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Endocannabinoid System Modulates Relapse to Methamphetamine Seeking: Possible Mediation by the Arachidonic Acid Cascade

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We clarified the modulating action of the endocannabinoid system, and its possible mediation by the arachidonic acid cascade, on the reinstatement of methamphetamine (METH)-seeking behavior, using the intravenous self-administration paradigm in rats. Following 12 days of self-administration of METH, the replacement of METH with saline resulted in a gradual decrease in lever press responses (extinction). Under extinction conditions, METH-priming or re-exposure to cues previously paired with METH infusion markedly increased the responses (reinstatement of drug-seeking). The cannabinoid CB1 receptor antagonist, SR141716A, blocked this behavior. Although the cannabinoid agonist, Δ^8-tetrahydrocannabinol (THC), had no effects by itself, coadministration of the agonist and METH at small doses reinstated the drug-seeking behavior. THC attenuated the effects of the reinstatement-inducing dose of METH, but enhanced the effect of cues. Either given repeatedly during the extinction or singly, 24 h before the first METH-priming or cues challenge, THC suppressed the reinstatement. In another set of experiments, we found that diclofenac, a cyclooxygenase inhibitor, also attenuated the reinstatement induced by exposure to cues or drug-priming. These results suggest that the endocannabinoid system, through possible mediation by the arachidonic acid cascade, serves as a modulator of the reinstating effects of METH-priming and cues. Extending the current view on the treatment of drug dependence, these results indicate that endocannabinoid-activating substances as well as cyclooxygenase inhibitors may be promising as antirelapse agents.
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INTRODUCTION

Methamphetamine (METH) is a highly addictive psychostimulant (Woolverton *et al*, 1984; Cho, 1990; Hart *et al*, 2001), with reinforcing properties that are comparable to those of cocaine (Peltier *et al*, 1996; Shimosato and Ohkuma, 2000). Over the past several years, METH abuse has significantly risen worldwide, and is becoming increasingly problematic (Greberman and Wada, 1994).

Recently, cumulating evidence has indicated the involvement of the endocannabinoid system in reward circuit in the brain (Yamamoto and Takada, 2000; Gardner, 2002). Early studies have shown that the psychoactive ingredient of marihuana, Δ^9-tetrahydrocannabinol (THC), enhanced brain reward, as indicated by its effect of reducing electrical brain stimulation threshold levels in the medial forebrain bundle (Gardner *et al*, 1988). Furthermore, like other well-known habit-forming drugs, Δ^9-THC was demonstrated to enhance the extracellular dopamine efflux in reward-relevant brain loci, such as the striatum, nucleus accumbens, and medial prefrontal cortex (Ng Cheong Ton *et al*, 1988, Chen *et al*, 1990). The manifestation of the withdrawal syndrome has been clearly demonstrated following cessation of repeated intake of natural (Hutcheson *et al*, 1998) and synthetic (Aceto *et al*, 2001) cannabinoids as well as endogenous cannabinoid ligand (Costa *et al*, 2000) in dependent animals. Crossinteraction observed between cannabinoid and opioid systems (Navarro *et al*, 2001; Yamaguchi *et al*, 2001a) further supports the notion that the endocannabinoid system is a component of reward substrates in the brain.

Using the self-administration paradigm, the cannabinoid system has been indicated to be involved in the maintenance of drug-seeking and -taking behavior, as evidenced by the reduction in the intake of cocaine by pretreatment with the cannabioid agonist WIN55,212-2 in rats self-administering cocaine (Fattore et al, 1999). In addition, another cannabinoid agonist HU-210 was shown to reinstate the drug-seeking behavior in rats withdrawn from cocaine and heroin self-administration (De Vries et al, 2001; De Vries et al, 2003).

Δ^1-THC, via a receptor-mediated mechanism, stimulated the mobilization of arachidonic acid (Hunter and Burnstein, 1997) and induced activation of phospholipase (Hunter et al, 1986; Wartmann et al, 1995). In line with this finding, Shivachar et al (1996) showed that anandamide- and Δ^9-THC-evoked arachidonic acid mobilization were blocked by the CB1 receptor antagonist, SR141716A. Further, there was evidence showing that the endogenous cannabinoid ligand 2-arachidonoylglycerol is the substrate for cyclooxygenase-2 to generate substances belonging to the prostaglandins family (Kozak et al, 2000). We recently demonstrated that cannabinoid-induced behavioral suppression is due to the activation of the arachidonic acid cascade (Yamaguchi et al, 2001b), which is mediated by the prostanoid EP3 receptor (Yamaguchi et al, 2004). Moreover, we have shown that prostaglandin E_2 attenuated the expression of the SR141716A-precipitated withdrawal syndrome in THC-dependent mice (Anggadiredja et al, 2003). This evidence suggests that the arachidonic acid cascade regulates the intracellular action of cannabinoids, and consequently, takes the role of the endocannabinoid in the brain reward system.

Until recently, data on relapse, which is the main hurdle in the treatment of drug dependence, to METH as it relates to the endocannabinoid system have been scarce. To confirm the cannabinoid hypothesis of relapse to METH abuse, which may describe the key role of the endocannabinoid system in reinstating effects of METH and cues, we tested the effects of the psychoactive ingredient of marihuana, Δ^8-THC, administered during the expression of 'relapse' as well as extinction phases, in rats self-administering METH. Further, we tested the possible involvement of the arachidonic acid cascade, downstream of the endocannabinoid system, in this reinstating effect of METH and cues.

MATERIALS AND METHODS

Subjects and Apparatus

Male Wistar/ST (SLC, Hamamatsu, Japan) rats (250–350 g) were used. Under general anesthesia with sodium pentobarbital (35 mg/kg), silascone® catheters (inner and outer diameter: 0.5 and 1.0 mm, respectively; Kaneka Medix Corporation, Japan) were implanted into the jugular vein and threaded down towards the right atrium. The catheter passed through the skin in the mid-scapular region and was fixed by ligature. The distal portion of the catheter was anchored by a mesh button fixed under the skin on the back. 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. The distal end of the catheter was connected to a syringe-driven pump containing METH or saline solution. To allow free movement of the rat during experimental sessions, the catheter was connected to a swivel. For the maintenance of catheter patency, before and after each session, the catheter was flushed with 0.15 ml of heparin solution (30 U/ml). The operant chambers were equipped with an active lever programmed by computer software (Med Associates, Georgia, Vermont).

Drugs

Methamphetamine HCl (DaiNippon, Osaka, Japan) was dissolved in saline. THC (kindly provided by Professor Y Shoyama, Department of Medical Resources Regulation, Graduate School of Pharmaceutical Sciences, Kyushu University) was emulsified in 1% Tween-80 solution. SR141716A (Sanofi Recherche, France) was dissolved in a mixture of Tween-80 and DMSO (Tween-80 and DMSO content in vehicle, $\pm 1\%$ by volume) and diluted with distilled water. Diclofenac sodium (Sigma, St Louis, MO, USA) was dissolved in saline. Except for the intravenously self-administered METH, all drugs were administered intraperitoneally, and injected at a volume of 0.1 ml per 100 g of body weight.

METH-Induced Reinstatement of METH-Seeking Behavior

Self-administration training sessions started 2 days after surgery. Rats were allowed to administer METH (0.02 mg/ infusion over 6 s) during a 2-h session, under fixed-ratio 1 (FR-1) schedule of reinforcement, performed each day for 12 days. The chamber light was switched on throughout the session. When the rat pressed the lever, the infusion pump was switched on for 6 s. The subsequent 20 s was the time out period during which lever presses were still recorded but not accompanied with infusions, and the lever light (mounted 2 cm above the lever) was switched off. Subsequently, they entered an extinction phase, during which lever presses only resulted in saline infusions. Reinstatement tests started from day 6 of extinction. The test sessions, lasted for 2 h, were carried out under the same conditions as in the extinction phase, in which only saline infusion was available. All drug pretreatments began 30 min before the session. Drugs were administered in a counterbalanced order every 3–4 days.

METH-Associated Cue-Induced Reinstatement of METH-Seeking Behavior

Drug self-administration training was carried out on an FR-3 schedule of reinforcement. During this training, each earned METH infusion (0.02 mg over 6 s) was accompanied by the presentation of lever light (300 lux) and tone (70 dB/ 7kHz). The presentation of these cues continued through the subsequent 20 s time out period, during which lever presses were still recorded but not accompanied with infusions. The 12-day training phase was followed by extinction training, in which neither drug nor cues were

available. Test sessions began on day 6 of extinction. Each session lasted for 2 h in total, and was divided into two periods. During the first hour (non-cue phase), the conditions were similar to those in the extinction phase. In the second hour (cue phase), the conditions were similar to those in the self-administration training; however, only saline infusions were available. At the beginning of this cue phase, lever light was switched on for 20 s. Drug pretreatments, administered in a counterbalanced order every 3–4 days, began 30 min before the second hour of the test sessions.

Effects of THC Given During the Extinction Phase on Reinstatement

During the extinction phase, one group of rats received THC injection after each daily session for 5 days. On day 6 of the phase, they were then challenged with METH-priming or re-exposed to METH-associated cues. In another group, rats were given only a single THC pretreatment, 24 h prior to the challenge; and in this group, the challenges were repeated on days 12, 20, and 30 of extinction.

Effects of Diclofenac on Operant Task Performance

Initially, all subjects were trained to lever press for food on an FR-1 schedule of reinforcement consisting of one food pellet (45 mg; Bioserve®, Holton Industries Co., Frenchtown, NJ). Each session ended when a subject has delivered 30 food pellets or the 1200 s experimental time was over. When the rats have been able to deliver 30 pellets within 600 s, subsequently, they were trained on an FR-3 schedule of reinforcement. The rats were considered ready for tests when they have been able to deliver 30 food pellets within 300 s in three consecutive training sessions. Each test session ended when a rat has delivered 20 food pellets or the 900 s experimental time was over.

Statistical Analyses

In the statistical analyses, drug treatment was a between subjects variable. One-way ANOVA was used to compare means and Benferroni/Dunn tests were used for *post hoc*. In METH-associated cue-induced reinstatement tests, number of lever presses obtained from the first hour (non-cue phase) was compared with that of the second hour (cue phase) of each session using paired *t*-test. A difference was considered significant at $p < 0.05$.

RESULTS

METH Self-Administration and Extinction Training and Reinstatement of METH-Seeking Behavior

During the self-administration training, the responses gradually increased, and remained stable from day 8 of the training. On the first day of self-administration training the number of infusions was 11.46 ± 1.80. This number gradually increased, reaching 16.10 ± 0.98 on day 12 of the training. The total number of responses during this self-administration training was 172.62 ± 12.19 METH infusions. When the drug was replaced with saline, the lever press responses gradually decreased from 30.75 ± 6.57 on the first

day, reaching 7.42 ± 1.75 lever presses on day 5 of extinction. Under extinction condition, one-way ANOVA revealed a significant main effect of METH priming ($F(2,18) = 34.26$, $p < 0.0001$). *Post hoc* analyses (Benferroni/Dunn) showed that injection of METH at 1 mg/kg produced a significant increase in lever responses during the 2 h-test sessions (see Figure 1a). In the same way, paired *t* test showed that, compared with responses obtained during first hour (non-cue), cues previously paired with METH-infusion produced a significant increase in lever responses ($p < 0.0001$) during the second hour of the test sessions (see Figure 1b). Furthermore, both METH-priming and re-exposure to cues still significantly reinstated drug-seeking behavior when the animals were re-challenged on days 12, 20, and 30 of extinction (see Figure 5b and c).

Effects of SR141716A on METH-Priming- and Cue-Induced Reinstatement

There was a significant main effect of SR141716A on reinstatement of METH-seeking behavior induced by METH-priming ($F(2,18) = 30.67$, $p < 0.0001$). *Post hoc* analyses (Benferroni/Dunn) revealed that 3.2 mg/kg SR141716A produced a significant decrease in lever responses (see Figure 2a). As depicted in Figure 2b, one-way ANOVA also revealed a significant main effect of the antagonist on reinstatement induced by re-exposure to METH-associated cues ($F(1,14) = 49.41$, $p < 0.0001$). Subsequent pairwise analyses (Benferroni/Dunn) showed that 1 mg/kg SR141716A significantly decreased this cue-induced reinstatement.

Reinstatement-Inducing Effect of THC

As shown in Figure 3, THC by itself (priming) failed to produce reinstatement at any doses tested, albeit it tended
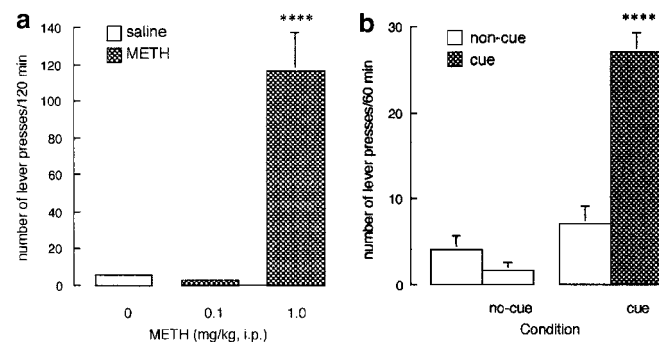
Figure 1 Reinstatement of METH-seeking behavior induced by METH-priming injection and METH-associated cues. Following METH self-administration training for 12 days, the rats entered extinction phase (only saline infusion was available). Reinstatement tests started on day 6 of extinction. (a) METH-priming injection 30 min prior to the test session (lasted for 2 h) reinstated METH-seeking behavior, indicated by the significant increase in the number of lever press responses. Data represent the average ± SE number of lever presses, with 5–8 rats per group; ****$p < 0.0001$ compared with saline-treated rats. (b) Re-exposure to METH-associated cues, also produced reinstatement of METH-seeking behavior, indicated by the increased responses during the second hour (cue phase) of the test session. Data represent the average ± SE number of lever presses, with 5–8 rats per group; ****$p < 0.0001$ compared with the first hour (non-cue phase) of the test session.
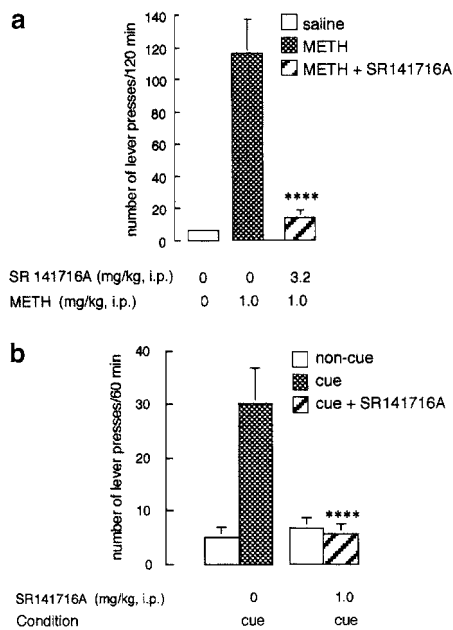
Figure 2 Effects of the selective CB1 receptor antagonist SR141716A on reinstatement of METH-seeking behavior in rats. (a) Coadministered with METH 30 min before the test session, 3.2 mg/kg SR141716A produced decreased number of lever press responses, which were significantly different from those produced by METH alone. Data represent the average $\pm$ SE number of lever presses, with 5–8 rats per group; ****$p < 0.0001$ compared with only METH-treated group. (b) This antagonist at 1.0 mg/kg also suppressed the reinstatement of METH-seeking behavior when administered 30 min prior to the second hour (cue phase) of the test session. Data represent the average $\pm$ SE number of lever presses, with 5–8 rats per group; ****$p < 0.0001$ compared with the cue session in only saline-treated rats.
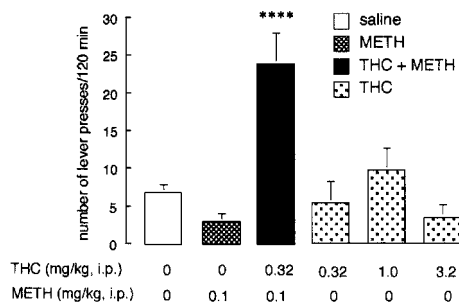

Figure 3 Effects of the cannabinoid agonist THC in METH self-administering rats. Although THC, in place of METH-priming, failed to induce reinstatement, there was a tendency for METH seeking after 1 mg/kg THC. While each of the drug by itself induced no behavioral consequences, coinjection of i.p. THC (0.32 mg/kg) and METH (0.1 mg/kg) significantly increased lever press responses. Data represent average $\pm$ SE number of lever presses. ****$p < 0.0001$ compared with only saline-, THC (0.32 mg/kg)- or METH (0.1 mg/kg)-treated group. There were 5–7 rats in each group.

to increase the number of responses at 1 mg/kg. However, coadministered with METH at 0.1 mg/kg, which induced no behavioral consequences, and even reinstatement, 0.32 mg/kg THC produced a significant increase in lever responses (F(3,22) = 16.56, $p < 0.0001$, one-way ANOVA).
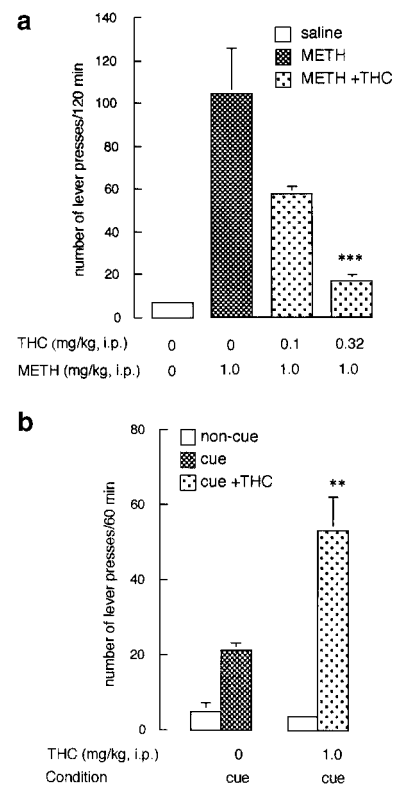

Figure 4 Effects of THC on reinstatement induced by METH-priming and that induced after re-exposure to METH-associated cues. (a) With METH-priming, 0.32 mg/kg THC showed reinstatement-attenuating effects, as represented by a significant decrease in the number of lever responses. Data represent the average $\pm$ SE number of lever presses, with 5–8 rats per group; ***$p < 0.001$ compared with only METH-treated group. (b) On the other hand, this agonist at 1.0 mg/kg enhanced the effects of cue on reinstatement. Data represent the average $\pm$ SE number of lever presses; **$p < 0.01$ compared with cue session in only saline-treated group. There were 5–8 rats in each group.

Effects of THC on METH-Priming- and Cue-Induced Reinstatement

The data shown in Figure 4a demonstrated the effect of THC on reinstatement of METH-seeking behavior induced by 1 mg/kg METH. The data were analyzed with a one-way ANOVA, which revealed a significant main effect of treatment (F(3,23) = 13.10, $p < 0.01$). Subsequent pairwise analyses (Benferroni/Dunn) showed that 0.32 mg/kg THC significantly attenuated METH-seeking behavior. In contrast to these results, however, THC at 1 mg/kg enhanced the effects of cues, as shown by the significant increase in lever press responses (F(1,10) = 12.20, $p < 0.01$) (see Figure 4b).

Effects of THC Administration During Extinction Phase on Reinstatement

When THC was given repeatedly after each session from day one through five of extinction, one-way ANOVA revealed a significant main effect of treatment (F(2,20) = 22.08, $p < 0.0001$). Subsequent pairwise analyses (Benferroni/Dunn) showed that 3.2 mg/kg THC significantly attenuated reinstatement of drug seeking induced by

METH-priming (see Figure 5a). After single administration of this agonist, 24 h before METH-priming, one-way ANOVA also revealed a significant effect of treatment $(F(2,19) = 14.6, p < 0.0001)$; and *post hoc* analyses (Benferroni/Dunn) showed that this treatment significantly decreased reinstatement of METH-priming-induced seeking behavior (see Figure 5b). The attenuating effect of THC was also noted after challenging the rats with drug-associated cues. One-way ANOVA revealed a significant main effect of treatment $(F(1,10) = 11.4, p < 0.01)$, and subsequent

pairwise analyses (Benferroni/Dunn) showed that THC significantly attenuated reinstatement of METH-seeking behavior (see Figure 5c). The significant reinstatement-attenuating effects of single THC could still be noted on day 12 of extinction, either after METH-priming injection $(F(2,15) = 5.7, p < 0.05)$ or re-exposure to cues $(F(1,7) = 6.7, p < 0.05)$. In addition, this THC treatment seemed to suppress reinstatement even after the third and fourth METH/cues challenges.

Effects of Diclofenac on Reinstatement of METH-Seeking Behavior

The drug-induced reinstatement data were analyzed by one-way ANOVA, which revealed a significant main effect of treatment $(F(3,22) = 4.3, p < 0.05)$. Subsequent pairwise analyses (Benferroni/Dunn) showed that diclofenac at 3.2 and 10 mg/kg produced significant attenuation of reinstatement (see Figure 6a). Significant main effect was also revealed in cue-induced reinstatement data, as analyzed by one-way ANOVA $(F(2,17) = 22.3, p < 0.001)$. *Post hoc* analyses (Benferroni/Dunn) showed that the same doses of diclofenac significantly attenuated reinstatement in rats re-exposed to cues (see Figure 6b). As demonstrated in Figure 6c, analyses with one-way ANOVA revealed no significant effect of diclofenac on the response rate of food-self administration $(F(1,12) = 0.12, p < 0.74)$.

DISCUSSION

Reinstatement of METH-seeking behavior was induced by drug-priming and re-exposure to the cues previously accompanying METH taking. SR141716A blocked the reinstatement, and furthermore, a small dose of THC enhanced the effects of a sub-threshold dose of METH. These results may describe hyperactivation of the endocannabinoid system during reinstatement. However, the findings after pretreatment with THC during the extinction phase indicate that prior to the expression of drug-seeking, the endocannabinoid system is inactivated/hyposensitized. Such a hyposensitization following METH self-administration may possibly be related with a decrease in the levels of endogenous cannabinoid ligands or cannabinoid receptors. Thus, chronic cocaine administration has been reported to produce a decrease in 2-arachidonoylglycerol levels in the limbic forebrain area (Gonzalez *et al*, 2002a). Cocaine was also demonstrated to produce a decrease in CB1 receptor mRNA in brain areas such as hypothalamus and cerebral cortex (Gonzalez *et al*, 2002b). Considering the close link between the cannabinoid and dopaminergic systems (Gorriti *et al*, 1999; Schlicker and Kathmann, 2001), these findings may be consistent with recent electrophysiological evidence of hyposensitivity and hypersensitivity of the dopaminergic system in the nucleus accumbens on day one and day 5 of withdrawal, respectively, following repeated administration of METH (Amano *et al*, 2002). Thus, pretreatment with THC (before the induction of reinstatement) may prevent further hyposensitization of the endocannabinoid system, and the consequent rebound hypersensitization of the system. This would be manifested
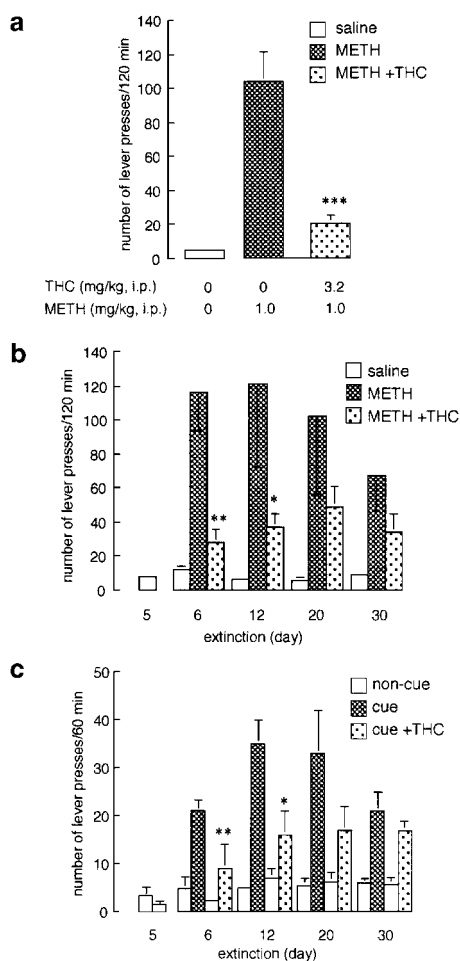

Figure 5 Effects of THC administered during the extinction phase on reinstatement of METH-seeking behavior. (a) Injecting the rats with THC after each session during extinction period (days 1–5 of extinction) suppressed reinstatement of METH-seeking behavior induced by METH-priming on day 6 of extinction. Data represent the average ± SE of number lever presses, with 5–8 rats per group; ***$p < 0.001$ compared with only METH-treated group. (b) Even single THC pretreatment after the session on day 5 of extinction, 24 h prior to METH-priming, blocked METH-seeking behavior. Data represent the average ± SE number of lever presses, with 5–8 rats per group; **$p < 0.01$ compared with only METH-treated group. (c) Similar results were found in the group challenged with METH-associated cues. Single THC pretreatment after the session on day 5 of extinction produced decreased number of lever press responses. Data represent the average ± SE number of lever presses, with 4–7 rats per group; ** $p < 0.01$ compared with only cue-treated group. In addition, the effect of such a THC pretreatment remained significant when the second challenges were repeated on day 12 of extinction, and had the tendency to remain after the third and fourth priming injections, on day 20 and 30 of extinction, respectively.
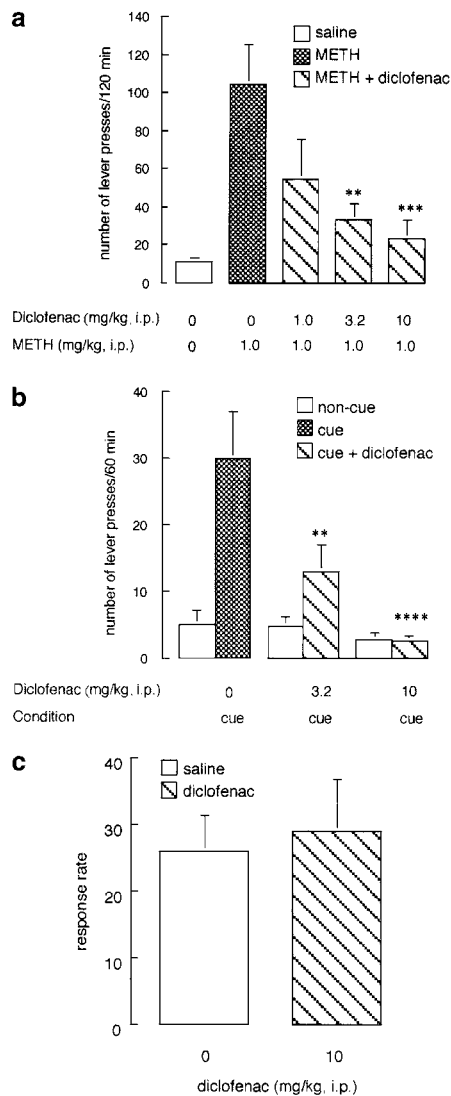
Figure 6 Effects of the cyclooxygenase inhibitor diclofenac on reinstatement of METH-seeking behavior. (a) Given along with METH-priming 30 min prior to test session, diclofenac suppressed the reinstatement of METH-seeking behavior. Data represent the average ± SE number of lever presses, with 5–8 rats per group; **$p < 0.01$, *** $p < 0.001$ compared with only METH-treated group. (b) Pretreatment with diclofenac 30 min before the second hour (cue phase) of the test session attenuated the reinstatement induced by METH-associated cues. Data represent the average + SE of number of lever presses. There were 5–8 rats in each group; **$p < 0.01$, ****$p < 0.0001$ compared with only cue-treated group. (c) Effect of diclofenac on food-reinforced operant responding in the rat. The data are represented as average ± SE of response rate, during the session lasted until 20 food pellets were delivered or the 15 min experimental time was over. There were 6–8 rats in each group.

in the attenuation of lever press responses (drug-seeking behavior).

In line with the hypothesis of a hypersensitized endo-cannabinoid system during the expression phase of seeking behavior, THC enhanced the effects of cues on drug-seeking behavior. In contrast to this result, however, THC depressed the reinstatement-inducing effects of 1 mg/kg METH. Early data indicated that THC at low doses could stimulate motor

activity, an effect that was amplified by amphetamine (Evans et al, 1976). In addition, combination of THC with METH or cocaine has been shown to induce stereotyped behaviors (Consroe et al, 1976). Judging from these data, in our study the combination of THC and METH might induce hyperactivity or stereotyped behaviors that masked the lever press behavior. Thus, there might be a possibility that this depressed reinstatement is due to a non-specific effect. Alternatively, this attenuation in reinstatement might be a consequence of the hypothesized inhibitory feedback mechanism that counters the striatal dopamine-cannabi-noid pathway (Giuffrida et al, 1999). Indeed, this mechanism might underlie the ameliorating effect of cannabinoid activation in animal models (Beltramo et al, 2000) as well as the patients (Muller-Vahl et al, 2003) of disorders related to the hyperactive dopaminergic tone.

Compelling is the result showing that seeking behavior suppression by THC pretreatment lasted for somewhat long period of time, as indicated by the persistently lower lever response after the second priming/cue presentation on day 12 of extinction. A close relationship between 'relapse' of psychostimulant-seeking behavior and its sensitization has been indicated (De Vries et al, 1998; Vanderschuren et al, 1999; De Vries et al, 2002). On the other hand, repeated cannabinoid agonist treatment has been found to induce sensitization to the psychomotor effects of amphetamine (Gorriti et al, 1999). These findings further indicate the relation between the state of abstinence from METH self-administration and that of cessation of chronic cannabinoid treatment. Moreover, the expression of cannabinoid with-drawal signs was blocked by THC pretreatment (Lichtman et al, 2001), which may be analogous to the blocking effect of THC pretreatment on the expression of seeking behavior as shown in our study. One recent report demonstrated that spontaneous cannabinoid withdrawal induced proopiome-lanocortin gene expression, which was augmented for at least 14 days (this time span coincides well with the duration of reinstatement-suppressing effects of THC in our study) after withdrawal (Oliva et al, 2003). Indeed, changes in gene transcription have been proposed as one of the factors responsible for the vulnerability to drug addiction, including relapse to drug taking (Koob et al, 1998). Meanwhile, THC has been shown to decrease cerebral metabolism, including that in the limbic forebrain areas (Freedland et al, 2002), the brain loci essential to motivational responses, and where many of the gene alterations associated with drug addiction take place (Koob et al, 1998). Decreased metabolism has been shown to parallel the decrease in opioid peptide gene expression, including prodynorphin and proopiomelanocortin (Girau-do et al, 1998; Kim et al, 1999). Considering all this evidence, one may suggest that the long-lasting suppressing effects of THC pretreatment on seeking behavior have a close link with its action on certain gene expression machinery, possibly that of the opioid peptides. The likelihood that such effects originate from its locomotor suppressing action may be ruled out, because the effects of an even higher dose of THC completely disappeared 24 h after its administration (Whitlow et al, 2002).

The mediating action of the arachidonic acid cascade in several effects of cannabinoids has been shown in a number of animal studies. Indeed, in human subjects, the cycloox-

ygenase inhibitor, indomethacin, has been demonstrated to antagonize psychological effects of marihuana, including the subjective 'high' and time estimation delay (Perez-Reyes *et al*, 1991). Furthermore, there is evidence for the involvement of the cascade in the development of stimulant sensitization. The phospholipase A2 inhibitor, quinacrine (Reid *et al*, 1996), or cyclooxygenase inhibitor, indomethacin (Reid *et al*, 2002), co-administered during the development phase of sensitization, has been shown to block the behavioral expression of amphetamine and cocaine. A recent study by Kita *et al* (2000) revealed that treatment with METH induced cyclooxygenase-2 protein expression in the mice striatum; and in turn, ketoprofen prevented against METH-induced striatal neurotoxicity (Asanuma *et al*, 2003). Furthermore, more recent study indicated a decreased striatal activity of Ca-stimulated phospholipase A2 in autopsied brain of chronic cocaine and METH users (Ross *et al*, 2002). The latter finding is in line with an earlier result showing that pretreatment with citicoline, which increases the rate of membrane phospholipids synthesis, for two weeks attenuated some measures of craving in cocaine-dependent subjects (Renshaw *et al*, 1999). Together with the data showing rich distribution of cannabinoid CB1 (Bidaut-Russel *et al*, 1990) and prostanoid EP3 (Nakamura *et al*, 2000) receptors in forebrain loci, it is likely that following repeated METH taking, the striatal arachidonic acid cascade is hyposensitized. This is followed by a rebound hypersensitization of the cascade during the expression of seeking behavior. Such changes in the arachidonic acid cascade may be a down stream consequence of the adaptation of the endocannabinoid system. While this finding gives further support to the important role of the cascade in reinforcing properties of psychostimulants, there have been no data on the effects of diclofenac on operant behavioral performance. To determine the specificity of effect of diclofenac on METH-priming and cue-induced reinstatement of METH-seeking behavior, we assessed the effect of the cyclooxygenase inhibitor on the self-administration of food pellets. The lack of effect of diclofenac on the response rate of food self-administration, as result showed, rules out the non-specificity of its effect. Taken together, these results suggest that the arachidonic acid cascade mediates the role of the endocannabinoid system in the reinstatement of METH-seeking behavior.

In summary, with the results presented here, we demonstrated several new insights into the mechanisms behind the reinforcing effects of METH priming and cues as viewed from the standpoint of the endocannabinoid-arachidonic acid pathway. Further extending the current view on drug addiction treatment, these results indicate that, besides the potential use of cannabinoid antagonist for the maintenance of drug abstinence, substances which induce the activation of the endocannabinoid tone may be useful for the prevention of relapse to drug use as well as treating drug intoxication in addicts. Yet, the data suggesting reinstatement-blocking effect of THC, if it is considered for clinical application, should be judged in light of some limitations. This is because, like other habit-forming drugs, THC may also produce dependence (Jones *et al*, 1981; Haney *et al*, 1999a, b). Furthermore, cannabis consumption has been associated with the risk for psychotic syndromes (Andreasson *et al*, 1987; Thomas, 1996). It may be

necessary, therefore, to develop endocannabinoid-activating substances devoid of such characteristics of THC. Probably, the most applicable in the near future is the use of cyclooxygenase inhibitors as anti-relapse agents.

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