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# Reducing endocannabinoid metabolism with the fatty acid amide hydrolase inhibitor, URB597, fails to modify reinstatement of morphine-induced conditioned floor preference and naloxone-precipitated morphine withdrawal-induced conditioned floor avoidance

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ABSTRACT

The potential of the fatty acid amide hydrolase (FAAH) inhibitor, URB597, to modify drug prime-induced reinstatement of morphine-induced conditioned floor preference or naloxone-precipitated morphine withdrawal-induced conditioned floor avoidance was evaluated. In Experiment 1, morphine-induced conditioned floor preference was established across 4 conditioning trials. Following extinction training (4 trials), rats were pretreated with URB597 or vehicle prior to a morphine prime or a saline prime. Morphine reinstated the previously extinguished floor preference, but URB597 did not modify the strength of the reinstated preference. In Experiment 2, naloxone-precipitated morphine withdrawal-induced conditioned floor avoidance was established across 2 conditioning trials. Following extinction training (14 trials), rats were pretreated with URB597 or vehicle prior to a saline prime or a morphine withdrawal prime. The morphine withdrawal prime reinstated the previously extinguished floor avoidance, but URB597 did not modify the strength of reinstated avoidance. These results suggest that under the conditions in which URB597 promotes extinction (e.g., Manwell et al. (2009)) it does not interfere with drug-induced reinstatement of either conditioned floor preference or avoidance. That is, although activation of the endocannabinoid (eCB) system promotes extinction of aversive learning, it may not prevent reinstatement of that aversion by re-exposure to the aversive treatment.



## 1. Introduction

The endogenous cannabinoid (eCB) system has been implicated in extinction of previously learned aversive behaviors (Marsicano et al., 2002). Marsicano et al. (2002) initially reported that $CB_1$ receptor knockout mice and wild-type mice administered the $CB_1$ inverse agonist/antagonist, Rimonabant (SR141716), showed impaired extinction in classical auditory fear-conditioning tests, with memory acquisition and consolidation remaining unaffected. Using the aversively motivated Morris water maze task, Varvel and Lichtman (2002) reported that $CB_1$ knockout mice and wild-type mice showed similar acquisition rates in learning to swim to a fixed platform; however, the $CB_1$ deficient mice demonstrated deficits during a subsequent reversal task in which the mice were required to inhibit their previously learned behavior. On the other hand, $CB_1$ agonists have been reported to enhance the rate of extinction of aversively motivated tasks (Chhatwal et al., 2005; Pamplona et al., 2006). These effects on extinction are selective to aversive memories (e.g., Lutz, 2007), but not those produced by rewarding stimuli (Harloe et al., 2008; Holter et al., 2005).

Of most interest to the current study, Manwell et al. (2009) found that pretreatment with a fatty acid amide hydrolase (FAAH) inhibitor, URB597, selectively enhanced extinction of a conditioned floor avoidance produced by a naloxone-precipitated morphine withdrawal (see Azar et al., 2003; Parker and Joshi, 1998), but not a conditioned floor preference produced by morphine. By deactivating FAAH, URB597 selectively prolongs the duration of action of the eCB, anandamide, at the sites at which it is produced 'on demand.' The $ID_{50}$ for FAAH inhibition by URB597 in rats ex vivo is 0.15 mg/kg ip (Kathuria et al., 2003; Fegley et al., 2005). At the dose (0.3 mg/kg) employed by Manwell et al. (2009), URB597 produces maximal inhibition of FAAH within 15 min of administration and persists for at least 16 h (Fegley et al., 2005). This effect is associated with a parallel increase in brain anandamide content which attained peak levels 1 to 6 h following the injection. Furthermore, at the dose of 0.3 mg/kg, URB597 produces maximal efficacy in anyxiolytic-like (Kathuria et al., 2003; Patel and Hillard, 2006; Scherma et al., 2008a), anti-depressant-like (Gobbi et al., 2005) and anti-nausea-like effects (Cross-Mellor et al., 2007; Rock et al., 2008) in rats. The enhanced extinction of the floor avoidance was most likely produced by action of anandamide on

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the $CB_1$ receptor, because Rimonabant prolonged the duration of extinction relative to vehicle controls (Manwell et al., 2009).

Although URB597 facilitated extinction of conditioned floor avoidance, this facilitated extinction did not prevent the potential of a subsequent prime of naloxone-precipitated morphine withdrawal to reinstate the previously extinguished floor avoidance (Manwell et al., 2009). Such drug-induced reinstatement effects provide evidence that extinction is not unlearning (Bouton, 2002), but instead new inhibitory learning. These results suggest that eCB system manipulation by means of FAAH inhibition during extinction does not result in an elimination of the aversive memories given that they can be reinstated. However, it is not known under these conditions whether URB597 given prior to the actual reinstatement trial will result in attenuation of the reinstated aversive memory, as it has previously been reported to prevent reinstatement of a nicotine-induced conditioned place preference (Scherma et al., 2008b). It is conceivable that elevated levels of anandamide at the time of reinstatement of either a morphine-induced floor preference or a morphine withdrawal-induced floor avoidance would suppress the reinstated memory. Therefore, the present study was designed to evaluate the potential of URB597, at the maximally effective dose reported in other studies (Forget et al., 2009; Manwell et al., 2009; Scherma et al., 2008b) administered during reinstatement testing to interfere with reinstatement of both a previously extinguished morphine-induced floor preference [Experiment 1] and a naloxone-precipitated morphine withdrawal-induced conditioned floor avoidance [Experiment 2].

## 2. Materials and method

### 2.1. Subjects

The subjects in Experiment 1 and 2 were male Sprague–Dawley rats. The animals were maintained on an *ad libitum* schedule of food and water and were pair-housed in shoebox cages in the colony room at an ambient temperature of 21 degrees Celsius with a 12 h/12 h reverse light/dark schedule (lights off at 7:00 h). Experimental procedures began at least 3 h after the beginning of the dark cycle and were completed within 2 h prior to the end of the dark cycle. All procedures adhered to the guidelines of the Canadian Council of Animal Care and were approved by the Animal Care Committee of the University of Guelph.

### 2.2. Drugs

Morphine was prepared in physiological saline at concentrations of either 10 mg/ml [Experiment 1] or 20 mg/ml [Experiment 2] and administered subcutaneously (sc) in a volume of 1 ml/kg at 10 min prior to conditioning [Experiment 1] or 4 h before conditioning [Experiment 2]. Naloxone was prepared in physiological saline in a concentration of 1 mg/ml and administered (sc) in a volume of 1 ml/kg at 10 min prior to conditioning. URB597 (Cayman Chemicals) was prepared in 2-hydroxypropyl-ß-cyclodextrin (2-HPBCD, 45%) at a concentration of 0.3 mg/kg (ip) 2 h prior to reinstatement. At a dose of 0.3 mg/kg, URB-597 has been shown to produce a slow and consistent accumulation of anandamide in the brain with maximal effect occurring 2 h post injection (Fegley et al., 2005).

### 2.3. Apparatus

The conditioning apparatus used was a black Plexiglas rectangular box (60×25×25 cm) with a wire-mesh lid (as previously described in Manwell et al., 2009). During conditioning trials, the tactile cues on both sides of the box were identical. However, during pretest and choice tests, one side of the chamber had a metal hole floor and the other side had a metal grid floor (counterbalanced orientation), and the intersection of the two floors was defined as a neutral zone (9×25 cm) which was not included in the analysis. The amount of time (seconds) each rat spent on each of the floors was recorded and later analyzed by the Noldus Ethovision activity monitoring system (Noldus Information Technology, Sterling, VA.) Pretests did not reveal a significant difference between time spent on the hole or grid floors demonstrating that the apparatus provides an unbiased test of conditioned preference and aversion.

### 2.4. Procedure

#### 2.4.1. Experiment 1: effect of URB-597 on reinstatement of morphine-induced conditioned preference

A 10-min pretest was administered and the amount of time spent on each floor was measured. The rats were subsequently assigned to groups matched on the basis of their pretest score. The rats received 4 conditioning trials. During conditioning cycles all rats were injected with morphine or saline 10 min before placement in the box with a distinctive floor for 20 min. Therefore, each conditioning cycle was comprised of one morphine trial and one saline trial separated by 24 h. Additionally, each of the cycles was separated by 48–72 h. The order of the morphine trial within a cycle and the floor paired with morphine were counterbalanced among rats. Forty-eight hours after the 4th conditioning cycle, the rats were given a 10-min test. Starting 24 h after the test, the rats were given repeated 10-min extinction choice test trials, each separated by 24 h. The trials continued until there was no significant difference in preference for the morphine-paired floor and the saline-paired floor on at least 2 trials. One week subsequent to the final extinction trial, the rats received a reinstatement cycle. They were assigned-matched for drug-paired floor and order of morphine trial during conditioning, to group URB597 ($n=12$) and VEH ($n=11$). On each of the next two trials the rats were injected (ip) with either Vehicle (VEH) or 0.3 mg/kg URB597 at 2 h prior to a 10-min test trial. Ten minutes prior to one reinstatement test trial the rats were injected (sc) with saline (saline reinstatement trial), and 10 min prior to the other test trial (24 h later), the rats were injected (sc) with 5 mg/kg of morphine (morphine reinstatement trial). The order of morphine and saline trials were counterbalanced.

#### 2.4.2. Experiment 2: effect of URB-597 on reinstatement of naloxone-precipitated morphine withdrawal-induced conditioned avoidance

The assignment of rats to groups was matched according to the results of a 10-min pretest. They were then given 2 conditioning trial cycles (separated by 72 h), each comprised of a 2-day schedule separated by 24 h. For each conditioning cycle, on Day 1 the rats were injected with saline (sc) 10 min before being placed in the conditioning box with distinctive floor for 20 min. On Day 2, rats were administered 20 mg/kg of morphine (sc) at 4 h, and 1 mg/kg of naloxone (sc) at 10 min before placement in the conditioning box with the opposite distinctive floor (as on Day 1) for 20 min (see Azar et al., 2003). Extinction trials began 72 h after the final conditioning cycle. On each trial, the rats received drug-free access to both floors for 10 min. The trials occurred every 24 h for 14 days until there was no longer a significant difference between the morphine withdrawal-paired floor and saline-paired floor for two consecutive days.

A reinstatement cycle commenced one week after the final extinction trial. On Day 1 rats were injected (ip) with either VEH ($n=12$) or 0.3 mg/kg URB597 ($n=12$), and then with 1 ml/kg saline (sc) 10 min prior to a 10-min choice test. On Day 2, the rats were administered 10 mg/kg of morphine (sc) at 2 h prior to an injection (ip) of 0.3 mg/kg URB597 or VEH. Two hours later they all received an injection (sc) of naloxone (0.5 mg/kg) 10 min prior to a 10-min reinstatement choice test. The mean amount of time spent on each floor and the overall distance (cm) traveled in the conditioning chamber was analyzed by the Noldus Ethovision activity monitoring system.