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ARTICLE

Cannabinoid CB_1 receptor neutral antagonist AM4113 inhibits heroin self-administration without depressive side effects in rats

Xiang-hu He[1,2], Chloe J. Jordan[1], Kiran Vemuri[3,4], Guo-hua Bi[1], Jia Zhan[1,2], Eliot L. Gardner[1], Alexandros Makriyannis[3,4], Yan-lin Wang[2] and Zheng-xiong Xi[1]

Cannabinoid CB_1 receptors (CB_1Rs) have been shown to be a promising target in medication development for the treatment of addiction. However, clinical trials with SR141716A (rimonabant, a selective CB_1R antagonist/inverse agonist) for the treatment of obesity and smoking cessation failed due to unwanted side effects, such as depression, anxiety, and suicidal tendencies. Recent preclinical studies suggest that the neutral CB_1R antagonist AM4113 may retain the therapeutic anti-addictive effects of SR141716A in nicotine self-administration models and possibly has fewer unwanted side effects. However, little is known about whether AM4113 is also effective for other drugs of abuse, such as opioids and psychostimulants, and whether it produces depressive side effects similar to SR141716A in experimental animals. In this study, we demonstrated that systemic administration of AM4113 (3 and 10 mg/kg) dose-dependently inhibited the self-administration of intravenous heroin but not cocaine or methamphetamine, whereas SR141716A (3 and 10 mg/kg) dose-dependently inhibited the self-administration of heroin and methamphetamine but not cocaine. In the electrical brain-stimulation reward (BSR) paradigm, SR141716A (3 and 10 mg/kg) dose-dependently increased the BSR stimulation threshold (i.e., decreased the stimulation reward), but AM4113 had no effect on BSR at the same doses, suggesting that SR141716A may produce aversive effects while AM4113 may not. Together, these findings show that neutral CB_1R antagonists such as AM4113 deserve further research as a new class of CB_1R-based medications for the treatment of opioid addiction without SR141716A-like aversive effects.

Keywords: CB_1 receptors; AM4113; SR141716A; CB_1 neutral antagonist; CB_1 inverse agonist; drug reward; aversion; depression; self-administration; brain-stimulation reward

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INTRODUCTION

Drug addiction is characterized by persistent drug-taking and drug-seeking behaviors, despite serious negative physiological, medical, or social consequences [1, 2]. Although great progress has been made in understanding the neural mechanisms underlying drug addiction, treatments remain limited in their ability to successfully reduce addiction-related behaviors [2, 3]. Accumulating evidence suggests that the cannabinoid system plays a vital modulatory role in diverse functions that may contribute to drug abuse [4].

Two types of cannabinoid receptor subtypes, cannabinoid CB_1 receptor (CB_1R) and cannabinoid CB_2 receptor, have been cloned and identified in the brain [5–8]. Since the CB_1Rs are found in the mesocorticolimbic system [8, 9] and activation of CB_1Rs has been shown to excite midbrain dopamine neurons and increase dopamine release in the nucleus accumbens [10–13], it has been proposed that CB_1R antagonists may have therapeutic effects in the treatment of drug abuse and addiction [14, 15]. In support of this hypothesis, CB_1R antagonists have been shown to attenuate addiction-related behaviors for several classes of abused drugs [16], such as cocaine [17], heroin [18–21], methamphetamine [22], and nicotine [23], as assessed in intravenous drug self-administration [19, 22, 24], conditioned place preferences [25], and reinstatement of drug-seeking behavior [17, 22, 26, 27] models.

SR141716A (rimonabant) was the first selective CB_1R antagonist to be developed, and it has been shown to be promising in the treatment of obesity, smoking cessation, and other drug addictions [28–32]. However, clinical studies suggest that SR141716A has significant adverse effects such as nausea and emesis and, more seriously, depression and suicidal tendencies [33, 34]. As a consequence, SR141716A has been withdrawn from clinical trials worldwide. As other CB_1R antagonists/inverse agonists, such as AM251 and taranabant, have similar adverse side effects [35, 36], almost all CB_1R inverse agonist-related research projects in major pharmaceutical companies worldwide have been terminated [14, 15].

The reasons underlying the adverse psychiatric effects observed with SR141716A treatment are unclear, but they may be related to its inverse agonist profile [14, 15]. Therefore, it was proposed that

[1]Intramural Research Program, National Institute on Drug Abuse, Baltimore, MD 21224, USA; [2]Department of Anesthesiology, Zhongnan Hospital of Wuhan University, Wuhan 430071, China; [3]Center for Drug Discovery, Department of Pharmaceutical Sciences, Northeastern University, Boston, MA 02115, USA and [4]Department of Chemistry and Chemical Biology, Northeastern University, Boston, MA 02115, USA
Correspondence: Zheng-xiong Xi (zxi@mail.nih.gov) or Yan-lin Wang (wyl0342@sina.com)

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neutral CB_1R antagonists without inverse agonist profiles should retain the therapeutic anti-addictive effects without the unwanted psychiatric effects. Accordingly, several neutral CB_1R antagonists have been developed, including AM4113, AM6257, NESS0327, LH-21, and PIMSR [37–41]. AM4113 is a pyrazole-3-carboxamide analog of SR141716A [37]. Unlike SR141716A and AM251, AM4113 does not alter forskolin-stimulated cAMP formation in vitro [35], suggesting a lack of an inverse agonist profile. In experimental animals, AM4113 does not produce significant side effects, such as nausea, malaise, or anxiety-like effects [35, 37, 42], suggesting an improved safety profile over inverse CB_1R agonists [43, 44]. Strikingly, recent studies in rodents and non-human primates indicated that AM4113 significantly inhibits nicotine and Δ^9-THC self-administration, ethanol consumption, and nicotine-induced, cue-induced, or yohimbine-induced reinstatement of nicotine-seeking behavior [45–47], suggesting the possible utility of AM4113 in the treatment of nicotine and cannabis addiction as well as alcoholism.

Although evidence supporting the efficacy of AM4113 for addiction is accumulating, little is known regarding whether the therapeutic effects produced by AM4113 can be generalized to other drugs of abuse, such as opioids or illicit psychostimulants such as cocaine and methamphetamine. Given the currently global epidemic in opioid abuse and overdose deaths [48] and the lack of approved medications for the treatment of psychostimulant addiction [49], the development of novel pharmacotherapies for opioid and psychostimulant abuse is urgently and desperately needed. In addition, although some evidence has suggested that AM4113 may have fewer unwanted effects than SR141716A [46, 50], there is a paucity of key evidence indicating whether AM4113 produces similar aversive or depressive effects as SR141716A, which caused clinical trial termination. Therefore, in the present study, we first explored the potential utility of AM4113 in the treatment of opioid or psychostimulant abuse and addiction by using the "gold standard" intravenous drug self-administration paradigm. We then used the electrical brain-stimulation reward (BSR), the most commonly used paradigm to evaluate drug reward vs. aversion [51], to examine, and compare the effects of AM4113 and SR141716A on brain-reward function.

MATERIALS AND METHODS

Animals

Male Long–Evans rats (Charles River Laboratories, Raleigh, NC) weighing 280–320 g were used for all experiments. All animals were housed individually in a climate-controlled room on a reversed light–dark cycle with free access to food and water. Experimental procedures were carried out in accordance with the Guide for the Care and Use of Laboratory Animals of the United States National Academy of Sciences and were approved by the Animal Care and Use Committee of the National Institute on Drug Abuse (NIDA).

Drugs and chemicals

Methamphetamine HCl, cocaine HCl, and heroin HCl were obtained from the NIDA and were dissolved in sterile 0.9% physiological saline for all treatments. AM4113 and SR141716A were obtained from the Center of Drug Discovery at Northeastern University, Boston, and were dissolved in 5% cremophor.

EXPERIMENT 1: INTRAVENOUS DRUG SELF-ADMINISTRATION

Intravenous catheter surgery

Under standard aseptic surgical techniques, all animals were prepared for experimentation by surgical catheterization of the right external jugular vein, as described previously [17, 52]. Briefly, animals were anesthetized by an intraperitoneal (i.p.) injection of sodium pentobarbital (65 mg/kg), and catheters constructed of microrenathane (Braintree Scientific Inc., Braintree, MA, USA) were inserted into the right jugular vein. After being sutured into place, the catheter was passed subcutaneously to the top of the skull and excited to a connector (a modified 24-g cannula; Plastics One, Roanoke, VA, USA), which was then mounted to the skull with jeweler's stainless-steel screws and dental acrylic. To prevent clogging, the catheters were flushed daily with a heparin–saline solution (30 IU/mL heparin; ICN Biochemicals, Cleveland, OH, USA).

Apparatus

Intravenous drug self-administration experiments were conducted in operant chambers ($32 \times 25 \times 33$ cm) from Med Associates Inc. (Georgia, VT, USA). Each chamber included two levers, one designated as active and one designated as inactive, which were located 6.5 cm above the floor. A cue light and a speaker were located 12 cm above the active lever. A house light was turned on during each 3-h test session. Depression of the active lever activated an infusion pump located outside the chamber; depression of the inactive lever was recorded but had no scheduled consequence. To promote acquisition and maintenance of drug self-administration behaviors, each drug infusion was paired with a conditioned cue light and sound (tone).

General self-administration procedure

After recovery from surgery, animals were placed into standard operant chambers for drug self-administration under a fixed-ratio 1 (FR1) reinforcement schedule. Animals were allowed to respond with the active lever to receive one of the following drugs: heroin (1 mg/kg/infusion), methamphetamine (0.05 mg/kg/infusion), or cocaine (1 mg/kg/infusion). All drugs were delivered in 0.08 mL over 4.6 s. During the 4.6-s infusion time, additional responses were recorded but did not lead to additional infusions. Inactive lever presses were counted but had no consequence. After stable FR1-reinforced responding was established, animals were then switched to a fixed-ratio 2 (FR2) reinforcement schedule to promote higher levels of drug-seeking behaviors. Self-administration training continued with heroin (0.5 mg/kg/infusion), methamphetamine (0.05 mg/kg/infusion), or cocaine (0.5 mg/kg/infusion). To avoid drug overdose during the self-administration period, each animal was limited to a maximum of 50 injections per 3-h session. The following criteria were used to determine when stable drug-maintained responding had been established: less than 10% variability in infusions earned and active lever presses and an active:inactive lever response ratio of at least 2:1 for a minimum of 3 consecutive days.

Effect of AM4113 or SR141716A pretreatment on drug self-administration

After stable heroin, methamphetamine, or cocaine self-administration under FR2 reinforcement was established as described above (requiring ~21–33 sessions), each rat randomly received one of two doses of AM4113 (3 and 10 mg/kg, i.p.), one of two doses of SR141716A (3 and 10 mg/kg), or vehicle (equal volume of 5% cremophor solution) 30 min prior to self-administration test sessions. After each drug test, animals were given an additional 5–7 days of self-administration until baseline responding was reestablished prior to testing the next dose of drug. The order of testing for the various doses of AM4113 or SR141716A was arranged according to a Latin square design.

EXPERIMENT 2: INTRACRANIAL ELECTRICAL BRAIN-STIMULATION REWARD

Surgery

Intracranial electrical BSR was performed as described previously [17]. Briefly, animals were anesthetized by an i.p. injection of sodium pentobarbital (65 mg/kg). Under standard aseptic surgical techniques, animals were stereotaxically implanted with unilateral monopolar stainless-steel stimulating electrodes (Plastics One, Roanoke, VA, USA) into the medial forebrain bundle at the

anterior–posterior level of the lateral hypothalamus (from Bregma, AP-2.56, ML ± 1.9, and DV –8.6; Paxinos and Watson 1998), and the electrodes were mounted to the skull with jeweler's screws and dental acrylic. A wire leading from the electrode was wrapped around a skull screw to serve as a current return.

Apparatus and general procedure

All training and testing were conducted in standard operant chambers (Med Associates, Inc., Georgia, VT, USA), which were enclosed in ventilated, sound-attenuating cabinets.

The general procedures for electrical BSR were identical to those described previously [17]. Briefly, after 7 days of recovery from surgery, rats were allowed to self-train (autoshape) to lever-press for rewarding BSR. Each press on the lever resulted in a 500-ms train with 0.1-ms rectangular cathodal pulses through the electrode implanted into the rat's medial forebrain bundle, followed by a 500-ms "timeout" in which further presses did not produce brain stimulation. Initial stimulation parameters were set at 72 Hz and 200 μA. If an animal did not learn to lever-press, the stimulation intensity was increased daily by 50 μA until the animal acquired responding (45–60 responses/30 s) or a maximum of 800 μA was reached. Animals that did not lever-press at 800 μA or that exhibited unwanted effects from the stimulation (e.g., head or body movements or vocalization) were removed from the experiment.

Rate–frequency BSR procedure

After the establishment of lever-pressing for BSR, animals were presented with a series of 16 different pulse frequencies, ranging from 141 to 25 Hz, in descending order. At each pulse frequency, animals were allowed to respond for two 30-s time periods (bins), after which the pulse frequency decreased by 0.05 log units. After each 30-s bin, the lever retracted for 5 s. Throughout the experiment, the animals underwent three sessions a day. The response rate for each frequency was defined as the mean number of lever responses during two 30-s bins. Because lever-pressing behavior tended to be variable during the first session (the "warm-up" session) but was stable during the second and third sessions, data from the first session were discarded, and the data from the second and third sessions were designated as the baseline-session data and test-session data, respectively. The BSR threshold (θ_0) was defined as the minimum frequency at which the animal responded for rewarding stimulation. The Y_{max} was defined as the maximal operant response (lever presses/30 s) for BSR. The BSR θ_0 and Y_{max} were mathematically derived for each "baseline" run and each "drug" test run by analyzing each rate–frequency BSR function generated by a given animal over a given descending series of pulse frequencies using "best-fit" mathematical algorithms [17].

Effects of AM4113 or SR141716A on BSR

After a stable θ_0 value or Y_{max} value was established (<10% variation over 5 continuous days), the effects of AM4113 or SR141716A on BSR were assessed. On each test day, the animals were randomly assigned to treatment groups. Either vehicle or a dose of AM4113 (3 and 10 mg/kg, i.p.) or SR141716A (3 and 10 mg/kg, i.p.) was administered. Following each test session, animals received an additional 5–7 days of BSR restabilization until

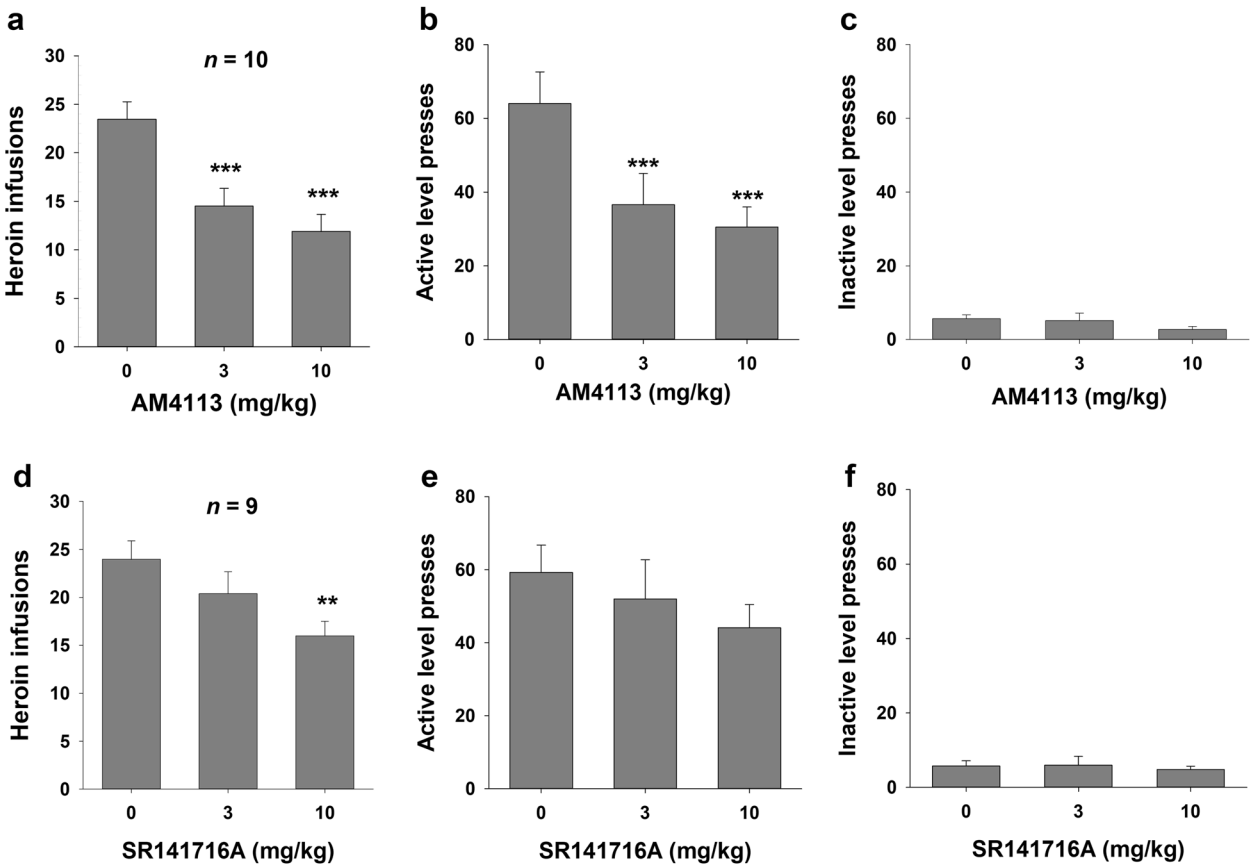

Fig. 1 Effects of AM4113 or SR141716A on heroin self-administration under a fixed-ratio 2 (FR2) reinforcement schedule. Administration of AM4113 (3 and 10 mg/kg, i.p.) significantly decreased the number of heroin infusions (**a**) and active lever presses (**b**), but had no effect on inactive lever presses (**c**). Administration of SR141716A (3 and 10 mg/kg, i.p.) dose-dependently decreased the number of heroin infusions (**d**), but did not alter active lever presses (**e**) or inactive lever presses (**f**). Data are presented as the mean ± SEM. $^{**}P < 0.01$ and $^{***}P < 0.001$ vs. vehicle (0 mg/kg, i.p.)

a new baseline θ_0 value or Y_{max} value was established. The order of testing for various doses of AM4113 or SR141716A was counterbalanced according to a Latin square design. The effect of AM4113 or SR141716A on BSR was evaluated by comparing alterations in θ_0 or Y_{max} values in the presence or absence of each dose of drug pretreatment.

Data analyses

All data are presented as the mean ± SEM. One-way repeated-measures analysis of variance (ANOVA) was used to analyze the effects of different doses of AM4113 or SR141716A on drug self-administration and BSR. Post hoc individual group comparisons were carried out using the Student–Newman–Keuls (SNK) test. The minimally acceptable statistical significance was set at a probability level of $P < 0.05$ for all tests.

RESULTS

AM4113 and SR141716A inhibit heroin self-administration

Figure 1 shows the effects of AM4113 (3 or 10 mg/kg, i.p.) or SR141716A (3 or 10 mg/kg, i.p.) on heroin self-administration under an FR2 reinforcement schedule. Pretreatment with AM4113 or SR141716A significantly and dose-dependently inhibited heroin self-administration. For AM4113, one-way repeated-measures ANOVA indicated statistically significant effects for heroin infusions and active lever presses ($F_{2,\ 18} = 20.64$, $P < 0.001$ and $F_{2,\ 18} = 19.60$, $P < 0.001$, respectively). Post hoc testing further revealed statistically significant reductions in infusions and active lever presses for heroin after 3 mg/kg ($q = 8.66$, $P < 0.001$ or $q = 6.80$, $P < 0.001$) and 10 mg/kg AM4113 ($q = 6.71$, $P < 0.001$ or $q = 8.31$, $P < 0.001$) compared with the vehicle treatment group.

For SR141716A, one-way repeated-measures ANOVA indicated statistically significant effects on heroin infusions ($F_{2,\ 16} = 7.43$, $P < 0.01$). Post hoc individual group comparisons revealed a statistically significant reduction in heroin infusions after 10 mg/kg AM4113 ($q = 5.44$, $P < 0.01$) but not after 3 mg/kg SR141716A ($q = 2.44$, $P =$ NS) compared with the vehicle treatment group. No statistically significant effects were observed in active lever presses following SR141716A ($F_{2,\ 16} = 2.61$, $P =$ NS). Moreover, neither AM4113 nor SR141716A affected inactive lever responding ($F_{2,\ 18} = 1.43$, $P =$ NS and $F_{2,\ 16} = 0.27$, $P =$ NS, respectively).

AM4113, but not SR141716A, inhibits methamphetamine self-administration

Figure 2 shows the effects of AM4113 (3 or 10 mg/kg, i.p.) and SR141716A (3 or 10 mg/kg, i.p.) on methamphetamine self-administration under an FR2 reinforcement schedule. Pretreatment with AM4113 (3 or 10 mg/kg, i.p.) had no significant effects on methamphetamine self-administration (infusions: $F_{2,\ 14} = 0.07$, $P =$ NS; active lever presses: $F_{2,\ 14} = 3.63$, $P =$ NS). In contrast, pretreatment with SR141716A dose-dependently decreased responding maintained by methamphetamine. One-way repeated-measures ANOVA indicated statistically significant effects of SR141716A on infusions and active lever presses for methamphetamine ($F_{2,\ 16} = 5.84$, $P < 0.01$ and $F_{2,\ 16} = 4.14$, $P < 0.05$, respectively). Post hoc individual group comparisons revealed statistically

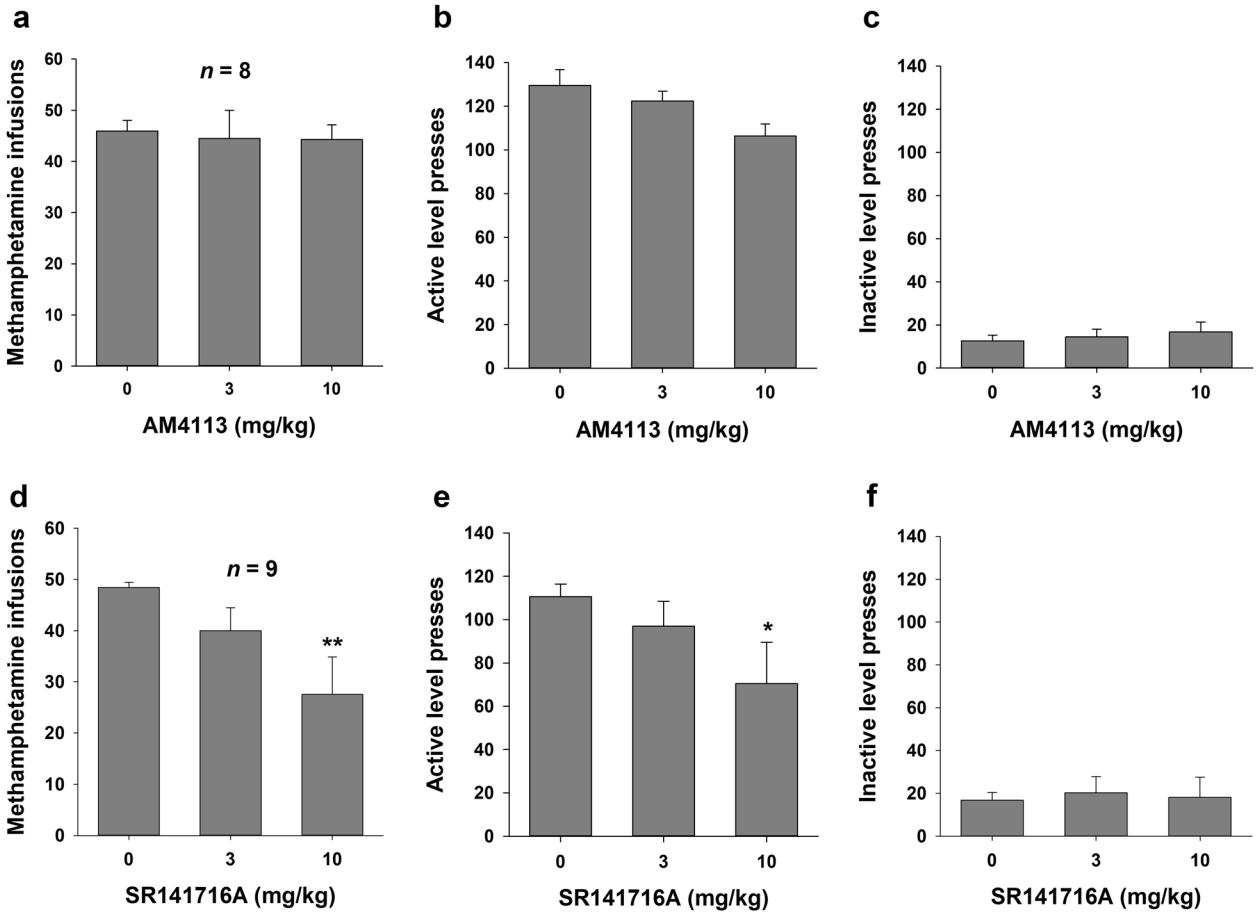

Fig. 2 Effects of AM4113 or SR141716A on methamphetamine self-administration under a FR2 reinforcement schedule. Administration of AM4113 did not alter the number of methamphetamine infusions (**a**), active lever presses (**b**), or inactive lever presses (**c**). Administration of SR141716A (10 mg/kg, i.p.) significantly decreased the number of methamphetamine infusions (**d**) and active lever presses (**e**), but had no effect on inactive lever presses (**f**). Data are presented as the mean ± SEM. $^{*}P < 0.05$ and $^{**}P < 0.01$ vs. vehicle (0 mg/kg, i.p.)

significant reductions in infusions and active lever presses for methamphetamine after 10 mg/kg AM4113 ($q = 4.80$, $P < 0.01$ and $q = 4.00$, $P < 0.05$) but not after 3 mg/kg SR141716A ($q = 1.94$, $P =$ NS and $q = 1.36$, $P =$ NS) compared with the vehicle treatment group. Neither AM4113 nor SR141716A pretreatment altered inactive lever presses ($F_{2,\ 14} = 0.73$, $P =$ NS and $F_{2,\ 16} = 0.13$, $P =$ NS, respectively).

AM4113 and SR141716A have no effect on cocaine self-administration

Figure 3 shows the effects of AM4113 (3 or 10 mg/kg, i.p.) or SR141716A (3 or 10 mg/kg, i.p.) on cocaine self-administration under a FR2 reinforcement schedule. One-way repeated-measures ANOVA indicated that neither AM4113 (3 or 10 mg/kg, i.p.) nor SR141716A (3 or 10 mg/kg, i.p.) pretreatment significantly altered cocaine self-administration ($F_{2,\ 14} = 3.22$, $P =$ NS and $F_{2,\ 14} = 2.41$, $P =$ NS, respectively). Similarly, neither AM4113 nor SR141716A had significant effects on active lever pressing ($F_{2,\ 14} = 3.55$, $P =$ NS and $F_{2,\ 14} = 2.14$, $P =$ NS, respectively) or inactive lever pressing ($F_{2,\ 14} = 1.13$, $P =$ NS or $F_{2,\ 14} = 1.89$, $P =$ NS, respectively) for cocaine reward.

SR141716A, but not AM4113, inhibits electrical BSR

Figure 4a and b shows representative stimulation–response rate curves from individual animals after each dose of AM4113 or SR141716A, indicating BSR thresholds (θ_0) and maximal operant response (Y_{max} level). AM4113 had no significant effect on these measures, while SR141716A significantly and dose-dependently shifted the stimulation–response rate curve to the right and increased the BSR stimulation threshold (θ_0). These results suggest that there is a reduction in BSR in the presence of SR141716A, such that higher stimulation intensity (or frequency in this study) is required to achieve a given level of reward. Figure 4c and d shows the mean effects of AM4113 and SR141716A on BSR. One-way repeated-measures ANOVA indicated that pretreatment with AM4113 (Fig. 4c, $F_{3,\ 14} = 0.30$, $P =$ NS) had no significant effect on θ_0 values or the Y_{max} level (Fig. 4e, $F_{3,\ 14} = 0.62$, $P =$ NS).

In contrast to AM4113, one-way repeated-measures ANOVA for SR141716A data indicated that pretreatment with SR141716A significantly decreased BSR, as assessed by increased θ_0 values (Fig. 4d, $F_{3,\ 16} = 5.84$, $P < 0.01$). Post hoc individual group comparisons using the SNK revealed statistically significant reductions in θ_0 values after 10 mg/kg SR141716A ($q = 4.80$, $P < 0.01$) but not after 3 mg/kg AM4113 ($q = 1.94$, $P =$ NS) compared with the vehicle treatment group. In addition, administration of SR141716A significantly altered Y_{max} levels (Fig. 4f, $F_{3,\ 16} = 5.84$, $P < 0.01$). Post hoc individual group comparisons using the SNK revealed a statistically significant reduction in the Y_{max} level after 10 mg/kg SR141716A ($q = 4.80$, $P < 0.01$) but not after 3 mg/kg AM4113 ($q = 1.94$, $P =$ NS) compared with the vehicle treatment group.

DISCUSSION

The present study is the first to demonstrate that the neutral CB_1R antagonist AM4113 (3 and 10 mg/kg) dose-dependently inhibits opioid self-administration in the drug self-administration paradigm. This finding is consistent with previous reports, indicating that AM4113 also reduced nicotine self-administration and reinstatement of nicotine-seeking behavior [46, 47]. However, at the same drug doses (3–10 mg/kg) that were effective in suppressing heroin self-administration, AM4113 altered neither methamphetamine nor cocaine self-administration. In contrast,

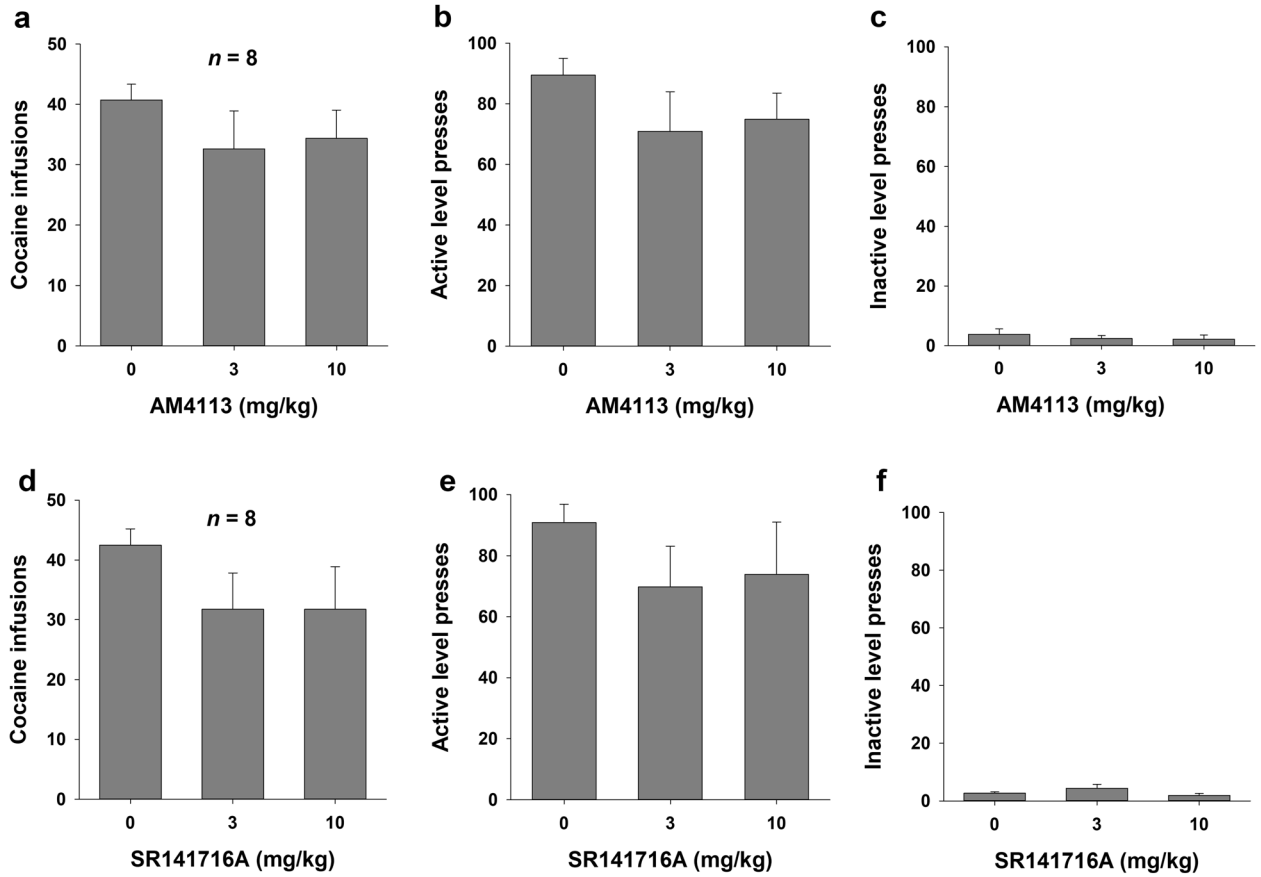

Fig. 3 Effects of AM4113 or SR141716A on cocaine self-administration under a FR2 reinforcement schedule. Administration of AM4113 did not alter the number of cocaine infusions (**a**), active lever presses (**b**), or inactive lever presses (**c**). Administration of SR141716A did not alter the number of cocaine infusions (**d**), active lever presses (**e**), or inactive lever presses (**f**). Data are presented as the mean ± SEM

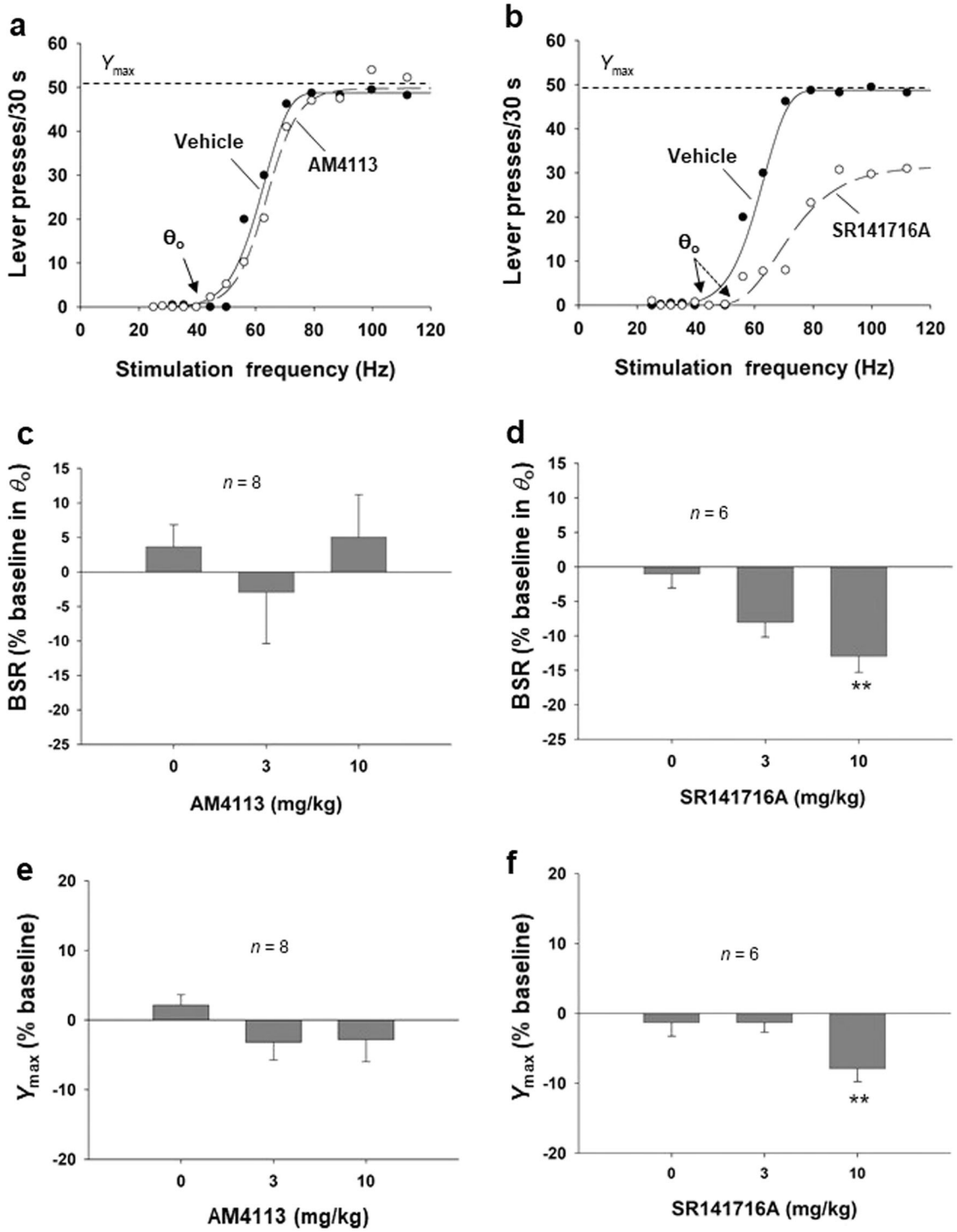

Fig. 4 Effects of AM4113 and SR141716A on brain-stimulation reward (BSR). **a**, **b** Representative stimulation–response rate curves indicating that AM4113 had no effect on BSR, while SR141716A dose-dependently shifted the curve to the right and increased the BSR threshold (θ_0). **c** AM4113 pretreatment did not affect the BSR threshold (θ_0) at either dose tested. **d** SR141716A (10 mg/kg, i.p.) significantly decreased BSR, as assessed by the increased stimulation threshold (θ_0) value. **e** AM4113 did not alter maximal operant responses (Y_{max} level). **f** SR141716A dose-dependently decreased the maximal operant responses (Y_{max} level). Data are presented as the mean ± SEM. $^{**}P < 0.01$ vs. vehicle (0 mg/kg, i.p.)

SR141716A significantly inhibited both heroin and methamphetamine intake at the higher dose tested (10 mg/kg). These results suggest that AM4113 retains the significant therapeutic anti-addictive effects of SR141716A. Importantly, SR141716A also significantly inhibited electrical BSR (as assessed by increased BSR stimulation threshold), suggesting that this drug has aversive or depressive effects, while AM4113 did not, indicating that AM4113 may not have SR141716A-like adverse psychiatric effects. Compared to SR141716A, AM4113 appeared to be more effective at reducing heroin self-administration at a lower dose, suggesting that AM4113 may have an efficacy as a pharmacotherapy for opioid addictions without the risk of depressive side effects.

Intravenous drug self-administration is one of the most commonly used animal models to study drug reward and relapse

[53]. Under this model, accumulating evidence has indicated that CB_1R antagonists/inverse agonists (such as SR141716A and AM251) are effective in reducing heroin-seeking and heroin-taking behaviors [18, 20, 54–56], as well as nicotine-seeking and nicotine-taking behaviors [57–60]. Consistent with these findings, in the present study, we reported that the neutral CB_1R antagonist AM4113 was also effective in suppressing heroin self-administration in rats, suggesting that this neutral CB_1R antagonist could act as an alternative to SR141716A in the development of a medication for the treatment of opioid abuse and addiction.

The decrease in heroin self-administration produced by AM4113 was unlikely due to non-specific sedation or locomotor impairment because AM4113 neither altered inactive lever responses during the self-administration experiments nor altered active lever responses in electrical BSR (as assessed by Y_{max}). It also failed to alter cocaine or methamphetamine self-administration at the same drug doses used in the heroin self-administration testing. Therefore, we exclude the possibility that AM4113 inhibits heroin self-administration by non-specific inhibition of locomotor activity.

There are some conflicting findings regarding CB_1 involvement in a psychostimulant reward. In the present study, we found that neither AM4113 nor SR141716A altered cocaine self-administration under the FR2 schedule at the same drug doses. This finding is consistent with previous reports that CB_1Rs were not critically involved in cocaine reward and dependence [6] and that AM4113 significantly inhibited nicotine, but not cocaine, self-administration in non-human primates [47]. Similarly, other studies have indicated that CB_1R antagonists/inverse agonists (SR141716A and AM251) also failed to alter cocaine self-administration under FR reinforcement in rodents [26, 52, 61, 62] and non-human primates [47].

With respect to methamphetamine, our data indicated that AM4113 (3 and 10 mg/kg) did not inhibit methamphetamine self-administration under FR2 reinforcement conditions, while SR141716A, at 10 mg/kg, significantly inhibited methamphetamine intake but not active lever responding. This finding is consistent with previous reports that SR141716A and AM251 attenuated methamphetamine self-administration [22, 63] and reinstatement of methamphetamine-seeking behavior [64, 65]. However, at least one group has reported negative findings of AM251 on methamphetamine-induced reinstatement [66]. These observations suggest that the brain CB_1Rs may be differentially involved in cocaine vs. methamphetamine reward processes. The reduction in methamphetamine self-administration may also be related to the inverse agonist effects of SR141716A or AM251 at the CB_1Rs.

Electrical BSR is a reliable and sensitive method for studying the effects of drugs directly on the neural circuitry that underlies brain reward [67]. In BSR, the lowering of thresholds by a test compound is interpreted as the potentiation of the mesolimbic activity subjectively perceived as rewarding. Conversely, raising BSR thresholds is interpreted as aversion or depreciation of the rewarding value of electrical stimulation [68]. Within this framework, our data indicate that systemic administration of AM4113 had no significant effect on BSR compared to vehicle, suggesting that AM4113 has neither rewarding nor aversive effects on its own. These findings are consistent with earlier reports that showed chronic treatment with AM4113 did not alter performance on the elevated plus maze or forced swim test, which are preclinical assays of anxiety and depression, respectively [46]. In contrast, we found that SR141716A produced significant inhibition of BSR at the higher dose tested (10 mg/kg), which is in accordance with clinical findings that SR141716A produces mood-depressant side effects.

In conclusion, the present study demonstrates that the neutral CB_1R antagonist AM4113 produced significant inhibitory effects on heroin self-administration but not on methamphetamine or cocaine self-administration. Unlike the CB_1R inverse agonist/antagonist SR141716A, AM4113 alone has no significant effect on the BSR threshold, suggesting that it will have fewer mood-depressant-like side effects. These results suggest that AM4113 or other more potent neutral CB_1R antagonists may be effective in the treatment of opioid abuse and addiction without adverse psychiatric effects.

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AUTHOR CONTRIBUTIONS

ELG, AM, Y-lW, and Z-xX designed the experiments. X-hH, KV, G-hB, and JZ performed the experiments. X-hH, CJJ, and Z-xX analyzed the data and wrote the manuscript with help from all other co-authors.

ADDITIONAL INFORMATION

Competing interests: The authors declare no competing interests.

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