

## REGULAR RESEARCH ARTICLE

# Inhibition of Monoacylglycerol Lipase Reduces the Reinstatement of Methamphetamine-Seeking and Anxiety-Like Behaviors in Methamphetamine Self-Administered Rats

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## Abstract

**Background:** Methamphetamine is a highly addictive psychostimulant with reinforcing properties. Our laboratory previously found that  $\Delta^8$ -tetrahydrocannabinol, an exogenous cannabinoid, suppressed the reinstatement of methamphetamine-seeking behavior. The purpose of this study was to determine whether the elevation of endocannabinoids modulates the reinstatement of methamphetamine-seeking behavior and emotional changes in methamphetamine self-administered rats.

**Methods:** Rats were tested for the reinstatement of methamphetamine-seeking behavior following methamphetamine self-administration and extinction. The elevated plus-maze test was performed in methamphetamine self-administered rats during withdrawal. We investigated the effects of JZL184 and URB597, 2 inhibitors of endocannabinoid hydrolysis, on the reinstatement of methamphetamine-seeking and anxiety-like behaviors.

**Results:** JZL184 (32 and 40 mg/kg, i.p.), an inhibitor of monoacylglycerol lipase, significantly attenuated both the cue- and stress-induced reinstatement of methamphetamine-seeking behavior. Furthermore, URB597 (3.2 and 10 mg/kg, i.p.), an inhibitor of fatty acid amide hydrolase, attenuated only cue-induced reinstatement. AM251, a cannabinoid CB<sub>1</sub> receptor antagonist, antagonized the attenuation of cue-induced reinstatement by JZL184 but not URB597. Neither JZL184 nor URB597 reinstated methamphetamine-seeking behavior when administered alone. In the elevated plus-maze test, rats that were in withdrawal from methamphetamine self-administration spent less time in the open arms. JZL184 ameliorated the decrease in time spent in the open arms.

**Conclusion:** We showed that JZL184 reduced both the cue- and stress-induced reinstatement of methamphetamine-seeking and anxiety-like behaviors in rats that had self-administered methamphetamine. It was suggested that a decrease in 2-arachidonoylglycerol in the brain could drive the reinstatement of methamphetamine-seeking and anxiety-like behaviors.

**Keywords:** methamphetamine, drug-seeking behavior, anxiety-like behavior, endocannabinoid, MAGL inhibitor

## Introduction

Methamphetamine (METH) is a highly addictive psychostimulant that has reinforcing properties (Radfar and Rawson, 2014). Drug-seeking behavior in drug addicts is elicited by different

kinds of stimuli, including stress and drug-associated cues (Sinha et al., 2003). Drug addicts also show persistent emotional dysfunctions such as anxiety and/or depression (Fox et al.,

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## Significance Statement

Methamphetamine (METH) is a highly addictive psychostimulant. METH addicts also show persistent emotional dysfunctions such as anxiety and/or depression, and increased anxiety is demonstrated to increase craving risk and worsen treatment outcomes among METH addicts. Our laboratory has found that  $\Delta^8$ -tetrahydrocannabinol, an exogenous cannabinoid, suppresses the reinstatement of METH-seeking behavior. The purpose of this study was to determine whether the activation of endocannabinoids by an endocannabinoid hydrolysis inhibitor modulates the reinstatement of METH-seeking and anxiety-like behaviors after METH self-administration in rats. JZL184, an inhibitor of monoacylglycerol lipase, reduced the cue- and stress-induced reinstatement of METH-seeking and anxiety-like behaviors in METH self-administered rats. It was suggested that a decrease in the endocannabinoid 2-AG in the brain could drive the reinstatement of METH-seeking and anxiety-like behaviors.

2008; Glasner-Edwards and Mooney, 2014), and this high anxiety response is accompanied by significantly higher levels of both stress- and cue-induced cravings (Fox and Sinha, 2014).

Cannabis is the most commonly abused illegal drug in the world, and its main psychoactive ingredient,  $\Delta^9$ -tetrahydrocannabinol ( $\Delta^9$ -THC), produces rewarding effects in humans and experimental animals (Justinova et al., 2005). Moreover, experimental findings have suggested a major involvement of the endocannabinoid system in reward functions (Yamamoto and Takada, 2000; Yamamoto et al., 2004; Justinova Z et al., 2009; Parsons and Hurd, 2015). Our laboratory has also reported that  $\Delta^9$ -THC suppresses the reinstatement of METH-seeking behavior when given repeatedly during withdrawal or once at 24 hours before a reinstatement test (Anggadiredja et al., 2004). Thus, it is suggested that the endocannabinoid system plays a critical role in METH abuse.

N-arachidonoyl ethanolamine (anandamide; Devane et al., 1992) and 2-arachidonoylglycerol (2-AG; Mechoulam et al., 1995; Sugiura et al., 1995) are lipid transmitters that serve as endogenous ligands of cannabinoid receptors. In the nervous system, anandamide and 2-AG are degraded primarily by the serine hydrolase enzyme fatty acid amide hydrolase (FAAH) and monoacylglycerol lipase (MAGL), respectively (Blankman and Cravatt, 2013). URB597, a selective FAAH inhibitor, significantly increases the brain levels of anandamide but does not alter the levels of 2-AG (Gobbi et al., 2005; Wiskerke et al., 2012). In contrast, JZL184, a selective MAGL inhibitor, increases the brain levels of 2-AG but does not alter the levels of anandamide (Long et al., 2009a; Wiskerke et al., 2012). These inhibitors were used in a recent study that reported the differential role of anandamide and 2-AG in memory and anxiety-like responses (Bansquets-Garcia et al., 2011).

The purpose of this study was to determine whether JZL184 and URB597 can modulate the reinstatement of METH-seeking behavior using drug self-administration in rats. We also investigated the effect of JZL184 on anxiety-like behavior in METH withdrawal.

## MATERIALS AND METHODS

### Animals

Seventy-two adult male Wistar/ST rats (10 weeks old, Nippon SLC, Hamamatsu), weighing 250 to 300 g, including 19 rats for the elevated plus-maze test, were used. Animals were housed in a temperature- and humidity-controlled environment under a 12-h-light/-dark cycle (lights on at 7:00 AM). The procedures used 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 the Faculty of Pharmaceutical Science using the Nagasaki International University Publication, which was enacted in 2009.

### Drugs

Methamphetamine HCl (METH; Dainippon Sumitomo Pharma, Osaka) was dissolved in saline. JZL184 (Cayman Chemicals, Ann Arbor, MI), a selective inhibitor of MAGL, was dissolved in a mixture of ethanol, Cremophor EL, and saline (1:1:8). URB597 (Cayman Chemicals), a selective FAAH inhibitor, and AM251 (Cayman Chemicals), a cannabinoid CB<sub>1</sub> receptor antagonist, were dissolved in a mixture of DMSO, Tween 80, and saline (1:1:18). Drugs were injected i.p. at a volume of 0.1 mL/100 g of body weight.

### Drug Self-Administration

The METH self-administration method was carried out as described previously (Anggadiredja et al., 2004; Hiranita et al., 2006; Nawata et al., 2012, 2016).

### Lever Response Training

Two days prior to the start of food training, rats received a restricted diet to achieve weight loss to approximately 90% of their normal weight and were trained to press levers for 45-mg food pellets (Bio-Serv, Frenchtown, NJ). The training occurred on a fixed ratio 1 schedule during which no stimuli were provided. Lever-pressing training ceased when the rats could obtain 30 pellets within 250 seconds for 3 consecutive sessions.

### Surgery

Following completion of the food training, the rats were anesthetized with isoflurane (Mylan Pharmaceuticals, Osaka, Japan) prior to surgical implantation of the indwelling i.v. catheters. Catheters were constructed from Silastic laboratory-grade tubing (0.5 mm i.d., 1.0 mm o.d., Kaneka Medix, Osaka, Japan). The catheter was implanted into the right jugular vein and secured in place with silk sutures around the silicon nodule. The Silastic tubing ran under the skin to an exit point in the mid-scapular region. Rats were i.v.-infused daily with 0.2 mL of heparinized saline (30 U/mL) during the experiment to prevent blockage of the catheter. Each rat was housed individually after surgery, and after 5 days for recovery, food was limited to 15 to 20 g/day/body.

### Apparatus

Self-administration chambers (30×20×24 cm; Neuroscience, Tokyo, Japan) were housed inside sound-attenuating cubicles fitted with a fan for airflow. Each chamber contained 2 fixed levers, 2 stimulus lights, a speaker for tone delivery, and a house light to provide general illumination. In addition, each chamber was equipped with a balanced metal arm and a spring leash attached to a swivel (Instech Laboratories, Plymouth Meeting, PA). The drug was delivered using a computer-controlled infusion pump (Med Associates, St. Albans, VT) located inside the sound-attenuating cubicle. The entire system was computer integrated using the MED PC 4 system (Med Associates).

### Intravenous METH Self-Administration

After at least 5 days of recovery, rats were allowed daily 2-hour sessions to self-administer METH using the fixed ratio 1 schedule on 10 consecutive days. The house light always signaled the beginning of a session and remained on throughout the session. During the sessions, a response on the active lever resulted in activation of the pump for a 6-second infusion (0.02 mg/0.1 mL/infusion) and presentation of a stimulus such as a drug-associated cue, consisting of a 6-second tone (85 dB, 2.9 kHz) and a white stimulus light over the active lever, followed by a 20-s timeout. Responses that occurred during the timeout and on the inactive lever were recorded but had no scheduled consequences.

### Reinstatement

After METH self-administration, rats were placed into extinction before testing carried out for 1-hour sessions for 5 days, and the responses on either lever had no scheduled consequences. After extinction criteria (<10 active lever responses on 2 consecutive days) were achieved, the reinstatement test was conducted under saline infusions. If necessary, additional days of extinction were included until the rats reached response criteria. Rats were tested for cue-induced or footshock-induced reinstatement. For cue-induced reinstatement, immediately after the session began, rats were reexposed to a drug-associated cue, and each press on the active lever resulted in the presentation of the cue in the same manner as during self-administration. For footshock-induced reinstatement, electrical footshocks (current intensity 0.8 mA; 1.0 seconds on) were delivered through a scrambler (Random Shocker version 7.1; Neuroscience, Tokyo) to the grid floor of the operant chamber. They were administered during a 15-minute period in an intermittent manner according to a variable interval schedule (mean interval: 40 seconds; range: 10–70 seconds).

JZL184 (32 or 40 mg/kg, i.p.) or URB597 (3.2 or 10 mg/kg, i.p.) were injected 2 hours or 1 hour prior to the test, respectively. For the antagonist experiments, AM251 was co-administered with JZL184 or URB597. The doses of JZL184 and URB597 were selected based on previous reports (Kinsey et al., 2009; Long et al., 2009a). The doses of AM251 were selected based on our previous reports (Nawata et al., 2016). We chose low doses of AM251 that clearly have no effect on reinstatement by itself. Forty-three rats were used for cue-induced reinstatement tests. Ten rats were used for footshock-induced reinstatement tests. The tests were conducted using the between-session reinstatement method previously reported (Hiranita et al., 2006; Nawata et al., 2012, 2016).

### Elevated Plus-Maze Test

The elevated plus-maze test was carried out as described previously (Nawata et al., 2012). The apparatus (Neuroscience, Tokyo) was constructed from black plastic and consisted of 2 open arms (50×10 cm) and 2 enclosed arms (50×10×50 cm) that extended from a central platform (10×10 cm). The maze was elevated 50 cm above the floor. Experiments began by placing a single rat on the central platform facing a closed arm. During the 10 minutes of free exploration, time spent on the open arms (defined as the animal placing its forepaws onto an arm) was recorded manually. In addition, the ambulation was measured with a digital tracking and computerized scoring system (LimeLight2, Actimetrics, Wilmette, IL). The maze was cleaned thoroughly with water between animals. This examination was carried out in 3 groups: control (measured 24 hours before the first day of METH self-administration), METH WD (measured on withdrawal day 10 after METH self-administration), and JZL184. JZL184

(40 mg/kg) was injected once on the test day. A different subject was used in each group.

### Data Analysis

For the self-administration, the number of lever responses was analyzed. For the elevated plus-maze test, the time spent on the open arms and the ambulation were analyzed. Data were analyzed by 1-way ANOVA, and the Bonferroni and Dunn post-hoc tests were used for follow-up analyses. Differences were considered significant at  $P < .05$ . All statistical analyses were performed using the Stat View software program (v. 5.0; SAS Institute, Cary, NC).

## RESULTS

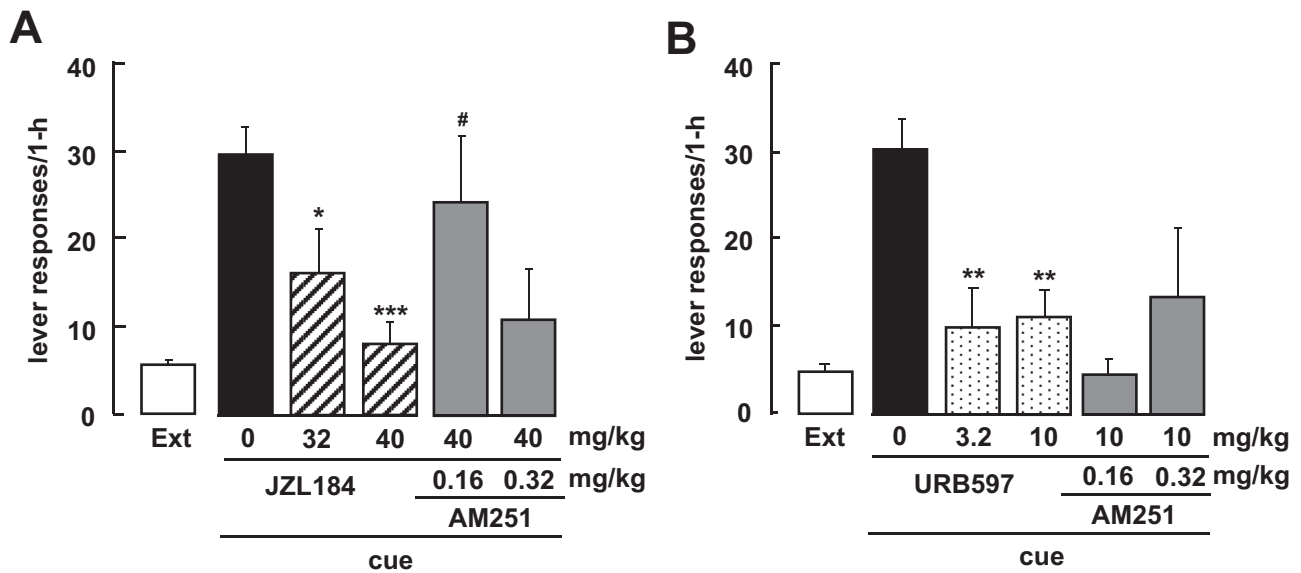
### Effects of JZL184 and URB597 on the Reinstatement of METH-Seeking Behavior

The number of active lever responses and METH infusions in the last training session (day 10) were  $30.6 \pm 2.1$  and  $24.2 \pm 1.6$ , respectively. When the drug was replaced with saline, the active lever responses gradually decreased from  $18.1 \pm 2.1$  on the first day, reaching  $5.2 \pm 0.6$  lever responses on the final extinction day.

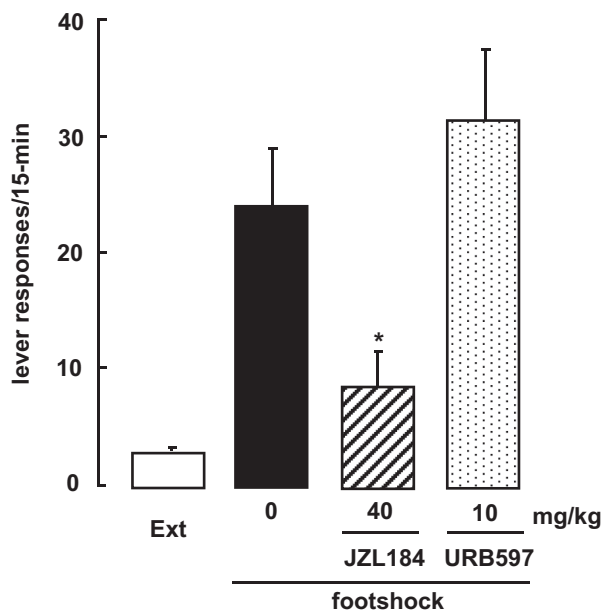
Under extinction conditions, reexposure to METH-associated cues significantly reinstated lever-pressing behaviors, which were considered so-called METH-seeking behaviors, compared with the final extinction (Ext) ( $5.3 \pm 0.5$  to  $29.3 \pm 3.1$ ;  $F [1, 26] = 99.0$ ,  $P < .0001$ ; Figure 1A). JZL184 (32 and 40 mg/kg, i.p.), a MAGL inhibitor, significantly attenuated cue-induced METH-seeking behavior in a dose-dependent manner ( $29.3 \pm 3.1$  to  $15.9 \pm 4.8$ ;  $F_{1,13} = 5.8$ ,  $P < .05$  and  $29.3 \pm 3.1$  to  $7.8 \pm 2.5$ ;  $F_{1,11} = 23.7$ ,  $P < .001$ ; Figure 1A). AM251 (0.16 mg/kg, i.p.), a CB<sub>1</sub> receptor antagonist, antagonized the attenuating effect of JZL184 (40 mg/kg, i.p.) on cue-induced reinstatement ( $7.8 \pm 2.5$  to  $23.8 \pm 7.2$ ;  $F_{1,7} = 6.8$ ,  $P < .05$ ; Figure 1A). We attempted to test higher doses of AM251, and lever-pressing behaviors in approximately one-half of the JZL184-treated rats were suppressed by AM251 (0.32 mg/kg), whereas AM251 (0.32 mg/kg) completely reversed the effects of JZL184 in the remaining rats. Moreover, almost all of the JZL184-treated rats with AM251 (3.2 mg/kg, i.p.) showed similar behavioral suppression. This behavioral suppression hampered the experiments with the higher doses of AM251. Both MAGL/FAAH inhibitors and AM251 alone did not induce behavioral suppression at the doses used in this study. Future studies should examine why the drug combination induced such behavioral suppression.

Figure 1B shows the effect of URB597, a FAAH inhibitor, on reinstatement. URB597 (3.2 and 10 mg/kg, i.p.) significantly suppressed METH-seeking behavior that was induced by reexposure to METH-associated cues ( $30.0 \pm 3.5$  to  $9.8 \pm 4.5$ ;  $F_{1,10} = 13.1$ ,  $P < .01$  and  $30.0 \pm 3.5$  to  $11.0 \pm 3.1$ ;  $F_{1,10} = 15.1$ ,  $P < .01$ ; Figure 1B). This attenuating effect of URB597 (10 mg/kg, i.p.) was not antagonized by either dose of AM251 (Figure 1B).

Figure 2 shows the effect of JZL184 or URB597 on the footshock-induced reinstatement of METH-seeking behavior. Under extinction conditions, the footshock significantly increased the active lever responses compared with Ext ( $2.8 \pm 0.6$  to  $24.0 \pm 5.0$ ;  $F_{1,13} = 37.8$ ,  $P < .0001$ ; Figure 2). JZL184 (40 mg/kg, i.p.) significantly attenuated the reinstatement of the footshock-induced METH-seeking behavior ( $24.0 \pm 5.0$  to  $8.8 \pm 3.0$ ,  $F_{1,8} = 6.9$ ,  $P < .05$ ; Figure 2). However, URB597 (10 mg/kg, i.p.) did not attenuate reinstatement (Figure 2). Neither JZL184 (40 mg/kg, i.p.) nor URB597 (10 mg/kg, i.p.) increased lever responses when administered alone (Figure 3).



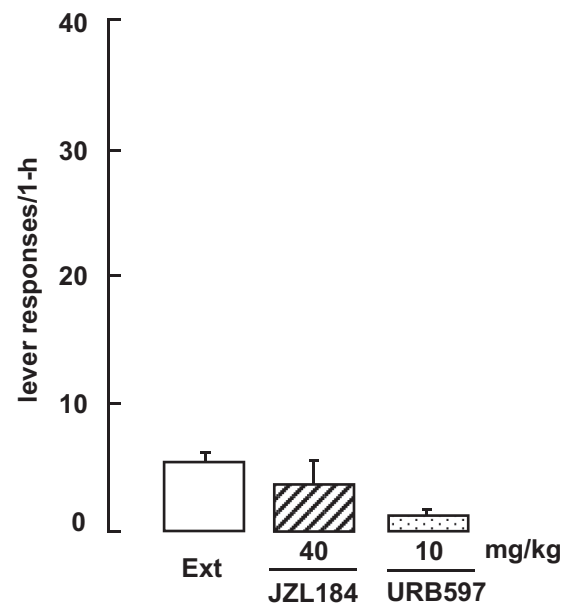
**Figure 1.** Effect of JZL184 and URB597 on the reinstatement of methamphetamine (METH)-seeking behavior by reexposure to cues. Rats were pretreated with a monoacylglycerol lipase (MAGL) inhibitor, JZL184 (32 or 40 mg/kg, i.p.,  $n=7$  and  $n=5$ , respectively) (A) or a fatty acid amide hydrolase (FAAH) inhibitor, URB597 (3.2 or 10 mg/kg, i.p.,  $n=5$  per group) (B). JZL184 and URB597 were injected 2 hours or 1 hour prior to the test, respectively. AM251 (0.16 or 0.32 mg/kg, i.p.) was coadministered with JZL184 or URB597 ( $n=4-6$ ). \* $P < .05$ , \*\* $P < .01$ , and \*\*\* $P < .001$  for cue- vs cue-JZL184/URB597-treated rats. # $P < .05$  for the cue-JZL184 vs cue-JZL184-AM251-treated rats. Each bar represents the mean ( $\pm$ SEM) number of presses on the previously active lever during the 1-hour reinstatement test.



**Figure 2.** Effects of JZL184 and URB597 on footshock-induced methamphetamine (METH)-seeking behavior. Rats were pretreated with JZL184 (40 mg/kg, i.p.) or URB597 (10 mg/kg, i.p.) 2 or 1 hour prior to the start of the session, respectively. \* $P < .05$  for footshock vs footshock-JZL184 (40 mg/kg, i.p.)-treated rats. Each bar represents the mean ( $\pm$ SEM) number of presses on the previously active lever during the 15-minute exposure to footshock (or Ext).  $n=5$  rats per group.

#### Effect of JZL184 on Anxiety-Like Behavior in METH Self-Administered Rats Upon Withdrawal

In the elevated plus-maze test, control rats spent  $167.8 \pm 33.7$  seconds on the open arms during the 10-minute test session (Figure 4A). However, on day 10 of withdrawal, METH self-administered rats spent less time on the open arms ( $167.8 \pm 33.7$  seconds to  $68.9 \pm 9.8$  seconds,  $F_{1,17} = 5.9$ ,  $P < .05$ ; Figure 4A). JZL184 (40 mg/kg, i.p.) ameliorated the decrease

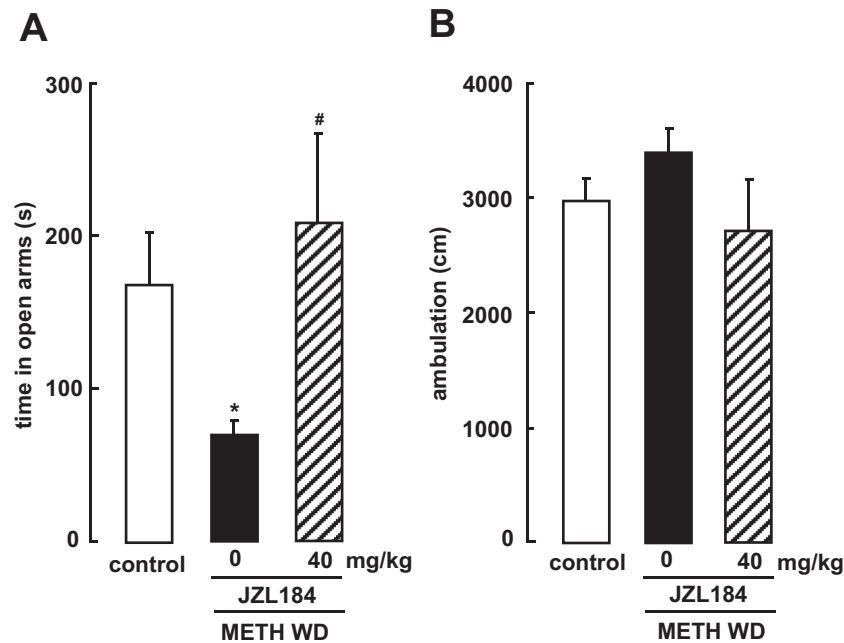


**Figure 3.** Effects of JZL184 and URB597 on lever pressing behavior in methamphetamine (METH) withdrawal rats. Rats were pretreated with JZL184 (40 mg/kg, i.p.) or URB597 (10 mg/kg, i.p.) 2 or 1 hour before the start of the session, respectively. Each bar represents the mean ( $\pm$ SEM) number of presses on the previously active lever during the 1-hour session.  $n=5-6$  rats per group.

in the time spent on the open arms ( $68.9 \pm 9.8$  seconds to  $207.4 \pm 58.7$  seconds,  $F_{1,14} = 5.4$ ,  $P < .05$ ; Figure 4A). There was no significant difference in ambulation of the maze among the 3 groups (Figure 4B).

#### Discussion

The major finding of this study was that the MAGL inhibitor JZL184 suppressed the reinstatement of METH-seeking



**Figure 4.** Effect of JZL184 on anxiety-like behavior in methamphetamine (METH) self-administered rats upon withdrawal. Anxiety-like behavior upon withdrawal after METH self-administration in the elevated plus-maze test. Each bar represents the mean ( $\pm$ SEM) total time spent on the open arms (A) and ambulation in the 10-minute test session (B) ( $n=8-11$ ). \* $P < .05$  for the control vs METH WD rats. # $P < .05$  for the METH WD vs JZL184 rats.

behavior in METH self-administered rats. The CB<sub>1</sub> receptor antagonist AM251 antagonized the suppressing effects of JZL184. These results indicated that JZL184 reduces cue-induced reinstatement through a CB<sub>1</sub> receptor-dependent mechanism. This study is the first to our knowledge to show that the MAGL inhibitor produced an anti-craving profile in experimental animals. JZL184 increased 2-AG levels in the prefrontal cortex, nucleus accumbens and amygdala (Wiebelhaus et al., 2015), which are the brain areas involved in the reinstatement of METH-seeking behavior (Hiranita et al., 2006). A recent study reported that both the 2-AG and anandamide levels in the limbic and subcortical areas decreased during extinction after cocaine self-administration in rats (Bystrowska et al., 2014). Taken together, these reports and our results suggest that a decrease in the level of endocannabinoids in the brain is a crucial factor for reinstating drug-seeking behaviors. The pharmacological mechanism underlying these effects of JZL184 is controversial. A previous study reported that the antinociceptive effects and hypomotility induced by JZL184 were mediated through CB<sub>1</sub> receptors in mice (Long et al., 2009b), while another study reported CB<sub>1</sub>-independent effects in rats (Seillier et al., 2014). This contradiction remains unclear, although there are differences in species and/or experimental conditions in these reports. Thus, further studies are required to clarify the pharmacological mechanism of JZL184. In the present study, we provide new evidence that JZL184 attenuated the reinstatement of METH-seeking behaviors mediated through CB<sub>1</sub> receptors.

It is now well established that endocannabinoids serve as retrograde messengers at various synapses in the central nervous system and contribute to different forms of short- and long-term synaptic plasticity (Maejima et al., 2001; Kano, 2014). Following synthesis and release from postsynaptic neurons, endocannabinoids activate presynaptic CB<sub>1</sub> receptors to suppress the release of both excitatory (e.g., glutamate) and inhibitory (e.g., GABA) neurotransmitters (Sidhpura and Parsons, 2011). Several lines of evidence have strongly

suggested that 2-AG, rather than anandamide, mediates the endocannabinoid-induced transient suppression of neurotransmitter release (Kano, 2014). It has also been reported that JZL184 but not the FAAH inhibitor PF3845 decreases neurotransmitter release in the hippocampus (Lee et al., 2015). Thus, the attenuating effect of JZL184 in the reinstatement procedure may result from its neuromodulatory effect via the stimulation of CB<sub>1</sub> receptors.

It is well documented that CB<sub>1</sub> receptor antagonists suppress drug-seeking behaviors (Panagis et al., 2014). There seems to be a discrepancy between our results and previous results using CB<sub>1</sub> receptor antagonists. For a neurochemical mechanism underlying the reinstatement of drug-seeking behavior, glutamatergic transmission has been shown to be required in rodent models of relapse (Torregrossa and Kalivas, 2008). Interestingly, AM251 prevents glutamate release induced by cocaine priming in the nucleus accumbens, while AM251 alone increases glutamate release (Xi et al., 2006). This inhibitory effect on glutamate release by AM251 under cocaine priming has been considered a mechanism for the prevention of reinstatement. Thus, AM251 appears to have both inhibitory and excitatory effects on glutamate release in cocaine self-administered rats. This biphasic action of AM251 may explain the contradiction that both the indirect cannabinoid receptor agonist JZL184 and a CB<sub>1</sub> receptor antagonist suppress the reinstatement of drug-seeking behaviors.

A previous study reported that JZL184 enhanced the cue-induced reinstatement of nicotine-seeking behavior (Trigo and Le Foll, 2016). JZL184 might show contradictory effects between METH- and nicotine-seeking behaviors. The direct cannabinoid receptor agonist, THC, is known to produce biphasic pharmacological effects on, for example, food intake or body temperature, depending on the dose (Taylor and Fennessy, 1977; Wiley et al., 2005). Trigo and Le Foll used a lower dose (16 mg/kg) of JZL184 than the one used in the present study. Thus, an indirect cannabinoid receptor agonist, JZL184, may have different effects on reinstatement depending on the dose. Furthermore,

the mechanisms underlying the reinstatement of METH-seeking behaviors might not necessarily correspond to those of nicotine-seeking behaviors based on previous neurobiological findings (see review; Bossert et al 2013). Therefore, as another possibility, the difference in training drugs may result in the contradictory effects of JZL184 between METH- and nicotine-seeking behaviors.

Few studies have described the role of the endocannabinoid system in stress-induced reinstatement. The critical role of the endocannabinoid system in stress responses has been reported as follows. The activation of the endocannabinoid system by WIN55,212-2, a cannabinoid receptor agonist, reduced stress-induced elevations in plasma corticosterone levels (Ganon-Elazar and Akirav, 2009). 2-AG was increased by stress, and this increased 2-AG signaling contributed to the termination of the stress response (Morena et al., 2016). It has also been reported that both JZL184 and URB597 reduced stress-related behaviors such as the anxiety- or depressive-like behaviors induced by chronic unpredictable stress (Zhong et al., 2014; Lomazzo et al., 2015). In addition, we previously reported that a corticotropin-releasing factor (CRF) receptor antagonist attenuated the reinstatement of footshock-induced METH-seeking behavior (Nawata et al., 2012). Moreover, the activation of endocannabinoids by a MAGL inhibitor reduced the CRF-induced stress response, including the activation of central sympatho-adrenomedullary outflow (Shimizu et al., 2010). Although it remains to be elucidated whether JZL184 directly inhibits CRF release, the suppression of footshock-induced METH-seeking behavior might be derived from a decrease in activation of the CRF system through 2-AG signaling elicited by JZL184.

Our results showed that URB597 attenuates cue-induced reinstatement but not footshock-induced reinstatement. It has been suggested that cue-induced drug-seeking behavior declines when drug memory reconsolidation is disrupted (Lee et al., 2006). Additionally, the endocannabinoid system is widely known to regulate memory (Puighermanal et al., 2012). In fact, our data show that URB597 but not JZL184 impaired cognitive function in a novel object recognition task (data not shown). Thus, there is a possibility that the attenuation of cue-induced reinstatement by URB597 in this study was due to cognitive dysfunction by increased levels of anandamide. Additionally, this attenuating effect was not antagonized by AM251. Therefore, the attenuation of cue-induced reinstatement by URB597 might be mediated through non-CB<sub>1</sub> receptors, at least using the present experimental conditions.

In the elevated plus-maze test, the duration of time spent in the open arms significantly decreased in the METH self-administered rats upon withdrawal, which indicated that anxiety-like behavior increased upon METH withdrawal. In addition, JZL184 had a significant anxiolytic-like effect on rats upon METH withdrawal. A recent study reported that mice with elevated MAGL levels at the glutamatergic terminal in the hippocampus exhibited a significant reduction in 2-AG and an increase in anxiety-like behavior (Guggenhuber et al., 2015). These mice also showed impaired depolarization-induced suppression of excitation, while depolarization-induced suppression of inhibition was not significantly changed (Guggenhuber et al., 2015). Thus, a possible mechanism underlying the anxiolytic-like effects of JZL184 on METH withdrawal may be the inhibition or normalization of glutamate release in the hippocampus.

Psychostimulant abusers have generally shown persistently high anxiety responses (Fox et al., 2008; Glasner-Edwards and

Mooney, 2014). A clinical study also showed that both stress- and cue-induced cocaine cravings were significantly associated with increased anxiety, anger, fear, and sadness in the cocaine addicts (Fox et al., 2008). In addition, increased anxiety has been demonstrated to increase craving risk and worsen treatment outcomes among METH addicts (Glasner-Edwards and Mooney, 2014). Judging from these reports and our results, the anxiolytic-like effects of JZL184 might contribute to the decrease in the reinstatement of METH-seeking behavior in our study. Thus, JZL184 could have strong therapeutic potential for METH addiction.

In summary, we showed that JZL184 reduced both the cue- and stress-induced reinstatement of METH-seeking and anxiety-like behaviors in METH self-administered rats. In particular, the stimulation of CB<sub>1</sub> receptors may contribute to the attenuation of the cue-induced reinstatement of METH-seeking behaviors. It has been suggested that a decrease in 2-AG in the brain could drive the reinstatement of METH-seeking and anxiety-like behaviors.

## Acknowledgments

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## Statement of Interest

None.

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