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Research report

The long-term effects of stress and kappa opioid receptor activation on conditioned place aversion in male and female California mice

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ABSTRACT

Psychosocial stress leads to the activation of kappa opioid receptors (KORs), which induce dysphoria and facilitate depression-like behaviors. However, less is known about the long-term effects of stress and KORs in females. We examined the long-term effects of social defeat stress on the aversive properties of KOR activation in male and female California mice (*Peromyscus californicus*) using a conditioned place aversion paradigm. Female California mice naïve to social defeat, formed a place aversion following treatment with 2.5 mg/kg of the KOR agonist U50,488, but females exposed to defeat did not form a place aversion to this dose. This supports the finding by others that social defeat weakens the aversive properties of KOR agonists. In contrast, both control and stressed males formed an aversion to 10 mg/kg of U50,488. We also examined EGR1 immunoreactivity, an indirect marker of neuronal activity, in the nucleus accumbens (NAc) and found that stress and treatment with 10 mg/kg of U50,488 increased EGR1 immunoreactivity in the NAc core in females but reduced activation in males. The effects of stress and U50,488 on EGR1 were specific to the NAc, as we found no differences in the bed nucleus of the stria terminalis. In summary, our data indicate important sex differences in the long-term effects of stress and indicate the need for further study of the molecular mechanisms mediating the behavioral effects of KOR in both males and females.

1. Introduction

Psychosocial stress induces the activation of kappa opioid receptors (KORs) and is an important risk factor for depression [1]. KORs are considered to be a promising target for new therapeutics to treat depression and anxiety because activation of KORs by agonists induces dysphoria [2–4]. The administration of KOR antagonists before psychosocial stress blocks behavioral phenotypes such as social withdrawal [5], behavioral despair [6–8], anxiety [9], and drug seeking behaviors [10–13]. A common theme in studies examining the therapeutic effects of KOR antagonists is that the behavioral experiments are conducted within 24 h or less after stress. However, psychosocial stress may induce important long-term neuroadaptations that affect KOR signaling.

Previous reports observed that the KOR agonist U50,488 decreases social interaction behavior [14] and that administration of the KOR antagonist norBNI before social defeat prevents decreases in social interaction [5]. However, stress may have different long term effects that alter the role of KORs in social behavior. In male mice that repeatedly lost aggressive interactions, U50,488 increased social interaction, but the same dose of U50,488 decreased social interaction in male mice with no experience in aggressive interactions [15,16]. In these studies, aggressive contests occurred over a 10-day period, a much longer time frame than studies examining effects of KOR antagonists in the context of stress. Similarly, administration of the long-lasting KOR antagonist JDTic before 10 days of social defeat stress did not prevent the development of decreased social interaction or anhedonia in male mice [17]. These data suggest that, over time, neuroadaptations induced by stress may alter the function of KORs.

Place conditioning studies provide a useful measure of the aversive properties of KOR agonists, and these studies suggest that long-term exposure to stress may alter the aversive properties of KORs. In male mice, short-term activation of KORs induces aversion [4,18] and restores drug-seeking behavior in the reinstatement model of stress-induced drug relapse [11,19–21]. For example, a conditioned place preference (CPP) for cocaine was induced in male mice and then extinguished [20]. In male mice naïve to stress, treatment with U50,488

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reinstated the CPP for cocaine, and a single episode of acute forced swim stress before U50,488 treatment potentiated this effect. However, U50,488 did not reinstate cocaine CPP in mice exposed to either 5 days of social defeat stress (completed the day before reinstatement testing) or 3 weeks of chronic mild stress (completed 10 days before reinstatement testing) [20]. These data suggest that stress has different short- and long-term effects on KOR activation.

Another gap in the literature is that most research on KOR function has been conducted on males. However, depression and anxiety are more common in women than men [22] and sex differences in physiological responses to stress are an important risk factor [23–25]. Shershen et al. [26] showed that the KOR agonist U62,066 potentiated cocaine-induced locomotor activity in female mice during the first 20 min of testing, but this difference dissipated over the next hour. However, Wang et al. [27] reported that the KOR agonist U50,488 decreased cocaine-induced locomotor activity in female but not male guinea pigs across 90 min. It is possible that KOR agonists affect locomotor activity differently in mice compared to guinea pigs. In contrast, female rats were less sensitive than males to the depressive-like effects of KOR agonist U50,488 in an intracranial self-stimulation paradigm [28]. Finally, no sex differences were observed in the effects of KOR facilitation of selective aggression toward novel conspecifics in male and female prairie voles [29]. To our knowledge, no previous study has examined the long-term effects of stress on the function of KORs in both males and females.

We studied *Peromyscus californicus* (California mouse), a monogamous species of rodent, in which both the male and the female are aggressive towards conspecifics [30]. This allowed us to observe the effects of social defeat stress in males, as well as females. In California mice, three episodes of social defeat induced social aversion in females but not males [25,31,32]. We used a place aversion assay to quantify the aversive properties of the KOR agonist U50,488 and to determine whether social defeat stress has different effects on aversion in males and females. Previously, males formed a place aversion to 10 mg/kg of U50,488, whereas females showed aversion to 2.5 mg/kg [14]. We hypothesized that long-term effects of defeat stress would reduce the aversive properties of U50,488. Here, we tested the effects of social defeat stress and two doses of U50,488 on conditioned place aversion (CPA) in males and females. We then examined how stress and U50,488 affected immediate early gene induction in nucleus accumbens (NAc) core and shell regions. The NAc is an important site of KOR action that is very sensitive to defeat stress [25] and also an important site mediating KOR-induced aversion [18]. Specifically, in male C57Bl6 mice optogenetic stimulation of the ventral NAc shell induced aversion, whereas stimulation of the dorsal NAc shell induced preference, as measured by real time conditioned place preference [18]. We quantified early growth response 1 (EGR1, also known as Zif268), an immediate early gene that, like c-fos, can be used as an indirect marker of neuronal activity. EGR1 protein activation is rapid and transient, peaking between 1 and 2 h after neuronal activation [33]. We also examined EGR1 activity in several subregions of the bed nucleus of the stria terminalis (BNST) because of its strong connections with the mesolimbic dopamine system [34] and involvement in reward and aversion [35]. We hypothesized that increased EGR1 expression would be observed in the ventral NAc of animals that formed stronger U50,488-induced aversion.

2. Methods

2.1. Animals and housing conditions

Adult male and female California mice (3–4 months old) were obtained from our breeding colony at UC Davis. They were group housed with 2–3 same-sex animals per cage. Animals were maintained in a temperature-controlled room (68–74 °F) on a 16L-8D cycle (lights off at 1400) with *ad libitum* water and food (Harlan Teklad 2016, Madison, WI). Mice were housed in Polycarbonate plastic cages containing Sanichip bedding, nestlets, and envirodri. All procedures were approved by the Institutional Animal Care and Use Committee (IACUC) and conformed to NIH guidelines. Social defeat stress was conducted during lights out (1400–1700) under dim red light (3 lx). Conditioned place aversion (CPA) testing was conducted during lights on (800–1400).

2.2. Social defeat stress

Male and female California mice were randomly assigned to social defeat stress or control handling for three consecutive days [32,36]. Mice assigned to social defeat were placed in the home cage of an aggressive, same-sex, sexually-experienced resident mouse. The experimental mouse remained in the resident's cage for either 7 min or 7 attacks, whichever occurred first. Control mice were introduced to a clean, empty cage for 7 min. Each experimental mouse was exposed to a different resident for each of the three episodes of defeat stress.

2.3. Place conditioning procedure

Two weeks after social defeat stress or control handling, conditioned place aversion (CPA) was conducted as described in Robles et al. [14] (Fig. 1). The apparatus consisted of three visually distinct interconnected standard sized mouse cages (28 × 17.5 × 11 cm). The center cage (black and white horizontal stripe background) was connected to a left cage (black and white vertical stripe background) and a right cage (black dots on a white background and textured plastic floor). After each test the apparatus was cleaned with Quatricide (1:64, Quatricide PV in water, Pharmacal Research Labs, Inc). Clear polypropylene lids were used to cover the apparatus during experiments to allow for video recording.

Conditioned place aversion was conducted over 4 days during the light cycle. The protocol was designed so that the mice learn to associate vehicle or drug injection with a given chamber. On day 1 (pretest) mice were placed in the apparatus and allowed to explore freely for 30 min while being tracked in real time with a visual tracking system (Any-maze Stoelting). For each mouse, initial place biases were corrected for by assigning drug conditioning to the preferred side chamber ([14,7]).

Mice were randomly assigned to be conditioned with either vehicle (10% Tween 80 in sterile PBS), 2.5 mg/kg, or 10 mg/kg of (±) U50,488 (Tocris, Ellisville, MO, USA) administered i.p. On days 2 and 3, each mouse received two training sessions. In the morning, each mouse received an i.p. injection of vehicle and was placed in the unconditioned chamber for 30 min. Afterwards mice were returned to their home cages. Three hours later, each mouse received an injection of either vehicle, 2.5 mg/kg, or 10 mg/kg of U50,488 and was placed in the conditioned chamber for 30 min. During training sessions, the entrance to the center chamber was closed so mice were confined to a single chamber. On day 4, each mouse was given free access to the entire apparatus for 30 min (post-test) and was tracked with the video tracking system. The time spent in each cage and the total distance traveled were both recorded. On day 5 mice were injected i.p. with their assigned conditioned drug, and, 1 h later, anesthetized with isoflurane and euthanized by decapitation. Brains were immediately

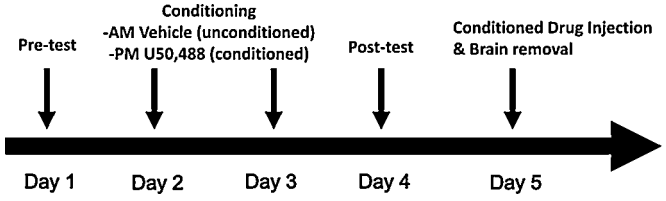

Fig. 1. Timeline for conditioned place aversion experiments.