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Original Article

A selective D₃ receptor antagonist YQA14 attenuates methamphetamine-induced behavioral sensitization and conditioned place preference in mice

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Aim: We have reported that a selective dopamine D₃ receptor antagonist YQA14 attenuates cocaine reward and relapse to drug-seeking in mice. In the present study, we investigated whether YQA14 could inhibit methamphetamine (METH)-induced locomotor sensitization and conditioned place preference (CPP) in mice.

Methods: Locomotor activity was monitored in mice treated with METH (1 mg/kg, ip) daily on d 4–13, followed by a challenge with METH (0.5 mg/kg) on d 21. CPP was examined in mice that were administered METH (1 mg/kg) or saline alternately on each other day for 8 days (METH conditioning). YQA14 was injected intraperitoneally 20 min prior to METH or saline.

Results: Both repetitive (daily on d 4–13) and a single injection (on the day of challenge) of YQA14 (6.25, 12.5 and 25 mg/kg) dose-dependently inhibited the acquisition and expression of METH-induced locomotor sensitization. However, repetitive injection of YQA14 (daily during the METH conditioning) did not alter the acquisition of METH-induced CPP, whereas a single injection of YQA14 (prior to CPP test) dose-dependently attenuated the expression of METH-induced CPP. In addition, the repetitive injection of YQA14 dose-dependently facilitated the extinction and decreased the reinstatement of METH-induced CPP.

Conclusion: Brain D₃ receptors are critically involved in the reward and psychomotor-stimulating effects of METH. Thus, YQA14 deserves further study as a potential medication for METH addiction.

Keywords: drug addiction; methamphetamine; YQA14; dopamine D₃ receptor, locomotor sensitization, conditioned place preference; reinstatement

Introduction

Methamphetamine (METH) is a highly addictive drug that is abused throughout the world. In China, METH users in the drug-abusing population rapidly increased from 6.7% in 2005 to 35.9% in 2013, and METH is predicted to be one of the most widely used addictive drugs in the future[1]. Despite extensive research in recent decades, no effective pharmacotherapy has been approved by the United States Food and Drug Administration for the treatment of METH addiction[2, 3]. To achieve this goal, various experimental animal models, such as locomotor behavior sensitization, conditioned place preference (CPP) and self-administration, have been developed to study

the mechanisms underlying METH addiction and to screen chemicals that may have therapeutic potential for the treatment of METH addiction[4].

The rewarding effects of METH are mediated by activation of mesolimbic dopamine (DA) transmission in the nucleus accumbens (NAc). Specifically, this action is mediated by METH re-uptake into presynaptic terminals via membrane DA transporters; METH inhibits DA re-uptake into vesicles via type 2 vesicular monoamine transporters (VMAT₂), thereby increasing cytoplasmic DA and DA release[5-7]. There are five subtypes of DA receptors, which are further classified as D₁-like (D₁ and D₅) and D₂-like (D₂, D₃, and D₄) based on their different intracellular G-protein-coupled signaling pathways. Thus, brain DA receptors are reasonable molecular targets for possible therapeutics to treat METH addiction[4]. Growing evidence suggests that DA D₃ receptors (D₃Rs) play a critically important role in mediating DA action and substance abuse,

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given the restricted distribution patterns of these receptors primarily within the mesolimbic DA system, including the ventral tegmental area (VTA), NAc, islands of Calleja, and olfactory tubercle[8-10]. Neuroimaging studies suggest that mesolimbic D_3Rs are up-regulated in both METH and cocaine abusers[11]. Accordingly, inducing normalization (antagonism) of D_3R functions using D_3R antagonists may effectively attenuate vulnerability to psychostimulant reward and relapse.

Various compounds with D_3R antagonist profiles, such as YQA14, SB-277011A, NGB2904, BP-897, S-33138, PG-01037, and SR21502, have been evaluated for the treatment of addiction to psychostimulants, particularly cocaine, in experimental animals[10, 12-15]. However, few studies have evaluated the potential use of these compounds for the treatment of METH addiction. The systemic administration of SB-277011A, NGB2904, BP-897 and PG01307 attenuates the reward effects of METH-enhanced electrical brain stimulation[13, 16]. SB-277011A also decreases the breaking point for METH self-administration and METH-induced reinstatement of drug-seeking behavior in rats[13, 16-18]. However, further development of SB-277011A as a therapeutic candidate for the treatment of drug addiction has been terminated due to its poor pharmacokinetic profile (an extremely short half-life in primates) and concerns about toxicity[10, 19]. Therefore, we have recently developed another novel D_3R antagonist, YQA14, which binds to two high-affinity binding sites on D_3Rs with K_i values of 0.68×10^{-4} nmol/L and 2.11 nmol/L. YQA14 displays more than 150-fold selectivity for D_3Rs over D_2Rs and more than 1000-fold selectivity for D_3Rs over other DA receptors[12]. Moreover, pharmacokinetic assays demonstrate that YQA14 has improved oral bioavailability (~20%) *in vivo* in dogs and a longer half-life (>1.5 h in human liver microsome enzymes) compared with SB-277011A (~20 min) *in vitro*[20]. YQA14 appears to have no rewarding or addictive potential because it does not sustain self-administration in rats that previously self-administered cocaine[21] or induce conditioned place preference (CPP) or conditioned place aversion (CPA)[22]. Furthermore, YQA14 has no effect on locomotor activity when administered acutely or chronically[21]. These data suggest that YQA14 displays several advantageous properties that are useful for potential therapeutic development for the treatment of drug addiction compared with other reported D_3R ligands, including SB-277011A.

Therefore, in the present study, we further evaluated the therapeutic potential of using YQA14 in experimental animals for the treatment of METH addiction. Specifically, we investigated whether chronic or acute administration of YQA14 significantly alters the acquisition and expression of METH-induced locomotor sensitization, METH-induced CPP, METH-induced CPP extinction, and METH-induced reinstatement of CPP.

Materials and methods
Drugs
Methamphetamine was obtained from the Beijing Public Security Bureau Forensic Medical Examination Center (China). YQA14 was synthesized by the Beijing Institute of Pharmacology and Toxicology and dissolved in 2-hydroxypropyl-β-cyclodextrin (Xi'an Deli Biological Chemical Co, Ltd, Xi'an, China) which was used as the vehicle.

Animals
Male Kun-Ming mice (Beijing Animal Center, Beijing, China) weighing 18–20 g were housed under environmentally controlled conditions at an ambient temperature maintained at 21±1°C and with humidity maintained at 50%–60%. All mice were maintained on a 12-h light/dark cycle (lights on at 7:00 AM and lights off at 7:00 PM) with food and water provided *ad libitum*. All animals were maintained in a facility fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International, and all experimental procedures were conducted in accordance with the guidelines established by the Institutional Review Committee for the Use of Animals.

Locomotor behavior sensitization experiment
The general procedure for sensitization was identical to a previously described procedure[21]. Briefly, the apparatus (JLBehv-LAG-9, Shanghai, China) was a locomotor activity monitor chamber.

Habituation phase (d 1–3)
Before receiving drug treatment, mice were habituated in the locomotor detection chambers for 1 h per day for 3 days.

METH-induced behavior sensitization and challenge phases (d 4–21)
Animals in the METH group and the saline group received METH (1 mg/kg, ip) or saline (10 mL/kg, ip), respectively, and were immediately placed in a locomotor detection chamber, where their activity was recorded for 1 h daily for 10 days (d 4–13). All animals were then returned to their home cages without METH or saline treatment for a 7-d withdrawal period (d 14–20). Finally, all of the mice were challenged with METH (0.5 mg/kg, ip) on d 21.

To evaluate the effects of chronic YQA14 administration on the acquisition of METH challenge-induced locomotor sensitization (Figure 1A), the animals in the METH group were assigned to 4 groups that received either vehicle or YQA14 (6.25, 12.5 and 25 mg/kg, ip, *n*=12 per dose group) 20 min before receiving METH (1 mg/kg, ip). The locomotor activity of the animals was then recorded for 1 h daily for 10 days'. The mice did not receive YQA14 during the withdrawal period or on the challenge day.

To assess the effects of acute YQA14 administration on METH challenge-induced locomotor sensitization (Figure 2A), the animals in the METH group were assigned to 4 groups that received either vehicle or YQA14 (6.25, 12.5 and 25 mg/kg, ip, *n*=12 per dose group) 20 min before receiving METH (0.5 mg/ kg, ip) on the challenge day, and their locomotor activity was recorded for 1 h. These mice did not receive YQA14 during the period of acquisition of METH-induced behavior sensitization or during the withdrawal period.

All data were recorded using a computerized video tracking sys-

tem (Jiliang Software Co, Ltd, Shanghai, China). Travel distance was used to evaluate the effects of YQA14 on locomotor behavior.

Conditioned place preference experiment

The general procedure for the unbiased CPP procedure was identical to a previously described procedure[23] as shown in Figure 3A. Briefly, a conventional CPP apparatus (CPP-VR01, Ningbo, China) was used. The apparatus had two compartments of equal size (25 cm×35 cm×64 cm) and a central tunnel (15 cm×35 cm×64 cm). One compartment was black, with a stainless steel grid in the floor that consisted of perpendicularly placed rods (cross-section: 0.3 cm×0.3 cm) placed to form empty centers (1.5 cm×1.5 cm). The other compartment was white with a 28.5 cm×22.5 cm stainless steel mesh floor. The center corridor was painted black on the floor and white on the two lateral walls. Each compartment had a LED light and an overhead camera. All data were recorded using a computerized video tracking system (AniLab Software & Instrument Co, Ltd, Ningbo, China).

Pre-conditioning phase (d 1–3)

The mice were first placed in the center corridor and allowed free access to the other two compartments for 15 min daily. The time spent in each compartment was recorded. This habituation period was used to exclude biased mice that spent more than 800 s in either compartment during the study.

METH-induced CPP (d 4–12)

METH conditioning was conducted for 8 days (4 drug sessions and 4 saline sessions). Each animal received METH (1 mg/kg, ip) or saline (10 mL/kg, ip) injections alternately on every other day. The mice were then immediately confined in the appropriate drug- or saline-paired compartments for 60 min.

To assess the effects of YQA14 on the acquisition of METH-induced CPP, naïve animals received vehicle or YQA14 (6.25, 12.5 and 25 mg/kg, ip, $n=12$ per group) 20 min prior to each METH (1 mg/kg, ip) or saline (10 mL/kg, ip) injection during the METH conditioning phase (Figure 3A). The CPP test was conducted 24 h after the last injection. There was no drug treatment on the test day.

To evaluate the effects of YQA14 on the expression of METH-induced CPP, either vehicle or YQA14 (6.25, 12.5 and 25.0 mg/kg, ip, $n=18$–19 per group) was administered 20 min prior to the CPP test to additional groups of naïve mice that did not receive chronic YQA14 treatment during METH conditioning (Figure 4A). The mice were placed in the center corridor and were allowed free access to the other two compartments for 15 min. The preference (*ie*, CPP score) was calculated by subtracting the time spent in the saline-paired compartment from the time spent in the drug-paired compartment.

CPP extinction (d 13–22)

Naïve animals were trained with METH. Following the establishment of METH-induced CPP, the animals were separated into 4 groups and then underwent daily extinction training for 10 days. During this time, they received injections of

vehicle or YQA14, but without METH, and were confined in the previously drug-paired compartment for 60 min ($n=23$–24 per group). The effect of chronic YQA14 on the extinction response to METH-induced CPP was evaluated after each 4-d period of extinction training during an extinction test 1 on d 17 and an extinction test 2 on d 22.

Reinstatement test (d 23)

After the completion of the above extinction tests, all mice were primed with an injection of METH (0.5 mg/kg, ip) to analyze the METH-induced reinstatement of the CPP response after chronic vehicle or YQA14 treatment during the extinction described above.

Statistical analysis

All data are presented as the mean±SEM. The effects of YQA14 on METH-induced behavioral sensitization and CPP were assessed using one-way or two-way ANOVA. Individual group comparisons were performed using Student-Newman-Keuls methods. A paired *t*-test was also used to analyze the statistical significance of effects on METH-induced CPP between pre-conditioning and post-conditioning

Results

Chronic administration of YQA14 inhibits the acquisition of METH-induced behavioral sensitization

Figure 1B shows the effects of repeated METH administration (1 mg/kg, ip) on locomotor behavior in the presence or absence of YQA14. Chronic METH treatment produced significant behavioral sensitization ($P<0.05$ on d 12 and $P<0.01$ on d 13 *vs* d 1) in METH group mice. Chronic YQA14 treatment significantly attenuated the acquisition of METH-induced behavioral sensitization. Two-way ANOVA for repeated measures revealed a significant YQA14 treatment main effect ($F_{4, 55}=14.22$, $P<0.01$), time main effect ($F_{9, 486}=2.83$, $P=0.003$), and a treatment×time interaction ($F_{36, 589}=2.00$ $P<0.01$). On the challenge day, METH (0.5 mg/kg, ip) produced a significant increase in locomotion only in the vehicle group but not in any of the YQA14 treatment groups ($t=-6.539$, with 22 degrees of freedom, $P<0.01$ *vs* the saline group, Figure 1C). One-way ANOVA revealed a significant main effect of YQA14 treatment ($F_{3,44}=4.752$, $P<0.01$). *Post hoc* individual group comparisons revealed a significant difference between the vehicle and YQA14 treatment groups at each dose ($P<0.05$, $P<0.01$ and $P<0.01$ for 6.25, 12.5 and 25 mg/kg, respectively).

A single injection of YQA14 inhibits the effects of METH challenge during behavioral sensitization

METH (0.5 mg/kg, ip) priming produced a significantly enhanced locomotor response in the vehicle treatment group ($t=4.70$, $P<0.01$, Figure 2B). However, pretreatment with a single administration of YQA14 significantly attenuated this effect on METH-induced locomotor sensitization ($F_{3, 44}=6.45$, $P<0.01$) in a dose-dependent manner. Individual group comparisons revealed a statistically significant reduction after the administration of 12.5 mg/kg or 25 mg/kg YQA14 ($P<0.01$).

Chronic pretreatment with YQA14 does not alter the acquisition of METH-induced CPP

We then further examined whether repeated administration of YQA14 would alter the acquisition of METH-induced CPP in mice. METH produced a robust place preference regardless of

YQA14 treatment ($P<0.01$, $P<0.01$, $P<0.05$ and $P<0.01$ for vehicle, 6.25, 12.5 and 25 mg/kg YQA14, respectively, Figure 3B), indicating that chronic administration of YQA14 had no significant effect on the acquisition of METH-induced CPP compared with the vehicle treatment group ($F_{3, 44}= 0.11$, $P>0.05$).

A single injection of YQA14 inhibits the expression of METH-induced CPP

We next examined the effect of YQA-14 on the expression of METH-induced CPP. YQA14 pretreatment (6.25, 12.5, and 25 mg/kg, ip, 20 min prior to test) significantly attenuated METH-induced CPP in a dose-dependent manner ($F_{3, 74}=3.34$, $P<0.05$, Figure 4B). *Post hoc* individual group comparisons revealed a statistically significant reduction in CPP after administration of 12.5 mg/kg or 25 mg/kg of YQA14 ($P<0.05$, *vs* vehicle).

Chronic administration of YQA14 facilitates the extinction and inhibits the reinstatement of METH-induced CPP

Before extinction training, mice were not pretreated with YQA14. METH (1 mg/kg, ip) produced significant CPP in the vehicle and YQA14 treatment groups compared with the responses in the pre-conditioning phase. During the extinction phase, the mice were treated daily with vehicle or YQA14 immediately before being placed into the formerly METH-paired compartment. Repeated pretreatment with YQA14
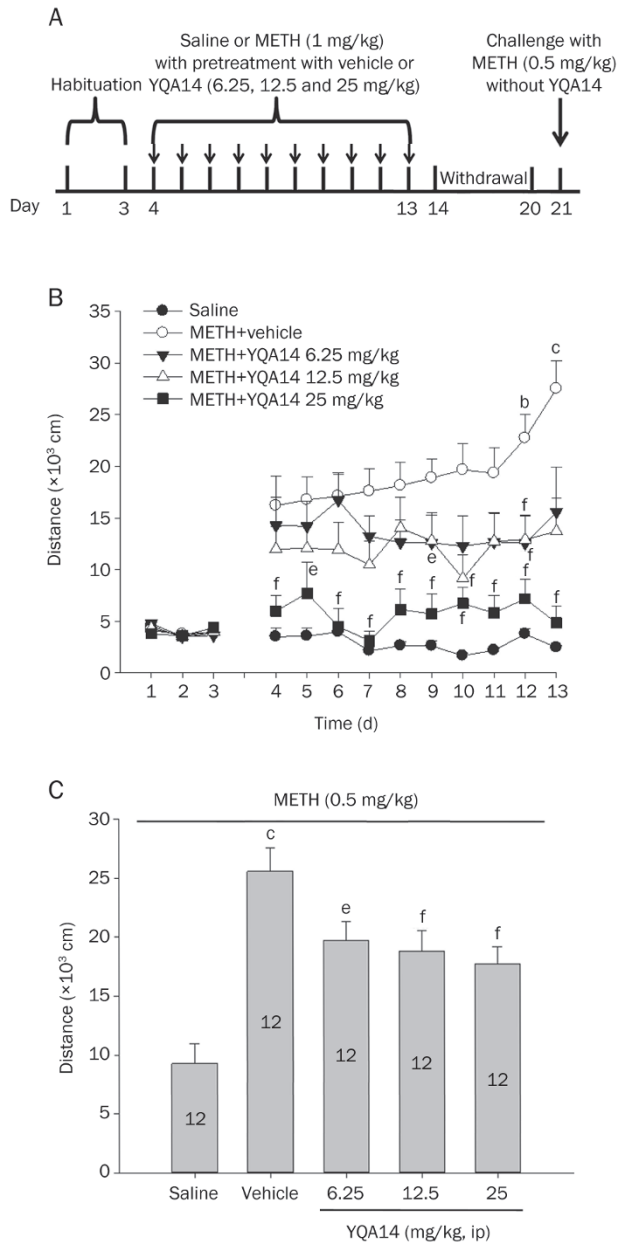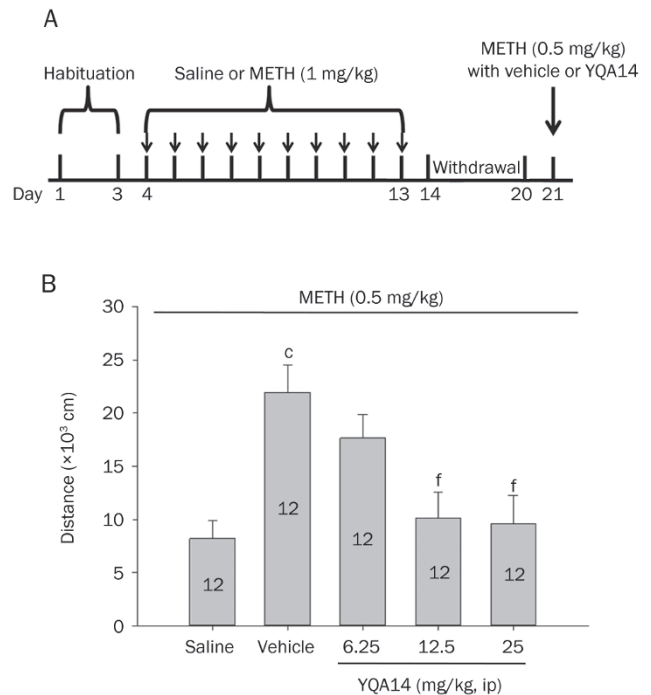

Figure 1. Effects of chronic administration of YQA14 on the acquisition of METH-induced behavioral sensitization in mice. (A) Experimental protocol for YQA14 and METH treatments. (B) Time courses of METH-induced hyperactivity and locomotor sensitization in the presence or absence of YQA14 pretreatment. Mean±SEM. $n=12$. [b]$P<0.05$, [c]$P<0.01$ *vs* day 1; [e]$P<0.05$, [f]$P<0.01$ *vs* METH treatment alone. (C) METH (0.5 mg/kg, ip) priming produced a significant increase in locomotor activity 7 days after the last METH injection ([c]$P<0.01$ *vs* the saline group). This effect was dose-dependently attenuated in the groups that received chronic treatment with YQA14 ([e]$P<0.05$, [f]$P<0.01$ *vs* METH treatment alone). Mean±SEM. $n=12$.

Figure 2. Effects of a single injection of YQA14 on METH challenge-induced behavior sensitization in mice. (A) Experimental protocol for YQA14 and METH treatments. (B) METH (0.5 mg/kg, ip) priming produced a significant increase in locomotor activity 7 days after the last METH injection ([c]$P<0.01$ *vs* the saline group). This effect was dose-dependently inhibited by a single injection of YQA14 ([f]$P<0.01$ *vs* vehicle group). Mean±SEM. $n=12$.
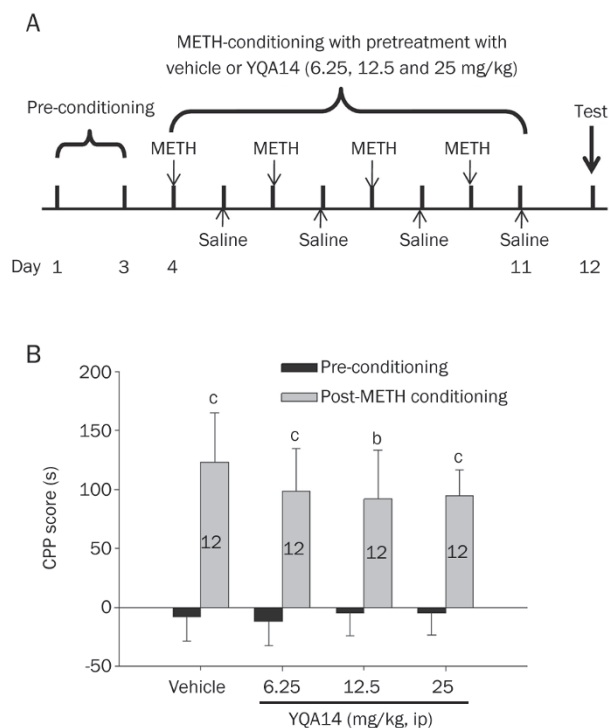
Figure 3. Effects of chronic administration of YQA14 on the acquisition of METH-induced CPP in mice. (A) YQA14 and METH treatment protocols. (B) CPP scores after vehicle or YQA14 administration illustrate that pretreatment with YQA14 did not produce a statistically significant reduction in METH-induced CPP. Mean±SEM. n=12. ^bP<0.05, ^cP<0.01 vs pre-conditioning.

Figure 4. Effects of a single injection of YQA14 on the expression of METH-induced CPP in mice. (A) The METH and YQA14 treatment protocols. (B) CPP scores after treatment with vehicle or YQA14 illustrate that pretreatment with YQA14 on the CPP test day dose-dependently inhibited the expression of METH-induced CPP. Mean±SEM. ^cP<0.01 vs pre-conditioning. ^eP<0.05 vs vehicle.

dose-dependently decreased reward-seeking during extinction, ie, the time spent in the METH-paired compartment on the first extinction test day compared with the vehicle treatment group ($F_{3, 90}$=2.96, P<0.05, Figure 5B). Because the CPP response was not completely extinguished on the first test day, we continued daily extinction using vehicle or YQA14 treatment for an additional 4-days. Next, we observed the effects of chronic YQA14 treatment on the METH-induced reinstatement of CPP. After 8 d of extinction training, METH-induced CPP was nearly completely extinguished in all vehicle and YQA14 treatment groups during test 2. On the reinstatement test day, priming with METH (0.5 mg/kg, ip) in the vehicle group induced a robust reinstatement of the METH-induced CPP (t=6.37, P<0.01) that was significantly attenuated by YQA14 (6.25, 12.5, and 25 mg/kg, ip) in a dose-dependent manner ($F_{3, 90}$=6.85, P<0.01, Figure 5B). Individual group comparisons revealed a statistically significant reduction after the administration of 6.25, 12.5 and 25 mg/kg YQA14.

Discussion

The major findings of the present study are that chronic or acute administration of the selective D_3R antagonist YQA14 significantly inhibits the acquisition and expression of METH-induced locomotor sensitization and the expression of METH-induced CPP in a dose-dependent manner. In addition, the
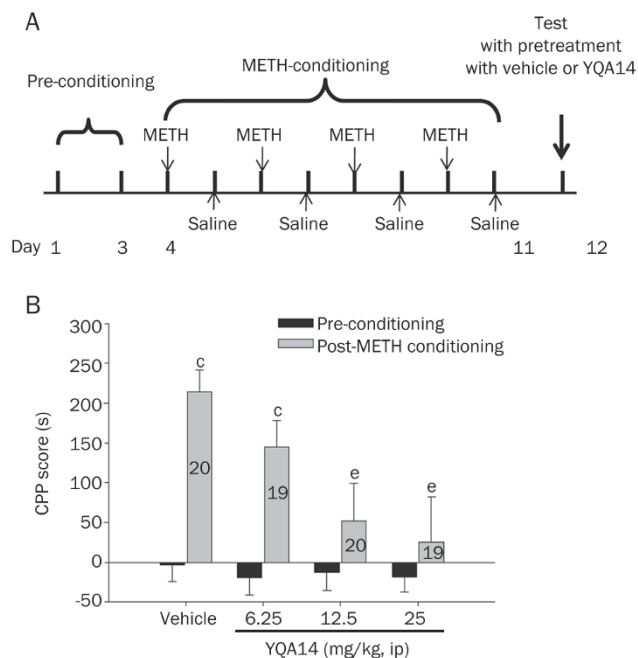
chronic administration of YQA14 also facilitates the extinction of reward-seeking and attenuates the METH-induced reinstatement of CPP. These data suggest that brain D_3Rs play a critical role in the stimulatory effects of METH. Thus, YQA14 or other D_3R antagonists warrant further research aimed at studying their effect as potential pharmacotherapies for METH addiction.

Locomotor hyperactivity is one of the most commonly used paradigms for studying the rewarding and psychostimulatory effects of drugs[24-29]. In the present study, we observed that chronic administration of METH induced gradually increasing locomotion after daily injection. A previous study reported that acute or chronic administration of YQA14 had no effect on locomotor activity[21]. However, acute injection of YQA14 prior to METH administration significantly inhibited METH-induced hyperactivity, suggesting a reduction in METH reward and psychomotor-stimulation. This is consistent with the significantly attenuated acute locomotor responses observed following administration of METH in $D_3R^{-/-}$ mice compared with the effects observed in wild-type mice[30]. These results are also consistent with the ability of YQA14 and other D_3R antagonists, such as SB-277011A, NGB2904, and PG-01037, to significantly inhibit METH or cocaine self-administration[12, 13, 18, 21, 22, 31-34] and METH- or cocaine-enhanced electrical brain stimulation rewards, two of the other most common animal models used to evaluate drug reward systems[14-16, 22, 33, 35].
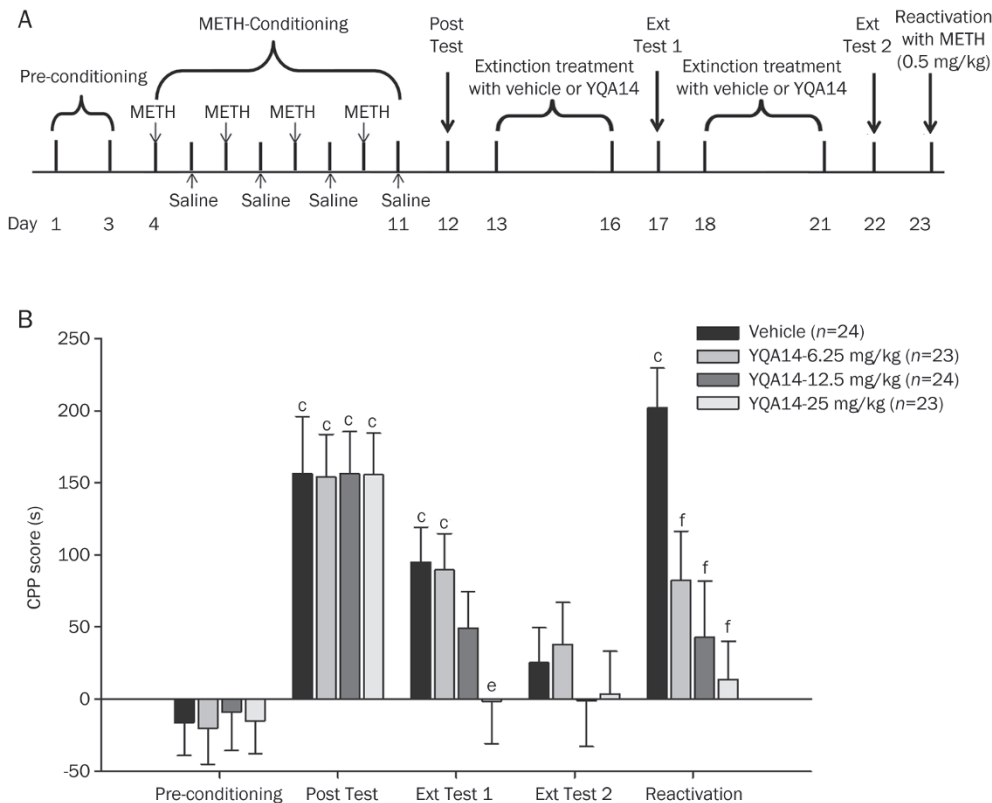
Figure 5. Effects of chronic administration of YQA14 on the extinction and reinstatement of METH-induced CPP in mice. (A) The complete experimental protocol for METH and YQA14 treatments. (B) CPP scores after treatment with vehicle or YQA14 during extinction illustrate that YQA14 dose-dependently facilitated the extinction of METH reward-seeking and the METH-primed reinstatement of METH-seeking behavior. Mean±SEM. *n*=23–24, [c]$P<0.01$ *vs* pre-conditioning; [e]$P<0.05$, [f]$P<0.01$ *vs* the vehicle control group.

Behavioral sensitization has been proposed as a useful paradigm for investigating the mechanisms involved in addiction to psychostimulants. Behavioral sensitization, which is the progressive and enduring augmentation of certain behaviors following repetitive drug use, alters rodent locomotion in a long-standing manner and is a well-studied model of behavioral plasticity[36]. The mechanisms underlying behavioral sensitization are thought to involve enhanced DA transmission[37-41]. In the present study, we observed that repeated administration of the same dose of METH produced significant locomotor sensitization, *ie*, an enhanced locomotor response, compared with responses during the first few days of METH injections. This effect was long-lasting and persisted for at least 7 days after the start of METH abstinence. This finding suggests that METH-induced behavioral sensitization may in part be involved in the enduring neural adaptations associated with drug cravings and relapses after chronic long-term use of METH. In addition, chronic administration of YQA14 significantly and dose-dependently inhibited both the development and the expression of METH-induced behavioral sensitization. This inhibition is congruent with the above DA hypothesis regarding behavioral sensitization and with previous findings showing that YQA14 or SB-277011A significantly attenuated cocaine-induced behavioral sensitization[12, 21]. The experimental results observed in the present study are also

consistent with findings in studies of other D_3R antagonists (*ie*, nafadotride, U99194A and GR103691) that have shown that blockade of D_3Rs attenuates the behavioral sensitization produced by repeated amphetamine administration[42, 43]. The D_3 receptor mutant ($D_3R^{-/-}$) mice also exhibited attenuated acute locomotor responses as well as the development of behavioral sensitization to METH compared with the results in wild-type mice[30]. Taken together, these results suggest an important role for D_3Rs in METH-induced locomotor sensitization and possibly in relapse toward METH-seeking behavior.

In support of these conclusions, we used the CPP paradigm to evaluate the effects of chronic *vs* acute administration of YQA14 on the acquisition, expression, extinction and reinstatement of METH-induced CPP. The CPP paradigm is based on Pavlovian stimulus learning, which is considered a reliable measure of the reinforcing properties of addictive drugs and is particularly useful for evaluating the reinforcing effect of paired addictive drugs and environmental cues[44]. In the present study, we observed that the chronic administration of YQA14 did not significantly block the development of METH-induced CPP. However, a single injection of YQA14 dose-dependently attenuated the expression of METH-induced CPP measured 7 days after chronic METH administration. Overall, these findings are consistent with previous findings showing that the D_3R-preferring agents BP-897 and nafadotride

also selectively block the expression, but not the acquisition, of amphetamine-induced CPP at the same drug doses[2, 45, 46]. Furthermore, these results are consistent with our recent finding that YQA14 selectively inhibits the expression, but not the acquisition, of morphine-induced CPP[23]. The precise mechanisms underlying the inability of YQA14 to disrupt the acquisition of METH-induced CPP are unclear. The simplest explanation is that the doses of YQA14 used here were not adequate to block METH-induced CPP. This possibility is supported by our finding that in the absence of METH, the same doses of YQA14 significantly inhibited the expression of METH-induced CPP (Figure 4B). Another reasonable explanation is that DA receptor mechanisms other than those related to D_3Rs may also be involved in the development of METH-induced CPP. The CPP model is viewed as an incentive learning process. Accordingly, during the conditioning sessions, the stimuli in the chamber was associated with the METH to display the perceived appetitive. However, on the expression day, the animals that did not receive METH spent more time in the drug-paired chamber because of this incentivized learning[47-49]. The results indicate that YQA14 blocks D_3Rs and impairs the capacity of the environmental cues that were associated with METH to elicit approach behaviors; however, YQA14 does not alter the perceived appetitive value of METH. These results suggest that D_3Rs play a more important role in the expression of conditioned behaviors than in their acquisition[47, 50]. Regardless of the mechanisms underlying the ineffectiveness of YQA14 at combatting the acquisition of METH-induced CPP, the present finding that YQA14 inhibits the expression of METH-induced CPP is consistent with previous reports that have shown that the blockade of D_3Rs by SB-277011A, YQA14 or SR21502 inhibits cocaine-induced CPP[14, 22, 51], cocaine and METH self-administration[12, 18, 21, 31], and cocaine- and METH-induced enhancement of electrical brain stimulation rewards[21, 33, 35]. Importantly, YQA14 itself does not induce CPP or CPA[22].

These findings suggest that blockade of D_3Rs impairs the capacity of the environmental cues that were associated with METH to elicit approach behaviors but does not alter the perceived appetitive value of METH. These results indicate that D_3Rs play a more important role in the expression of incentive learning than in the acquisition of METH-induced CPP[47, 50].

METH addiction is also characterized by persistent susceptibility to drug relapse[52]. Identifying effective medications that can prevent drug relapse is a key goal of the study of drug addiction. Drug-primed reinstatement is identified as one of the most important aspects of relapse in humans and has been modeled by the reinstatement of extinguished METH seeking or METH-induced CPP behavior in mice[4]. Using this model, we observed, for the first time, that chronic administration of YQA14 significantly facilitated the extinction of METH-induced CPP (*ie*, reward-seeking behavior) and the METH-induced reinstatement of drug-seeking behavior.

Extinction training of drug-associated cues has been proposed as a treatment called cue exposure therapy, which aims to prevent relapse by reducing the motivational properties of the cues. However, cue exposure therapy in clinical settings has not been efficacious[53, 54]. A modified extinction training procedure based on disruption of the reconsolidation of the memories associated with drugs of abuse was recently proposed as a potential strategy to decrease relapse[55-57]. In this study, we used cue-exposure therapy protocols combined with the use of selective D_3R antagonists such as YQA14. The results are similar to those in previous studies showing that the chronic administration of YQA14 inhibits morphine-induced reinstatement of CPP and that the acute administration of YQA14 inhibits cocaine- or cue-induced relapse to cocaine-seeking behavior[12, 21, 22, 58]. The neuronal mechanisms underlying these effects are unclear. Drug-associated cues can increase DA (and glutamate) release in the mesolimbic DA system, particularly in the NAc[59-63]. This effect may explain why the blockade of D_3Rs by YQA14 facilitated the extinction of METH-induced CPP and diminished reinstatement in the present study.

In addition, the extinction of reward-seeking is an active learning process that does not erase the memory of addiction but instead establishes a new memory[64]. Mesolimbic–mesocortical DA has been thought to play an important role in learning and memory[65]. Moreover, a series of studies has demonstrated the participation of D_3Rs. The moderately selective D_3R agonist 7-OH-DPAT impaired passive avoidance performance in mice and produced disturbances in an object discrimination task in marmosets[66]. The DA D_2/D_3 receptor antagonist raclopride alleviated the memory impairment induced by a compound that activates D_3Rs[67]. The relatively selective D_3R antagonist nafadotride antagonized scopolamine-induced memory deficits in rats[68]. The highly selective D_3R antagonists SB-277011A and RGH-1756, the moderately selective U-99194A and the partially selective D_3R agonist BP-897 also improved learning performance in memory-impaired rats[67]. These data suggest that DA D_3R antagonists produce cognition-enhancing effects that may be beneficial for learning and memory. This DA-D_3R hypothesis may also partially explain the YQA14 antagonism of the reinstatement of METH-seeking behavior observed in the present study. Clearly, further study is required to fully address this issue.

In conclusion, the chronic administration of YQA14, a selective D_3R antagonist, induces the persistent reversal of behavioral sensitization to METH. Furthermore, YQA14 significantly inhibits the expression of METH-induced CPP. Importantly, it also facilitates the extinction and reduces the METH-primed reinstatement of METH-induced CPP in mice in a dose-dependent manner. These findings suggest that YQA14 not only decreases the rewarding effect of METH but also attenuates relapse to METH-seeking behavior. Thus, chronic administration of D_3R antagonists in combination with cognitive behavioral therapy may provide a novel effective treatment for METH relapse.

Acknowledgements

Author contribution

Li SUN and Rui SONG conducted the experiments and analyzed the data. Rui SONG designed the experiments and wrote a draft of the manuscript. Ri-fang YANG synthesized YQA14. Ying CHEN performed the behavior sensitization experiments. Ning WU initiated this research project. Rui-bin SU supervised the experiments. Jin LI supervised the experiments and revised the manuscript.

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