I (2006) Dopamine D3 as well as D2 receptor ligands attenuate the cue-induced cocaine-seeking in a relapse model in rats. Drug Alcohol Depend 81:63–70.

Gilbert JG, Newman AH, Gardner EL, Ashby CR Jr, Heidbreder CA, Pak AC, Peng XQ, Xi ZX (2005) Acute administration of SB-277011A, NGB 2904, or BP 897 inhibits cocaine cue-induced reinstatement of drug-seeking behavior in rats: role of dopamine D3 receptors. Synapse 57:17–28.

Hamabe W, Fujita R, Yasusa T, Yoneda F, Yoshida A, Ueda H (2000) (−)1-(Benzofuran-2-yl)-2-propylaminopentane shows survival effect on cortical neurons under serum-free condition through sigma receptors. Cell Mol Neurobiol 20:695–702.

Henningfield J (1995) Nicotine medications for smoking cessation. N Engl J Med 333:1196–1203.

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.

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

Hiranita T, Soto PL, Newman AH, Katz JL (2009) Assessment of reinforcing effects of benztropine analogs and their effects on cocaine self-administration in rats: comparisons with monoamine uptake inhibitors. J Pharmacol Exp Ther 329:677–686.

Kato K, Hayako H, Ishihara Y, Marui S, Iwane M, Miyamoto M (1999) TAK-147, an acetylcholinesterase inhibitor, increases choline acetyltransferase activity in cultured rat septal cholinergic neurons. Neurosci Lett 260:5–8.

Katz JL, Izenwasser S, Kline RH, Allen AC, Newman AH (1999) Novel 3alpha-diphenylmethoxytropane analogs: selective dopamine uptake inhibitors with behavioral effects distinct from those of cocaine. J Pharmacol Exp Ther 288:302–315.

Katz JL, Kopajtic TA, Agoston GE, Newman AH (2004) Effects of N-substituted analogs of benztropine: diminished cocaine-like effects in dopamine transporter ligands. J Pharmacol Exp Ther 309:650–660.

Katz JL, Libby TA, Kopajtic T, Husbands SM, Newman AH (2003) Behavioral effects of rimcazole analogues alone and in combination with cocaine. Eur J Pharmacol 468:109–119.

Knoll J, Miklya I, Knoll B, Marko R, Racz D (1996) Phenylethylamine and tyramine are mixed-acting sympathomimetic amines in the brain. Life Sci 58:2101–2114.

Kreek M (1996) Opiates, opioids and addiction. Mol Psychiatry 1: 232–254.

Le Foll B, Goldberg SR (2005) Control of the reinforcing effects of nicotine by associated environmental stimuli in animals and humans. Trends Pharmacol Sci 26:287–293.

Loland CJ, Desai RI, Zou MF, Cao J, Grundt P, Gerstbrein K, Sitte HH, Newman AH, Katz JL, Gether U (2008) Relationship between conformational changes in the dopamine transporter and cocaine-like subjective effects of uptake inhibitors. Mol Pharmacol 73:813–823.

Magyar K, Lengyel J, Bolehovszky A, Knoll B, Miklya I, Knoll J (2002) The fate of (−)1-(benzofuran-2-yl)-2-propylaminopentane. HCl, (−)-BPAP, in rats, a potent enhancer of the impulse-evoked release of catecholamines and serotonin in the brain. Eur J Drug Metab Pharmacokinet 27:157–161.

Martin TJ, Kim SA, Buechler NL, Porreca F, Eisenach JC (2007) Opioid self-administration in the nerve-injured rat: relevance of antiallodynic effects to drug consumption and effects of intrathecal analgesics. Anesthesiology 106:312–322.

Miklya I, Knoll J (2003) Analysis of the effect of (−)-BPAP, a selective enhancer of the impulse propagation mediated release of catecholamines and serotonin in the brain. Life Sci 72:2915–2921.

Natesan S, Reckless GE, Barlow KB, Nobrega JN, Kapur S (2008) Amisulpride the "atypical" atypical antipsychotic—comparison to haloperidol, risperidone and clozapine. Schizophr Res 105:224–235.

Newman AH, Kline RH, Allen AC, Izenwasser S, George C, Katz JL (1995) Novel 4′-substituted and 4′,4″-disubstituted 3 alpha-(diphenylmethoxy)tropane analogs as potent and selective dopamine uptake inhibitors. J Med Chem 38:3933–3940.

Oei TP, Singer G, Jefferys D (1980) The interaction of a fixed time food delivery schedule and body weight on self-administration of narcotic analgesics. Psychopharmacology 67:171–176.

Perrault G, Depoortere R, Morel E, Sanger DJ, Scatton B (1997) Psychopharmacological profile of amisulpride: an antipsychotic drug with presynaptic D2/D3 dopamine receptor antagonist activity and limbic selectivity. J Pharmacol Exp Ther 280:73–82.

Platt DM, Rowlett JK, Spealman RD (2007) Noradrenergic mechanisms in cocaine-induced reinstatement of drug seeking in squirrel monkeys. J Pharmacol Exp Ther 322:894–902.

Rothman RB, Baumann MH (2003) Monoamine transporters and psychostimulant drugs. Eur J Pharmacol 479:23–40.

Schenk S, Gittings D (2003) Effects of SCH 23390 and eticlopride on cocaine-seeking produced by cocaine and WIN 35,428 in rats. Psychopharmacology 168:118–123.

Schneider LH, Gibbs J, Smith GP (1986) D-2 selective receptor antagonists suppress sucrose sham feeding in the rat. Brain Res Bull 17:605–611.

Schoemaker H, Claustre Y, Fage D, Rouquier L, Chergui K, Curet O, Oblin A, Gonon F, Carter C, Benavides J, Scatton B (1997) Neurochemical characteristics of amisulpride, an atypical dopamine D2/D3 receptor antagonist with both presynaptic and limbic selectivity. J Pharmacol Exp Ther 280:83–97.

Self DW, Barnhart WJ, Lehman DA, Nestler EJ (1996) Opposite modulation of cocaine-seeking behavior by D1- and D2-like dopamine receptor agonists. Science 271:1586–1589.

Self DW, Karanian DA, Spencer JJ (2000) Effects of the novel D1 dopamine receptor agonist ABT-431 on cocaine self-administration and reinstatement. Ann N Y Acad Sci 909:133–144.

Shalev U, Grimm JW, Shaham Y (2002) Neurobiology of relapse to heroin and cocaine seeking: a review. Pharmacol Rev 54: 1–42.

Shimazu S, Takahata K, Katsuki H, Tsunekawa H, Tanigawa A, Yoneda F, Knoll J, Akaike A (2001) (−)-1-(Benzofuran-2-yl)-2-propylaminopentane enhances locomotor activity in rats due to its ability to induce dopamine release. Eur J Pharmacol 421:181–189.

Shimazu S, Tamashiro A, Yoneda F, Knoll J (2003a) The L-DOPA-sparing effect of R-(−)-1-(benzofuran-2-yl)-2-propylaminopentane hydrochloride [(−)-BPAP] in reserpine-pretreated rats. Life Sci 72:1413–1419.

Shimazu S, Tsunekawa H, Yoneda F, Katsuki H, Akaike A, Janowsky A (2003b) Transporter-mediated actions of R-(−)-1-(benzofuran-2-yl)-2-propylaminopentane. Eur J Pharmacol 482:9–16.

Stefanski R, Justinova Z, Hayashi T, Takebayashi M, Goldberg SR, Su TP (2004) Sigma1 receptor upregulation after chronic methamphetamine self-administration in rats: a study with yoked controls. Psychopharmacology 175:68–75.

Stefanski R, Ladenheim B, Lee SH, Cadet JL, Goldberg SR (1999) Neuroadaptations in the dopaminergic system after active self-administration but not after passive administration of methamphetamine. Eur J Pharmacol 371:123–135.

Szerman N, Peris L, Mesias B, Colis P, Rosa J, Prieto A (2005) Reboxetine for the treatment of patients with cocaine dependence disorder. Hum Psychopharmacol 20:189–192.

Tong J, Ross BM, Schmunk GA, Peretti FJ, Kalasinsky KS, Furukawa Y, Ang LC, Aiken SS, Wickham DJ, Kish SJ (2003) Decreased striatal dopamine D1 receptor-stimulated adenylyl cyclase activity in human methamphetamine users. Am J Psychiatry 160:896–903.

Tsunekawa H, Noda Y, Miyazaki M, Yoneda F, Nabeshima T, Wang D (2008) Effects of (R)-(−)-1-(benzofuran-2-yl)-2-propylaminopentane hydrochloride [(−)-BPAP] in animal models of mood disorders. Behav Brain Res 189:107–116.

Volkow ND, Chang L, Wang GJ, Fowler JS, Franceschi D, Sedler M, Gatley SJ, Miller E, Hitzemann R, Ding YS, Logan J (2001a) Loss of dopamine transporters in methamphetamine abusers recovers with protracted abstinence. J Neurosci 21:9414–9418.

Volkow ND, Chang L, Wang GJ, Fowler JS, Leonido-Yee M, Franceschi D, Sedler MJ, Gatley SJ, Hitzemann R, Ding YS, Logan J, Wong C, Miller EN (2001b) Association of dopamine transporter reduction with psychomotor impairment in methamphetamine abusers. Am J Psychiatry 158:377–382.

Vorel SR, Ashby CR Jr, Paul M, Liu X, Hayes R, Hagan JJ, Middlemiss DN, Stemp G, Gardner EL (2002) Dopamine D3 receptor antagonism inhibits cocaine-seeking and cocaine-enhanced brain reward in rats. J Neurosci 22:9595–9603.

Weiss F, Markou A, Lorang MT, Koob GF (1992) Basal extracellular dopamine levels in the nucleus accumbens are decreased during cocaine withdrawal after unlimited-access self-administration. Brain Res 593:314–318.

Werner TE, Smith SG, Davis WM (1976) A dose-response comparison between methadone and morphine self-administration. Psychopharmacologia 47:209–211.

Wilson JM, Kalasinsky KS, Levey AI, Bergeron C, Reiber G, Anthony RM, Schmunk GA, Shannak K, Haycock JW, Kish SJ (1996) Striatal dopamine nerve terminal markers in human, chronic methamphetamine users. Nat Med 2:699–703.

Wu JC, Bell K, Najafi A, Widmark C, Keator D, Tang C, Klein E, Bunney BG, Fallon J, Bunney WE (1997) Decreasing striatal

6-FDOPA uptake with increasing duration of cocaine withdrawal. Neuropsychopharmacology 17:402–409.
Xi ZX, Gardner EL (2007) Pharmacological actions of NGB 2904, a selective dopamine D3 receptor antagonist, in animal models of drug addiction. CNS drug reviews 13:240–259.
Xi ZX, Newman AH, Gilbert JG, Pak AC, Peng XQ, Ashby CR Jr, Gitajn L, Gardner EL (2006) The novel dopamine D3 receptor antagonist NGB 2904 inhibits cocaine's rewarding effects and cocaine-induced reinstatement of drug-seeking behavior in rats. Neuropsychopharmacology 31:1393–1405.
Yoneda F, Moto T, Sakae M, Ohde H, Knoll B, Miklya I, Knoll J (2001) Structure-activity studies leading to (−)1-(benzofuran-2-yl)-2-propylaminopentane, ((−)BPAP), a highly potent, selective enhancer of the impulse propagation mediated release of catecholamines and serotonin in the brain. Bioorg Med Chem 9:1197–1212.

(Accepted 27 October 2009)
(Available online 31 October 2009)

Neuropsychopharmacology (2010) 35, 515–520
© 2010 Nature Publishing Group All rights reserved 0893-133X/10 $32.00
www.neuropsychopharmacology.org

A Cannabinoid CB_1 Receptor Antagonist Ameliorates Impairment of Recognition Memory on Withdrawal from MDMA (Ecstasy)

Yoko Nawata[1], Takato Hiranita[1,2] and Tsuneyuki Yamamoto*[,1]

[1]*Department of Pharmacology, Faculty of Pharmaceutical Sciences, Nagasaki International University, Sasebo, Nagasaki, Japan*

(+/−)-3,4-Methylenedioxymethamphetamine (MDMA, 'Ecstasy') abusers have persistent neuropsychiatric deficits including memory impairments after the cessation of abuse. On the other hand, cannabinoid CB_1 receptors have been implicated in learning/memory, and are highly expressed in the hippocampus, a region of the brain believed to have an important function in certain forms of learning and memory. In this study, we clarified the mechanism underlying the cognitive impairment that develops during MDMA withdrawal from the standpoint of the cannabinoid CB_1 receptors. Mice were administered MDMA (10 mg/kg, i.p.) once a day for 7 days. On the 7th day of withdrawal, a novel object recognition task was performed and the amount of cannabinoid CB_1 receptor protein was measured with western blotting. Recognition performance was impaired on the 7th day of withdrawal. This impairment was blocked by AM251, a cannabinoid CB_1 receptor antagonist, administered 30 min before the training trial or co-administered with MDMA. At this time, the level of cannabinoid CB_1 receptor protein increased significantly in the hippocampus but not the prefrontal cortex or striatum. This increase of CB_1 receptor protein in the hippocampus was also blocked by the co-administration of AM251. Furthermore, CB_1 receptor knockout mice showed no impairment of recognition performance on the withdrawal from MDMA. The impairment of recognition memory during withdrawal from MDMA may result from the activation of cannabinoid CB_1 receptors in the hippocampus.
Neuropsychopharmacology (2010) **35,** 515–520; doi:10.1038/npp.2009.158; published online 14 October 2009

Keywords: MDMA; cannabinoid CB_1 receptors; recognition memory; novel object recognition task; withdrawal; hippocampus

INTRODUCTION

(+/−)-3,4-Methylenedioxymethamphetamine (MDMA) is widely abused throughout the world. MDMA abusers have neuropsychiatric deficits including memory impairments (McCardle *et al*, 2004; Wareing *et al*, 2007). Recent studies suggest that this neuropsychiatric deficit persists after the cessation of abuse (Ward *et al*, 2006). In addition, cocaine, amphetamine, or opiate abusers also show cognitive impairment during long-term drug abstinence (Ersche *et al*, 2006; Pace-Schott *et al*, 2008).

Cannabis usage causes deficits in attention, executive functioning, and short-term memory (O'Leary *et al*, 2002; Medina *et al*, 2007). We showed earlier that repeated treatment with Δ^9-tetrahydrocannabinol (Δ^9-THC), the main psychoactive ingredient of marijuana (*cannabis*), impaired delayed matching-to-sample performance even 24 h after the administration (Miyamoto *et al*, 1995). An other study has also found that Δ^9-THC impairs spatial memory (Lichtman and Martin, 1996). These reports suggest that the activation of the brain cannabinoid system impairs working memory. Furthermore, it has been revealed that the cannabinoid system is involved in drug dependence (Yamamoto and Takada, 2000; Yamamoto *et al*, 2004). A cannabinoid CB_1 receptor antagonist, SR141716A, attenuated the reinstatement of methamphetamine-seeking behavior (Anggadiredja *et al*, 2004; Hiranita *et al*, 2008). Moreover, cannabinoid CB_1 receptor knockout mice failed to establish cocaine, morphine, and ethanol self-administration (Cossu *et al*, 2001; Soria *et al*, 2005; Thanos *et al*, 2005). In a biochemical study, Gonzalez *et al* (2002) reported that chronic exposure to morphine increased levels of cannabinoid CB_1 receptor mRNA and CB_1 receptor binding in the brain. In addition, the hippocampal cannabinoid system seems to be activated during withdrawal from ethanol, because both endogenous cannabinoids and CB_1 receptors levels increased (Mitrirattanakul *et al*, 2007). Despite the close involvement of the cannabinoid system in

*Correspondence: Dr T Yamamoto, Department of Pharmacology, Faculty of Pharmaceutical Sciences, Nagasaki International University, 2825-7, Huis Ten Bosch, Sasebo, Nagasaki 859-3298, Japan, Tel: +81 956 20 5629, Fax: +81 956 20 5629, E-mail: tyamamot@niu.ac.jp

[2]Current address: Psychobiology Section, Medications Discovery Research Branch, Intramural Research Program, National Institute on Drug Abuse, National Institutes of Health/Department of Health and Human Services, 251 Bayview Blvd., Suite 200, Biomedical Research Center, Baltimore, MD 21224, USA

Received 15 April 2009; revised 25 August 2009; accepted 25 August 2009

the reward system, it is still unclear whether the system is involved in cognitive impairment on withdrawal from chronic exposure to drugs of abuse.

Here, we clarified the role of the cannabinoid system in cognitive impairment during withdrawal from MDMA using the novel object recognition task. We also investigated that the effect of MDMA on the level of cannabinoid CB_1 receptor protein correlated with a behavioral test.

MATERIALS AND METHODS

Animals

Male CD1 (wild-type) mice (Charles River, Yokohama, Japan) and cannabinoid CB_1 receptor knockout (CB_1 KO) mice on a CD1 background, provided by Dr Catherine Ledent (Institut de Recherches en Biologie Humaine et Moléculaire, Université Libre de Bruxelles), and weighing 30–35 g, were used in the present experiment. There were 117 wild-type mice and 29 CB_1 KO mice used in all experiment. We conducted each experiment with a small control group of 2–3 mice each and these control group data were combined together in the end to represent the control values. The animals were housed in plastic cages and kept in a regulated environment (23 ± 1°C), with a 12/12 h light–dark cycle (lights on at 7:00 am). Food and water were available *ad libitum*. Procedures 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 Nagasaki International University Publication, enacted in 2006.

Drugs

(+/−)-3,4-Methylenedioxymethamphetamine HCl (MDMA; provided by Dr Tatsunori Iwamura, Matsuyama University) was dissolved in saline. AM251 (Sigma, St Louis, MO) was dissolved in a mixture of DMSO, Tween-80 and saline (1 : 1 : 18, respectively). All drugs were administered intraperitoneally (i.p.), and injected at a volume of 0.1 ml per 10 g of body weight. Saline or MDMA (10 mg/kg) was administered once or once daily for 7 days. AM251 (1.0 or 3.2 mg/kg) was co-administered with MDMA or singly administered 30 min before the training trial on the 7th day of withdrawal after the repeated administration of MDMA.

Behavioral Testing

Object recognition test. The object recognition test was carried out on the 1st or 7th day after the repeated administration of MDMA in separate groups. This test was performed in a Plexiglas open-field box (in cm 70 wide × 70 deep × 40 high) with black vertical walls and a floor. The objects to be discriminated were silver cone-shaped and bulb-shaped. Mice were habituated to the open field for 1 h (habituation trial). The next day, in the training trial, each mouse was placed in the open field and allowed to explore two identical objects for 10 min. The test trial was performed 3 h after the training trial. One familiar object and one novel object were placed in the same location as in the training trial. For the measurement on the 1st day of withdrawal, the habituation trial was conducted just before the last drug injection. The time spent exploring each object and the total amount of time spent exploring both objects were recorded. Exploration of an object was defined as placing the nose or a forepaw at or beyond marks put on the open-field at a distance of 1 cm from each object. A discrimination ratio was calculated as the difference in time spent exploring the novel and familiar object, expressed as a ratio of the total time spent exploring both objects in the test trial. Mice showing a total exploration time of <10 s during the training trial were excluded. The ambulation during the trial was measured with a digital tracking and computerized scoring system (LimeLight, Actimetrics). To determine whether the mice discriminated between novel and familiar objects, the discrimination ratios obtained under each condition were compared with those that would be expected by chance (ie, a ratio of 0.0), using one-sample *t* tests.

Biochemical Testing

Western blot analysis. Immediately after decapitation, the whole brain was removed from the skull, placed on ice, and the hippocampus, prefrontal cortex, and striatum were removed as described earlier (Yamaguchi *et al*, 2004). These tissues were immediately homogenized in a lysis buffer (10 mM Tris–HCl, pH 7.5, 5 mM $MgCl_2$, 5 mM EDTA, 250 mM Sucrose, 1 mM Dithiothreitol, 1% Triton X-100, 1% sodium cholate, and protease inhibitor cocktail). All samples were subjected to a BCA assay to adjust the amount of protein loaded before the sample buffer was added. The sample (10 μg) was applied to a 10% polyacrylamide gel (BioRad, Hercules, CA), and the proteins were transferred electrophoretically to nitrocellulose membranes (Bio Rad). The membrane was blocked with TBS-Tween 20 (0.1%) and 5% nonfat dry milk and incubated with the primary antibodies [anti-cannabinoid CB_1 (1 : 1000, Calbiochem, US and Canada) overnight at 4°C and anti-β-actin (1 : 2000, Sigma)] 1 h at room temperature. The antibodies were detected using HRP-conjugated anti-rabbit and anti-mouse IgG (GE Healthcare, Tokyo, Japan, 1 : 1000) secondary antibodies. The blots were detected using a chemiluminescence method (ECL system; GE Healthcare).

Data Analysis

Data are expressed as mean ± SE. A one-way ANOVA was used to compare means, and Bonferroni–Dunn tests were used for *post hoc* analysis. $p<0.05$ was accepted as statistically significant.

RESULTS

Novel Object Recognition Performance During MDMA Withdrawal

In the training trial, vehicle, single MDMA, and repeated (for 7 days) MDMA-treated mice on the 1st day after the last treatment spent 23.1 ± 1.8, 22.0 ± 5.6, and 21.3 ± 2.0 s exploring objects, respectively. Meanwhile, on the 7th day after treatment, the time spent exploring objects in the vehicle, single MDMA, and repeated MDMA-treated groups was 19.3 ± 2.7, 26.6 ± 5.2, and 21.4 ± 3.1 s, respectively.

Hence, the time spent exploring objects on the 1st day of withdrawal in the training trial in single MDMA- and repeated MDMA-treated groups was not significantly different from that of vehicle-treated group ($p = 0.06$ and $p = 0.55$ *vs* vehicle-treated group, respectively). In addition, on the 7th day of withdrawal, there was no significant difference in the time spent exploring objects among the three groups in the training trial ($p = 0.28$, $p = 0.94$ *vs* vehicle-treated group, respectively). In the test trial, the vehicle-treated mice spent significantly longer exploring the novel object (21.3 ± 2.3 s) than the familiar object (5.4 ± 1.0 s) ($F[1,28] = 30.8$, $p < 0.0001$ *vs* exploration time for the familiar object). On the 1st and 7th day after a single administration of MDMA, there was no significant change in the discrimination ratio ($p = 0.47$ and $p = 0.13$ *vs* control group on the 1st and 7th day, respectively). However, the discrimination ratio significantly decreased on the 1st and 7th days of withdrawal from repeated administration of MDMA (0.597 ± 0.071–0.26 ± 0.106%: $F[1,19] = 5.3$, $p < 0.05$ and 0.633 ± 0.048–0.048 ± 0.049%: $F[1,27] = 70.2$, $p < 0.001$ *vs* control group on the 1st and 7th days, respectively) (Figure 1). Discrimination ratios were significantly above chance in all groups except for mice on the 7th days of withdrawal from repeated MDMA ($p < 0.001$; control group on the 1st and 7th day, $p < 0.01$; on the 1st and 7th day of withdrawal from single MDMA, $p < 0.05$; on the 1st day of withdrawal from repeated MDMA). In this test trial, ambulation did not differ between the MDMA-treated and vehicle-treated groups ($p = 0.21$ and $p = 0.61$ on the 1st and 7th days, respectively). The decrease in the discrimination ratio on the 7th day of withdrawal was prevented by the co-administration of AM251, a cannabinoid CB_1 receptor antagonist, with MDMA in a dose-dependent manner (0.048 ± 0.049 to 0.592 ± 0.067%: $F[1,22] = 48.5$, $p < 0.001$ *vs* MDMA group) (Figure 2a). However, ambulation in AM251 co-administered group (4269 ± 269 cm) did not differ from ambulation in vehicle (4578 ± 354 cm) or MDMA (4334 ± 310 cm) groups ($p = 0.51$ and $p = 0.55$ *vs* vehicle and MDMA alone, respectively). On the other hand, a single administration of AM251 30 min before the training trial on the 7th day of withdrawal from repeated MDMA treatment stopped the reduction in the discrimination ratio in a dose-dependent manner (0.048 ± 0.049 to 0.661 ± 0.074%: $F[1,18] = 54.7$, $p < 0.001$ *vs* MDMA group) (Figure 2b). A single administration of AM251 on 7th day of withdrawal from repeated MDMA had no effect on ambulation ($p = 0.19$ and $p = 0.3$ *vs* vehicle and MDMA alone, respectively). Discrimination ratios were significantly above chance in mice co-administered and singly administered AM251 ($p < 0.001$). While, there was no significant difference in the time spent exploring objects and the discrimination ratio between vehicle-treated wild-type and CB_1 KO mice in the test trial. However, CB_1 KO mice did not exhibit a reduction in the discrimination ratio on both 1st and 7th day of withdrawal from repeated MDMA treatment (Figure 3). Discrimination ratios were significantly above chance in all groups of CB_1 KO mice ($p < 0.001$).

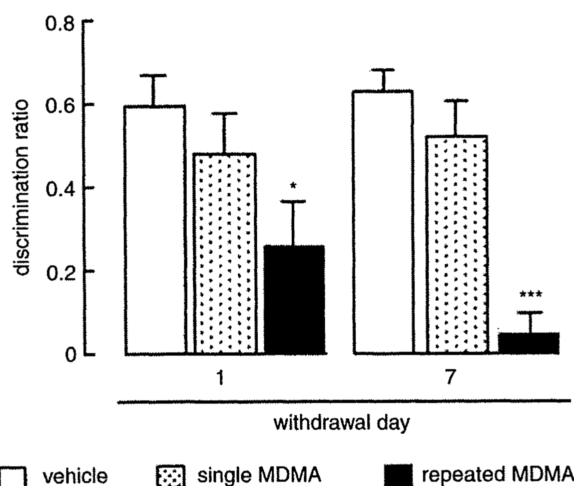

Figure 1 Novel object recognition performance in wild-type mice on the 1st or 7th day of withdrawal after single or repeated (daily for 7 day) MDMA treatment (10 mg/kg, i.p.). Each graph shows the discrimination ratio in the test trial. Data represent the mean ± SEM ($n = 5$–15). *$p < 0.05$, ***$p < 0.001$ *vs* vehicle-treated mice. Vehicle includes results for mice administered saline once or repeatedly for 7 days.

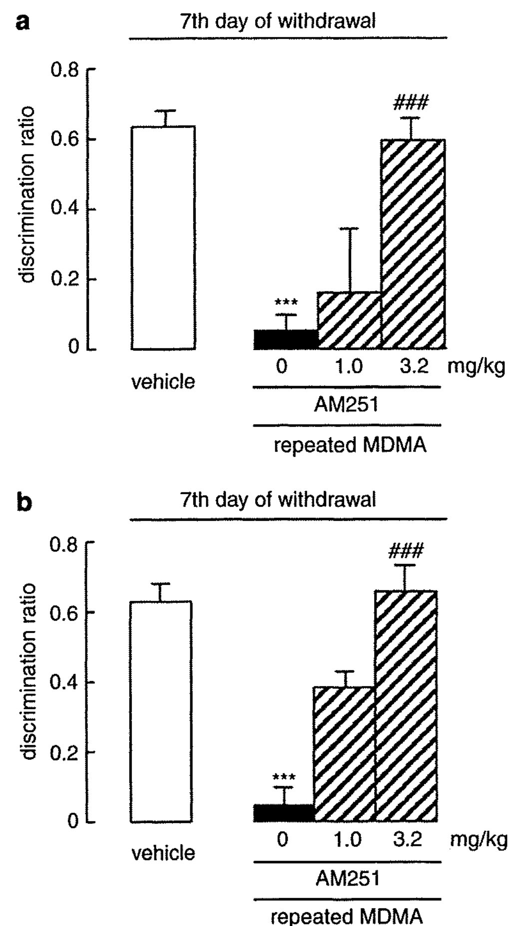

Figure 2 Effect of a cannabinoid CB_1 receptor antagonist, AM251, on cognitive impairment on the 7th day of MDMA withdrawal (10 mg/kg, i.p., daily for 7 days) in wild-type mice. (a) AM251 (1.0 or 3.2 mg/kg, i.p.) was co-administered with MDMA. Data represent the mean ± SEM ($n = 8$–15). ***$p < 0.001$ *vs* vehicle-treated mice; ###$p < 0.001$ *vs* MDMA (10 mg/kg)-treated mice. Vehicle means results for mice administered saline. (b) AM251 (1.0 or 3.2 mg/kg, i.p.) was administered 30 min before the training trial. Data represent the mean ± SEM ($n = 5$–15). ***$p < 0.001$ *vs* vehicle-treated mice; ###$p < 0.001$ *vs* MDMA-treated mice. Vehicle means results for mice administered saline.

Alteration of the Level of Cannabinoid CB_1 Receptor Protein During Withdrawal from Repeated MDMA Treatment

The level of cannabinoid CB_1 receptor protein did not change on the 1st day of withdrawal from repeated administration of MDMA in the hippocampus. On the 7th day of withdrawal, the level of CB_1 receptor protein in the hippocampus was significantly increased (0.48 ± 0.06–0.96 ± 0.07, $F[1,13] = 28.1$, $p < 0.001$ *vs* vehicle-treated group) (Figure 4a). This increase was prevented by co-administration of AM251 with MDMA (0.96 ± 0.07–0.60 ± 0.09, $F[1,14] = 9.6$, $p < 0.01$ *vs* MDMA-treated group) (Figure 4b). There was no significant change in the prefrontal cortex or striatum on both 1st and 7th day of withdrawal (Figure 4a).

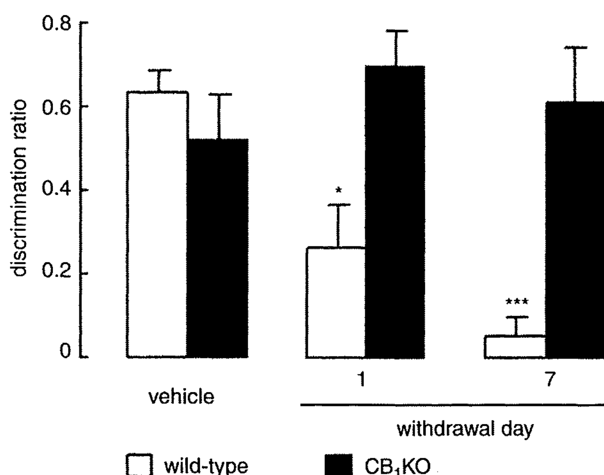

Figure 3 Comparison of novel object recognition performance in wild-type and CB_1 receptor knockout mice on the 1st and 7th day of MDMA withdrawal (10 mg/kg, i.p., daily for 7 days). Each graph shows the discrimination ratio in the test trial. Data represent the mean ± SEM ($n = 8$–15). $^{*}p < 0.05$, $^{***}p < 0.001$ *vs* vehicle-treated mice. Open and closed bars indicate wild-type and CB_1 receptor knockout mice, respectively. Vehicle means results for mice administered saline.

DISCUSSION

Object recognition memory in mice was impaired on withdrawal from repeated MDMA. This impairment was prevented by the co-administration of AM251, a cannabinoid CB_1 receptor antagonist, with MDMA in wild-type mice. In addition, a single treatment of AM251 on the 7th day of MDMA withdrawal ameliorated this recognition memory impairment. In CB_1 KO mice, recognition memory was not impaired on withdrawal from MDMA. These results suggest that the activation of cannabinoid CB_1 receptors is involved in the appearance of cognitive impairment on withdrawal from MDMA. In rats, it was also reported that object recognition memory is also impaired on withdrawal from MDMA similar to our present findings in mice (Morley *et al*, 2001; McGregor *et al*, 2003; Piper and Meyer, 2004). However, this is the first report to show the involvement of cannabinoid CB_1 receptors in the appearance of cognitive impairment on withdrawal from abusive drugs. Recently, Touriño *et al* (2008) indicated that CB_1 KO mice did not show the performance of MDMA self-administration. This finding suggests that the activation of CB_1 receptors is involved in the MDMA reinforcing effect.

The level of cannabinoid CB_1 receptor protein in the hippocampus was significantly increased on the 7th day of withdrawal but not on the 1st day while recognition

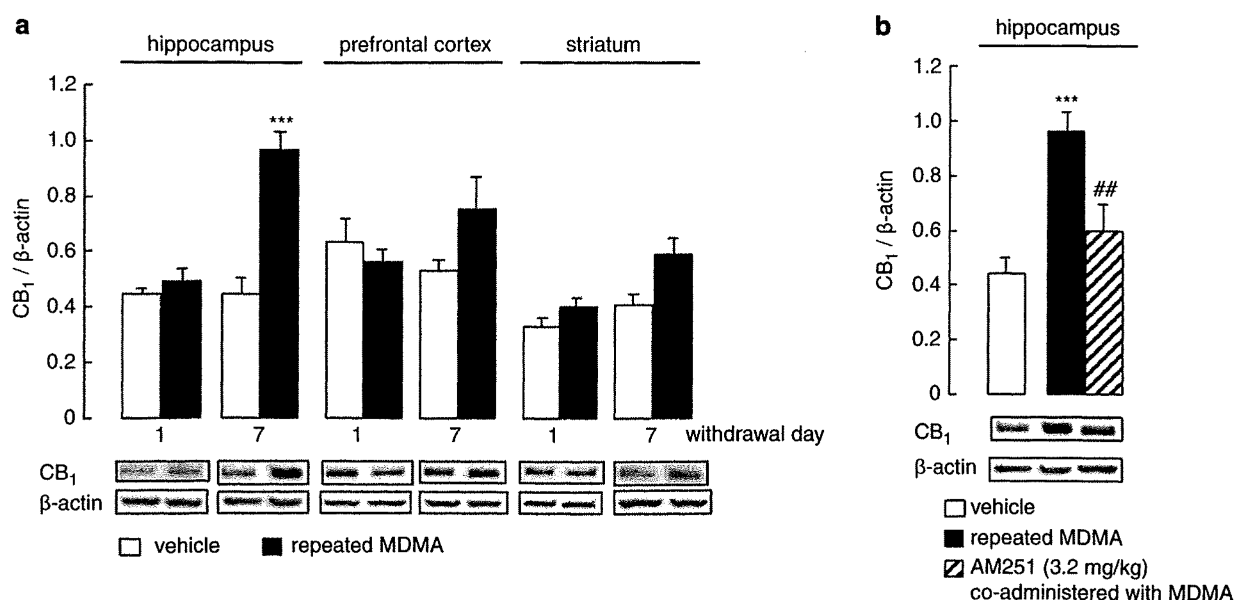

Figure 4 Effect of repeated administration of MDMA on the levels of CB_1 receptor protein in the brain in wild-type mice on the 1st and 7th day of MDMA withdrawal (10 mg/kg, i.p., daily for 7 days). (a) The levels of CB_1 receptor protein in the hippocampus, prefrontal cortex, and striatum were measured. Data represent the mean ± SEM ($n = 8$–11). (b) Effect of repeated administration of AM251 (3.2 mg/kg) with MDMA on MDMA-induced up-regulation of CB_1 receptor protein expression in the hippocampus. Open and closed bars indicate vehicle- and repeated MDMA-treated group. Data represent the mean ± SEM ($n = 5$–11). $^{***}p < 0.001$ *vs* vehicle-treated mice; $^{\#\#}p < 0.01$ *vs* MDMA-treated mice. Vehicle means results for mice administered saline.

memory was impaired on both the 1st day and 7th day of MDMA withdrawal. In this regard, cognitive impairment on the 1st day of MDMA withdrawal may be due to the increase in hippocampal endocannabinoid. Mitrirattanakul *et al* (2007) reported that the amount of endocannabinoid 2-AG in the hippocampus significantly increased in the early phase of ethanol withdrawal without any increase in CB_1 receptor expression. Our findings may be supported by this result. On the other hand, a single treatment with CB_1 receptor antagonist for MDMA withdrawal significantly ameliorated the recognition memory impairment on 7th day of MDMA withdrawal. Accordingly, CB_1 receptors may be activated with the increase in their expression at this time.

In addition, the activation of the brain cannabinoid system causes deficits in attention, executive functioning, and short-term memory (Lichtman and Martin, 1996; O'Leary *et al*, 2002; Medina *et al*, 2007). It is also demonstrated that object recognition memory was impaired by both endogenous cannabinoid Δ^9-THC and synthetic CB_1 receptor agonist WIN 55,212-2 (Schneider and Koch, 2002; Quinn *et al*, 2008). Our findings may be supported by the literatures above.

The important function of the hippocampus in cognitive functions including recognition memory is well established by earlier findings. Hippocampal damage from ibotenic acid disrupted recognition memory in the novel object recognition task and the visual paired comparison task (Clark *et al*, 2000; Broadbent *et al*, 2004). Furthermore, an intra-hippocampal WIN 55,212-2, also impaired performance of the novel object recognition task (Kosiorek *et al*, 2003; Suenaga and Ichitani, 2008).

Hampson and Deadwyler (2000) found that Δ^9-THC and WIN 55,212-2 act selectively to disrupt the encoding of events in the hippocampus during memory processing, on measuring the combined simultaneous multineuron firing rate. Recently, it was also suggested that the cannabinoids Δ^9-THC and a cannabinoid CB_1 receptor agonist CP55940 disrupted the temporal coordination of hippocampal neurons, and that this effect may correlate with memory deficits in individuals (Robbe *et al*, 2006).

Enocannabinoids are known to participate in forms of synaptic plasticity (Mackie, 2008). This phenomenon associated with endocannabinoids may help explain the mechanism by which cannabinoids impair memory. Long-term potentiation (LTP) is a form of synaptic plasticity thought to have functional roles in learning and memory processes. The importance of the hippocampal LTP in learning and memory has also been shown that hippocampal LTP is facilitated after following exposure to a novel environment but not by exposure to a familiar environment (Li *et al*, 2003). In addition, it has been shown that the cannabinoid system affects the hippocampal LTP by chronic Δ^9-THC blocking hippocampal LTP via CB_1 receptors after withdrawal (Hoffman *et al*, 2007). It has been shown that cannabinoids inhibit neurotransmitter release via presynaptic cannabinoid CB_1 receptors (Schlicker and Kathmann, 2001). Additionally, LTP disruption in the hippocampus by WIN 55,212-2 may be associated with an inhibition of hippocampal glutamatergic transmission (Misner and Sullivan, 1999). Accordingly, the appearance of cognitive impairment during MDMA withdrawal may result in dysfunction of hippocampal LTP via inhibition of glutamate release induced by an activation of CB_1 receptors. These reports strongly support our present finding that the activation of the hippocampal cannabinoid system disrupts recognition memory during MDMA withdrawal.

In conclusion, our results suggest the impairment of recognition memory during withdrawal from repeated administration of MDMA to be due to the activation of cannabinoid CB_1 receptors in the hippocampus. Moreover, these findings suggest that cannabinoid CB_1 receptor antagonists would have a therapeutic effect on cognitive dysfunction in MDMA abusers.

ACKNOWLEDGEMENTS

We thank Dr Catherine Ledent (Université Libre de Bruxelles) for donating CB_1 receptor knockout mice and Dr Tatsunori Iwamura (Matsuyama University) for providing MDMA. This study was supported by a grant from the Ministry of Health, Labor, and Welfare of Japan.

DISCLOSURE

The authors declare no conflict of interest.

REFERENCES

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

Broadbent NJ, Squire LR, Clark RE (2004). Spatial memory, recognition memory, and the hippocampus. *Proc Natl Acad Sci USA* **101**: 14515–14520.

Clark RE, Zola SM, Squire LR (2000). Impaired recognition memory in rats after damage to the hippocampus. *J Neurosci* **20**: 8853–8860.

Cossu G, Ledent C, Fattore L, Imperato A, Bohme GA, Parmentier M *et al* (2001). Cannabinoid CB1 receptor knockout mice fail to self-administer morphine but not other drugs of abuse. *Behav Brain Res* **118**: 61–65.

Ersche KD, Clark L, London M, Robbins TW, Sahakian BJ (2006). Profile of executive and memory function associated with amphetamine and opiate dependence. *Neuropsychopharmacology* **31**: 1036–1047.

Gonzalez S, Fernandez-Ruiz J, Sparpaglione V, Parolaro D, Ramos JA (2002). Chronic exposure to morphine, cocaine or ethanol in rats produced different effects in brain cannabinoid CB(1) receptor binding and mRNA levels. *Drug Alcohol Depend* **66**: 77–84.

Hampson RE, Deadwyler SA (2000). Cannabinoids reveal the necessity of hippocampal neural encoding for short-term memory in rats. *J Neurosci* **20**: 8932–8942.

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

Hoffman AF, Oz M, Yang R, Lichtman AH, Lupica CR (2007). Opposing actions of chronic Delta9-tetrahydrocannabinol and cannabinoid antagonists on hippocampal long-term potentiation. *Learn Mem* **14**: 63–74.

Kosiorek P, Hryniewicz A, Bialuk I, Zawadzka A, Winnicka MM (2003). Cannabinoids alter recognition memory in rats. *Pol J Pharmacol* **55**: 903–910.

Li S, Cullen WK, Anwyl R, Rowan MJ (2003). Dopamine-dependent facilitation of LTP induction in hippocampal CA1 by exposure to spatial novelty. *Nat Neurosci* **6**: 526–531.
Lichtman AH, Martin BR (1996). Delta 9-tetrahydrocannabinol impairs spatial memory through a cannabinoid receptor mechanism. *Psychopharmacology* **126**: 125–131.
Mackie K (2008). Signaling via CNS cannabinoid receptors. *Mol Cell Endocrinol* **286**: S60–S65.
McCardle K, Luebbers S, Carter JD, Croft RJ, Stough C (2004). Chronic MDMA (ecstasy) use, cognition and mood. *Psychopharmacology* **173**: 434–439.
McGregor IS, Gurtman CG, Morley KC, Clemens KJ, Blokland A, Li KM *et al* (2003). Increased anxiety and 'depressive' symptoms months after MDMA ('ecstasy') in rats: drug-induced hyperthermia does not predict long-term outcomes. *Psychopharmacology* **168**: 465–474.
Medina KL, Hanson KL, Schweinsburg AD, Cohen-Zion M, Nagel BJ, Tapert SF (2007). Neuropsychological functioning in adolescent marijuana users: subtle deficits detectable after a month of abstinence. *J Int Neuropsychol Soc* **13**: 807–820.
Misner DL, Sullivan JM (1999). Mechanism of cannabinoid effects on long-term potentiation and depression in hippocampal CA1 neurons. *J Neurosci* **19**: 6795–6805.
Mitrirattanakul S, Lopez-Valdes HE, Liang J, Matsuka Y, Mackie K, Faull KF *et al* (2007). Bidirectional alterations of hippocampal cannabinoid 1 receptors and their endogenous ligands in a rat model of alcohol withdrawal and dependence. *Alcohol Clin Exp Res* **31**: 855–867.
Miyamoto A, Yamamoto T, Watanabe S (1995). Effect of repeated administration of delta 9-tetrahydrocannabinol on delayed matching-to-sample performance in rats. *Neurosci Lett* **201**: 139–142.
Morley KC, Gallate JE, Hunt GE, Mallet PE, McGregor IS (2001). Increased anxiety and impaired memory in rats 3 months after administration of 3,4-methylenedioxymethamphetamine ('ecstasy'). *Eur J Pharmacol* **433**: 91–99.
O'Leary DS, Block RI, Koeppel JA, Flaum M, Schultz SK, Andreasen NC *et al* (2002). Effects of smoking marijuana on brain perfusion and cognition. *Neuropsychopharmacology* **26**: 802–816.
Pace-Schott EF, Morgan PT, Malison RT, Hart CL, Edgar C, Walker M *et al* (2008). Cocaine users differ from normals on cognitive tasks which show poorer performance during drug abstinence. *Am J Drug Alcohol Abuse* **34**: 109–121.
Piper BJ, Meyer JS (2004). Memory deficit and reduced anxiety in young adult rats given repeated intermittent MDMA treatment during the periadolescent period. *Pharmacol Biochem Behav* **79**: 723–731.
Quinn HR, Matsumoto I, Callaghan PD, Long LE, Arnold JC, Gunasekaran N *et al* (2008). Adolescent rats find repeated Delta(9)-THC less aversive than adult rats but display greater residual cognitive deficits and changes in hippocampal protein expression following exposure. *Neuropsychopharmacology* **33**: 1113–1126.
Robbe D, Montgomery SM, Thome A, Rueda-Orozco PE, McNaughton BL, Buzsaki G (2006). Cannabinoids reveal importance of spike timing coordination in hippocampal function. *Nat Neurosci* **9**: 1526–1533.
Schlicker E, Kathmann M (2001). Modulation of transmitter release via presynaptic cannabinoid receptors. *Trends Pharmacol Sci* **22**: 565–572.
Schneider M, Koch M (2002). The cannabinoid agonist WIN 55,212-2 reduces sensorimotor gating and recognition memory in rats. *Behav Pharmacol* **13**: 29–37.
Soria G, Mendizabal V, Tourino C, Robledo P, Ledent C, Parmentier M *et al* (2005). Lack of CB1 cannabinoid receptor impairs cocaine self-administration. *Neuropsychopharmacology* **30**: 1670–1680.
Suenaga T, Ichitani Y (2008). Effects of hippocampal administration of a cannabinoid receptor agonist WIN 55,212-2 on spontaneous object and place recognition in rats. *Behav Brain Res* **190**: 248–252.
Thanos PK, Dimitrakakis ES, Rice O, Gifford A, Volkow ND (2005). Ethanol self-administration and ethanol conditioned place preference are reduced in mice lacking cannabinoid CB1 receptors. *Behav Brain Res* **164**: 206–213.
Touriño C, Ledent C, Maldonado R, Valverde O (2008). CB1 cannabinoid receptor modulates 3,4-methylenedioxymethamphetamine acute responses and reinforcement. *Biol Psychiatry* **63**: 1030–1038.
Ward J, Hall K, Haslam C (2006). Patterns of memory dysfunction in current and 2-year abstinent MDMA users. *J Clin Exp Neuropsychol* **28**: 306–324.
Wareing M, Fisk JE, Montgomery C, Murphy PN, Chandler MD (2007). Information processing speed in ecstasy (MDMA) users. *Hum Psychopharmacol* **22**: 81–88.
Yamaguchi T, Kubota T, Watanabe S, Yamamoto T (2004). Activation of brain prostanoid EP3 receptors via arachidonic acid cascade during behavioral suppression induced by Delta8-tetrahydrocannabinol. *J Neurochem* **88**: 148–154.
Yamamoto T, Takada K (2000). Role of cannabinoid receptor in the brain as it relates to drug reward. *Jpn J Pharmacol* **84**: 229–236.
Yamamoto T, Anggadiredja K, Hiranita T (2004). New perspectives in the studies on endocannabinoid and cannabis: a role for the endocannabinoid-arachidonic acid pathway in drug reward and long-lasting relapse to drug taking. *J Pharmacol Sci* **96**: 382–388.

総説

日薬理誌（*Folia Pharmacol. Jpn.*）**130**，135～140（2007）

カンナビノイド受容体—中枢神経系における役割

山本　経之

要約：1980年代末に，カンナビノイドが特異的に結合する受容体が脳内に存在することが明らかにされ，主に中枢神経系にCB_1受容体また末梢神経系にCB_2受容体の2つのサブタイプが同定された．また内在性カンナビノイドとしてアナンダミドや2-AGが相次いで発見された．カンナビノイドCB受容体は，グルタミン酸，GABA，アセチルコリン（ACh）等の神経シナプス前膜に存在し，神経シナプス後膜から遊離さる内在性カンナビノイドを介して各種伝達物質の遊離を抑制する事が知られている．ここではカンナビノイドCB_1受容体ならびにその内在性カンナビノイドが中枢神経系の機能としての食欲・記憶・痛覚・脳内報酬系における役割について述べた．食欲はCB_1受容体の活性化により亢進し，逆に不活性化によって抑制される．"食欲抑制物質"レプチンとの相互作用が示唆されている．記憶・学習に重要な役割を演じている脳部位にCB_1受容体が高密度に分布し，その活性化によって記憶障害（"忘却"）が誘発される．その作用はACh神経からのACh遊離の抑制に基因する可能性が示唆されている．また内在性カンナビノイドには鎮痛や痛覚過敏の緩和作用があり，末梢神経系のCB_2受容体やバニロイドVR_1受容体との関連性が今後の課題である．一方，大麻が多幸感を起こす事から，脳内カンナビノイドは脳内報酬系との関与が強く示唆され，それを支持する知見もある．脳内カンナビノイドシステムの変容は，意欲や多幸感・満足感を創生する脳内報酬系の破綻をきたし，精神疾患を誘引している可能性がある．近年，統合失調症を初めとした精神疾患とCB_1受容体および内在性カンナビノイドとの関連性が指摘され，その是非は今後の研究に委ねられている．いずれにしても脳内オピオイドの発見の歴史を彷彿させる脳内"大麻様物質"の存在は，脳の多彩な機能の解明の新たな礎となることに疑いの余地はない．

1. カンナビノイド受容体と内因性カンナビノイド

1988年，DevaneとHowlett等は大麻（Cannabis sativa）の活性成分Δ^9-テトラヒドロカンナビノール（THC）の誘導体[^{3}H]-CP55,940を用いて，ラットの脳シナプトソームにカンナビノイドに対する受容体が存在することを初めて明らかにした(1)．Masuda等はウシのサブスタンスK受容体遺伝子の一部をプローブとしたラットの大脳皮質cDNAライブラリーのスクリーニングからカンナビノイドCB_1受容体を偶然発見し，クローニングした(2)．このCB_1受容体（ヒト472個，ラット473個のアミノ酸配列）は中枢神経と一部の末梢神経に存在する(3)．その脳内分布は，海馬，大脳皮質，線条体，黒質，前脳基底部，嗅脳および小脳に多い．カンナビノイド受容体にはサブタイプがあり，もう1つはヒト白血病細胞（HL-60）のcDNAライブラリーからクローニングされたCB_2受容体（ヒト・ラット共に360個のアミノ酸）であり末梢神経に存在する．CB_1受容体とCB_2受容体とのアミノ酸配列の相同性は，48%とされている．これらの両受容体は7回膜貫通型ドメインを持つ典型的なGTP結合タンパク質共役型の受容体で，アデニル酸シクラーゼを阻害し，MAPK（mitogen-activated protein kinase）を活性化させる．さらに，CB_1受容体は電位依存性カルシウムチャネル（N, L, PおよびQ型）を抑制し，また，電位依存性カリウムチャネルを活性化させる．

その内在リガンドとして，Devane等(4)によってブタ脳からアラキドン酸誘導体の1種であるN-アラキドニルエサノールアミド（アナンダミド）が，またSugiura(5, 6)およびMechoulamによってそれぞれラ

キーワード：カンナビノイド，中枢神経系，行動
長崎国際大学薬学部薬理学研究室（〒859-2198 長崎県佐世保市ハウステンボス町2825-7）
e-mail: tyamamot@niu.ac.jp
原稿受領日：2007年4月16日，依頼原稿
Title: Cannabinoid receptor: its critical role in the CNS.　Author: Tsuneyuki Yamamoto

ット脳およびイヌ小腸から2-アラキドニルグリセロール(2-AG) が相次いで発見された．さらに2002年には，カンナビノイド受容体アンタゴニストの性質を持つとされるビロダミン (virodhamine) も発見されている．これらの内因性カンナビノイドの産生はシナプス後膜でなされ，強い脱分極 (細胞内Ca濃度の上昇；Ca > 1μM) およびGqタンパクの活性化後のPLCβの活性化の2つの経路により行われる(7)．またその遊離は，担体輸送系 (carrier-mediated transport) を介して行われる．その後速やかに酵素的に不活性化されるのでシナプス前膜でのカンナビノイドCB_1受容体での生理的作用は，制限される事になる．脂肪酸アミノ脱水素酵素 (FAAH) はアナンダミドのような内在性シグナル脂質である脂肪酸ファミリーを加水分解する．この酵素は基質特異性に欠ける面があり，脂肪酸エタノールアミドのみならず，2-AGのような脂肪酸エステルにも加水分解を起こす．しかし，最近の報告では2-AGはFAAHよりも主に脳内モノグリセリドリパーゼにより分解される事が知られている(8)．

これらの内在性カンナビノイドは，シナプス前膜において逆行性の抑制性モジュレーターとして働き，興奮性神経伝達物質グルタミン酸や抑制性神経伝達物質GABA等の遊離を抑制すると考えられているが(9, 10)，GABAの取り込み阻害作用によりGABA神経の活性化を起こすとの報告もある．

2. 中枢神経における機能的役割

1) 食欲

Δ^9-THCやアナンダミド等のCB_1受容体アゴニストは，ゲッ歯類の餌の摂食量を増強させる(11)．CB_1受容体アンタゴニストSR141716A (rimonabant：リモナバン) はこれらのカンナビノイドアゴニストによる摂食亢進作用に拮抗すると同時に，正常摂食量をも減少させる．CB_1受容体欠損マウスでも餌の摂食量は減少している．一方，内在性カンナビノイドの脳内濃度は絶食によって増加し，逆に摂食によって減少する．また餌ペレット強化のオペラント行動も選択的なCB_1受容体アンタゴニストにより抑制されるが，AM630のような選択的CB_2受容体アンタゴニストでは抑制されない．一連のCB_1受容体アンタゴニストには自発運動の低下や記憶障害を起こす作用がないことから，このオペラント行動の抑制作用も食欲減退作用に基づくとされている．一方，これまでのCB_1受容体アンタゴニストはアンタゴニスト作用と共に，大部分がインバースアゴニスト作用を有している．従ってこの食欲減退作用がCB_1受容体の拮抗作用そのものに基因するのか否かは明らかではなかった．しかし，最近，アゴニストならびにインバースアゴニスト作用を持たないCB_1受容体の"サイレントアンタゴニスト"としてO-2050が合成された．このO-2050は絶食をかけない条件下で，SR141716Aと同様にラットの餌摂取量を著しく抑制する(12)．従って，CB_1受容体アンタゴニストの食欲減退作用はCB_1受容体の拮抗作用そのものに基づく事が分かる．

CB_1受容体アゴニストのラットでの摂食亢進作用は，中脳辺縁系および視床下部への微量注入により誘発されることも明らかにされている．これらの事から，視床下部を中心とする食欲のコントロールには，内在性カンナビノイドが促進的に関与している事が分かる．

一方，食欲を抑制する神経ホルモンとして知られているレプチンとの関係も指摘されている(13)．レプチンは視床下部での内在性カンナビノイド濃度を減少させ，逆にレプチン欠損ラットでは視床下部での内在性カンナビノイド濃度は増加している．このことは，食欲が内在性カンナビノイドとレプチンとの相互によって調節されていることを示している．

Van Gaal等は，SR 141716A（リモナバン）の1年間に渡る臨床試験データから肥満の治療薬としてのCB_1受容体アンタゴニストの可能性を指摘している(14)．現在，このリモナバン（フランスのSanofi-Aventis社）はacompliaの商品名で"抗肥満薬"として，2006年6月から英国で，その後EU各国その他で発売されている．

一方，逆に，カンナビノイドは臨床的に食欲を亢進させ，体重増加を起こす事からエイズや癌での消耗性疾患症候群を呈する患者の延命効果が期待されている．

2) 記憶

CB_1受容体や内在性カンナビノイドは記憶・学習に関与している脳部位に多く存在している．大麻由来ならびに合成されたカンナビノイドは認知機能を障害する事が知られている．コンピューター操作によって出し入れ可能なレバーが3つ装着されたオペラント装置を用いての我々の遅延レバー位置合わせ課題 (delayed-matching-to-lever position task) を図1-Aに示した．前面パネルに1つのレバーが提示される（見本試行；30秒間）．ラットがこのレバーを押せば，レバーが格納され，同時に後方パネルにあるフィーダーから餌ペレットが得られる．3秒間のインターバル後，見本レバーを含む3つのレバーが提示される（テスト試行）この時見本レバーを押せばレバー格納と同時に再度餌ペレットが得られる（正反応）．しかし，他の

レバーを押せば餌ペレットの獲得は出来ない(誤反応)．この２つの試行を１単位として，15 回繰り返す．Δ^9-THC 投与により，見本試行のレバー押し行動には影響を与えることなくテスト試行での正解率を低下させ，作業記憶障害が認められる（図 1-B)．さらにΔ^9-THC の背側海馬内微量注入でもこの作業記憶障害が同様に出現する．さらに合成カンナビノイドアゴニストである WIN55,212-2，CP55,940 および R-メトアナンダミド，また内在性カンナビノイドであるアナンダミドや 2-AG の投与でも，遅延見本合わせ又は非見本合わせ課題での作業記憶は，障害される事が分かっている(15, 16)．水迷路での作業記憶も合成カンナビノイドアゴニスト HU210 によって障害される．また海馬への合成カンナビノイドの微量注入も著しい作業記憶障害を起こし，その作用はカンナビノイド CB_1 受容体アンタゴニスト SR141716A によって拮抗される．

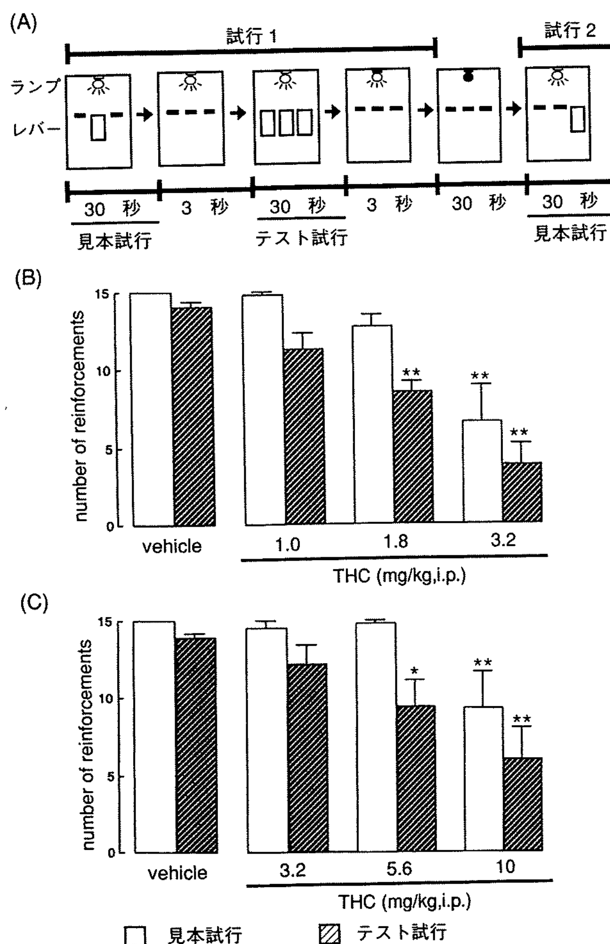

図１ 作業記憶に対する CB_1 受容体アゴニストΔ^9-テトラヒドロカンナビノール（THC）の作用
(A)3-lever オペラント装置を用いての遅延レバー位置合わせ課題（delayed matching-to-lever position task；作業記憶）の実験プロトコール，(B) Δ^9-THC 末梢投与による作業記憶障害．(C)背側海馬内Δ^9-THC 微量注入による作業記憶障害．$^*P < 0.05$，$^{**}P < 0.01$ および $^{***}P < 0.001$；対照（vehicle）群との比較．

逆にカンナビノイドアンタゴニストは記憶のプロセスを増強し，その作用は抗コリン剤スコポラミンによって拮抗される．この事から，脳内カンナビノイド系とアセチルコリン（ACh）神経伝達との関連性が示唆される．海馬や線条体切片での電気刺激による ACh 遊離は合成カンナビノイドアゴニスト CP55,940 や WIN55,212-2 で抑制され，逆に CB_1 受容体アンタゴニスト SR141716A によって増強される．また SR141716A 自身で ACh 遊離を増強させる．また海馬と同様，学習・記憶に重要な役割を演じている内側前頭前野でのマイクロダイアリシスを用いて無拘束ラット実験でも，WIN55,212-2 および CP55,940 は ACh 遊離を抑制し，その作用は SR141716A によって拮抗される．これらの事から，内在性カンナビノイドは内側前頭前野や海馬での ACh 神経活動に対して常に抑制的制御をかけている事が分かる．この事から，カンナビノイドによる作業記憶障害は，ACh 遊離の抑制に基づく可能性が示唆される．

一方，不快な記憶の獲得とその維持は，中枢神経系の重要な基本的役割の１つである．また学習獲得後の消去過程（強化子の除去）では，行動反応は次第に減少し，最終的には消去される．電撃ショックによるすくみ反応を指標とした聴覚性恐怖条件付け試験において，CB_1 受容体欠損マウスは記憶の獲得過程や保持過程には変化がなく，短期的および長期的な消去が著明に阻害されている．さらに野生型マウスでの CB_1 受容体アンタゴニスト SR141716A の作用態度は CB_1 受容体欠損マウスのそれと類似している(14)．痛みの閾値・自発運動量・不安水準そのものおよび無条件下でのすくみ反応には両者間には差がない．これらの事から，CB_1 受容体は不快記憶の消去に促進的な役割を演じている事が分かる．また不快記憶に関与する脳部位として知られる扁桃体基底外側部でのアナンダミドや 2-AG は，消去過程での音刺激提示によって増加する．この基底外側部では内在性カンナビノイドや CB_1 受容体は，GABA を介する抑制性電流の長期的抑制に促進的な役割を演じている．Marsicano 等は内在性カンナビノイドは扁桃体にある抑制性ネットワークにおいて選択的抑制効果を通して不快記憶の消去を促進させていると結論付けている(17)．

内在性カンナビノイドの“忘却を司る因子”としての意義とその応用は，今後の興味ある課題である．

3）痛覚

カラゲニン誘発炎症反応（浮腫）および熱刺激による疼痛反応（熱板試験および tail-flick 試験）は，Δ^9-THC，合成カンナビノイド HU210 や CP55,940 およ

び内因性カンナビノイドであるアナンダミドの脊髄内および脳室内投与により，鎮痛および痛覚過敏の緩和作用が認められる(18)．さらにカンナビノイド受容体アゴニストは視床の側部後方と中央下部領域，A5 領域，扁桃体，腹側中脳水道周囲灰白質（PAG）および延髄吻側腹側部への微量注入によっても，鎮痛作用を発現しこれらの脳部位は作用点の1つと考えられている．アジュバント注入で誘発されるラットのアロディニア（非侵害性の触・熱刺激で発痛する）も，カンナビノイド受容体アゴニスト WIN55, 212-2 の脊髄内投与により，抑制される(19)．興味深い事には，CB_1 受容体遺伝子欠損マウスでは，Δ^9-THC の熱板試験での鎮痛作用は認められないが，アナンダミドの鎮痛作用は出現する点である(20)．このようにアナンダミドの鎮痛作用の発現には，少なくとも CB_1 受容体以外の作用点もあるようである．アナンダミドは FAAH によって失活する．この FAAH 欠損マウスは脳内アナンダミド量が正常マウスのそれに比べて 15 倍も高く，侵害刺激に対する痛覚閾値も高い事が報告されている(21)．

一方，末梢性の侵害刺激によって，腹側中脳水道周囲灰白質においてアナンダミドが遊離される．また，Seigling 等は，ラットの末梢神経損傷後のその末梢神経と対側の視床において，CB_1 受容体のアップレギュレーションが起こっている事を報告した(22)．この事は，慢性疼痛の動物モデルにおけるカンナビノイドの疼痛緩和作用の発現が CB_1 受容体のアップレギュレーションに基づく可能性を示唆している．

また炎症性物質カラゲニンによって誘発される痛覚過敏はアナンダミドの末梢投与により抑制され，ホルマリン誘発疼痛反応もアナンダミドや CB_2 受容体の選択的アゴニストであるパルミチルエタノールアミドによって抑制される．カンナビノイドによる一連の疼痛抑制作用は，CB_1 受容体および CB_2 受容体アンタゴニストで拮抗される．さらに Malan 等は CB_2 受容体の選択的アゴニスト AM1241 の末梢投与により，CB_1 受容体アゴニストと同様に熱刺激に基づく疼痛反応（tail-flick 試験）が減弱される事を明らかにし，カンナビノイドの鎮痛作用が CB_2 受容体活性化を介しても起こる事を指摘している(23)．また最近 Bel-

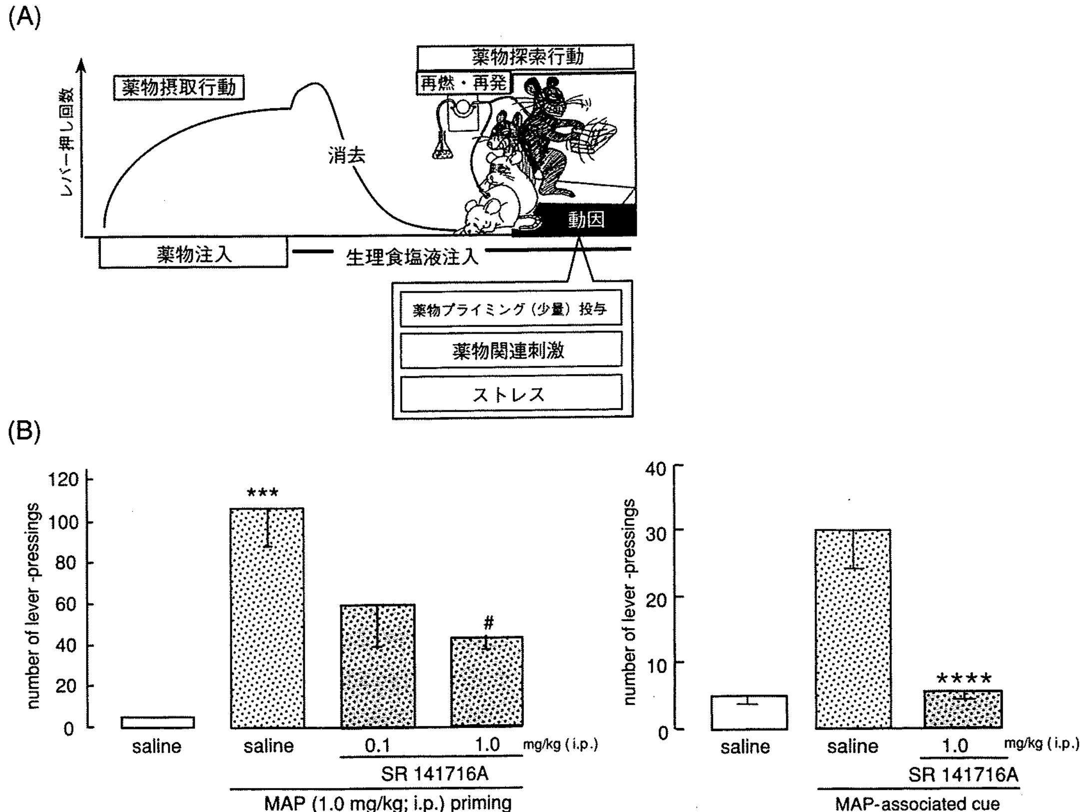

図2　ラットのメタンフェタミン探索行動に対する CB_1 受容体アンタゴニスト SR141716A の作用
(A)薬物自己投与実験の概念図（覚せい剤精神病と麻薬依存．東北大学出版会; 2004. p.101. より改変），(B)MAP プライミング投与ならびに MAP 関連刺激によって誘発される MAP 探索行動に対する SR141716A の抑制作用．***$P < 0.001$；退薬時の対照群との比較．また #$P < 0.05$；MAP- プライミング時での対照群との比較．****$P < 0.0001$；MAP 関連刺激提示時での対照群との比較．（文献 31 より改変）

tram 等も，慢性疼痛における痛みの軽減には CB_2 受容体の抹消神経系のみならず，中枢神経系（脊髄でのミクログリアの不活性化）での関与を明らかにしている(24).

今後，CB_1 および CB_2 受容体の痛覚神経路での機能的役割の解明が急がれる．さらに，各種侵害刺激によって活性化されるバニロイド受容体と CB 受容体の各受容体リガンドは共通する化学構造を持ち，アナンダミド・トランスポーターに対しても共に親和性を有しているが，痛覚に対しては相反する作用を示す事が知られている．両受容体のクロストークの解明が進めば，"痛み" の制御機構の解明に新たな糸口を与えてくれるものと期待される.

4）脳内報酬系

陽性の報酬効果として，自然環境から生まれる摂食・摂水などの "自然的な報酬" と依存性薬物など特殊な外来刺激によって誘発される "報酬" とがある．これらの報酬効果は内在性オピオイドや腹側被蓋野(VTA)を起始核とし前頭前野や中脳辺縁系に走行する DA 神経を中心とする脳内報酬系によって制御されている．大麻およびその活性成分 Δ^9-THC も他の依存性薬物と同様に，知覚効果（subjective）として多幸感や幸福感を起こし，ラットでの自己投与行動が成立する事も報告されている．これらの知見から，内在性カンナビノイドと脳内報酬系との関連性が指摘されている(25-28)．モルヒネの自己投与行動は CB_1 ノックアウトマウスでは成立せず(29)，さらにヘロイン自己投与行動は SR141716A により抑制される．これらの事は，オピオイドの報酬効果の発現にも CB1 受容体の活性化が介在している事を示唆している．また逆に，THC 自己投与行動もオピオイド受容体拮抗薬ナルトレキソンで抑制される事(30) から，カンナビノイドの報酬効果発現にもオピオイド受容体が関わっている事が分かる．

一方，依存性薬物の長期使用後の退薬時に，精神的苦痛を避ける為に薬物再摂取への渇望（craving）が誘引される．その為，如何なる犠牲を払っても，薬物を入手しようとするようになり，その行動を薬物探索行動と呼ぶ．薬物探索行動の動因には，1) 興奮性薬物の少量使用（薬物プライミング投与)，2) 薬物摂取時を連想させる環境刺激（薬物関連刺激）および 3) ストレスが知られている．図 2-A に実験動物を用いての薬物自己投与実験の概念図を書いた．覚せい剤メタンフェタミン（MAP）自己投与行動が成立したラットを用い，MAP から生理食塩液注入に切り替えるとレバー押し行動は減弱する．この時，MAP の少量投与または薬物関連刺激（音・光）を与えるとレバー押し行動が出現し，ヒトでの薬物探索行動を彷彿させる類似の行動と考えられている．我々はこれまで，CB_1 受容体アンタゴニスト SR141716A を投与すると，これら 2 つの刺激により誘発される薬物探索行動が有意に抑制される事を明らかにした(31)（図 2-B).

また，ヘロイン探索行動の発現も，SR141716A により抑制される．De Vries ら(2001）も，コカイン探索行動が SR141716A により抑制され，CB_1 受容体作動薬 HU210 それ自体で誘発される事を明らかにした(32)．これらの知見は，薬物探索行動の発現には脳内カンナビノイド系の活性化が必須である事を示唆している．しかし，我々のメタンフェタミン探索行動は Δ^8-THC 単独投与で誘発しなかった事から，作用機序は必ずしも同一ではなく依存性薬物によって一部異なる事が分かる．一方，メタンフェタミン探索行動は，消去過程での Δ^9-THC 投与により抑制される (33)．辺縁系における 2-AG 量は，コカイン反復投与後に減少する事も明らかにされている (34)．これ等の知見から，脳内カンナビノイド系の不活性な状態が薬物探索行動の "準備状態" を形成している可能性が示唆される．

これとは別に，ナロキソンによるモルヒネ退薬症候が 2-AG の脳室内注入により抑制され(35)，モルヒネ退薬症候発現時にも脳内カンナビノイド系の機能低下が起こっている可能性が示唆される．また，我々はアスピリンやジクロフェナクといった COX 阻害薬が THC によるレバー押し行動（餌強化）の抑制作用に拮抗する事(36)，THC の退薬症候を誘発する事，またプロスタグランジン PGE_2 が CB_1 受容体拮抗薬 SR141716A による THC 退薬症候を抑制する事を報告した(37)．これらの知見はカンナビノイドとアラキド

表 1 カンナビノイド CB_1 受容体，バニロイド VR_1 受容体およびアナダマイドトランスポーター（AMT）のリガンドとその受容体／トランスポーターに対する親和性

リガンド	親和性		
	CB_1	AMT	VR_1
olvanil	+	+ +	+ +
AM404	+	+ + +	+ +
arvanil	+ +	+ + +	+ +
anandamide	+ +	+ +	+
2-arachidonoyl-glycerol	+ +	+	—
resiniferatoxin	N.T.	N.T.	+ + + +
capsaicin	−	−	+ +

親和性の相対的強度を＋／−で表した．N.T.：not test
（文献 25 より改変）