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The corticotropin-releasing factor receptor-2 mediates the motivational effect of opiate withdrawal

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ABSTRACT

Altered motivational processes are key features of drug dependence and withdrawal, yet their neural mechanisms remain largely unknown. The present study shows that genetic disruption of the corticotropin-releasing factor receptor-2 ($CRF_2-/-$) does not impair motivation for palatable food in drug-naïve mice. However, CRF_2 receptor-deficiency effectively reduces the increase in palatable food-driven motivation induced by opiate withdrawal. Indeed, both in male and female wild-type mice, withdrawal from escalating morphine doses (20–100 mg/kg) induces a dramatic and relatively long-lasting (6 days) increase in palatable food-driven operant behavior under a progressive ratio (PR) schedule of reinforcement. In contrast, either male or female morphine-withdrawn $CRF_2-/-$ mice show smaller and shorter (2 days) increases in motivation than wild-type mice. Nevertheless, CRF_2 receptor-deficiency does not impair the ability to discriminate reinforced behavior prior to, during the partial opiate withdrawal periods occurring between morphine injections and following drug discontinuation, indicating preserved cognitive function. Moreover, CRF_2 receptor-deficiency does not affect the ambulatory or body weight effects of intermittent morphine injections and withdrawal. These results provide initial evidence of a gender-independent and specific role for the CRF_2 receptor in the motivational effects of opiate withdrawal.

1. Introduction

Opiate dependence is a major health problem across the world (www.who.int/substance_abuse). Studies show an alarming raise in the recreational use of opiate drugs among adolescents, indicating that the incidence rate of opiate dependence may dramatically increase in the next years (Compton and Volkow, 2006). Altered motivational processes are key features of opiate dependence. In opiate-dependent individuals, the primary excessive motivation is for opiate drugs (Cooper et al., 2008; Kenny et al., 2006; Negus, 2006). However, studies also show altered motivation for food or non-opiate drugs, especially during opiate withdrawal periods. For instance, opiate-withdrawn individuals show heightened preference and craving for sweet food (Morabia et al., 1989; Weiss, 1982). Accordingly, opiate-withdrawn rats show decreased or increased motivation for food, as assessed by operant behavior or home-cage food intake paradigms (Cooper et al., 2010; Ford and Balster, 1976; Langerman et al., 2001; Steinfels and Young, 1981). Opiate withdrawal also increases cocaine seeking and intake in rats and monkeys, although no effect is also reported (Cooper et al., 2010; Gerak et al., 2009; He and Grasing, 2004). More recently, we report long-lasting increases in the motivation for palatable food in opiate-withdrawn mice (Rouibi and Contarino, 2012). Thus, opiate withdrawal may increase behavior directed to intake of non-opiate substances. This suggests profound and generalized alterations in motivational processes, which might dramatically reduce the ability to overcome drug dependence. Nevertheless, the neural mechanisms underlying the motivational effects of opiate withdrawal remain largely unknown.

The corticotropin-releasing factor (CRF) system coordinates behavioral, neuroendocrine and autonomic responses to stressors (Koob, 1999; Rivier et al., 1982). The CRF system may also be implicated in substance dependence. Indeed, CRF receptor antagonists attenuate stress-induced reinstatement of ethanol- or

Abbreviations: ANOVA, analysis of variance; CRF, corticotropin-releasing factor; CRF_1, corticotropin-releasing factor receptor-1; CRF_2, corticotropin-releasing factor receptor-2; CTL, control; FR, fixed ratio; i.p., intraperitoneally; OW, opiate-withdrawn; PR, progressive ratio.

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cocaine-seeking behavior and decrease ethanol self-administration and anxiety-like behavior in ethanol- or cocaine-withdrawn rats (Basso et al., 1999; Erb et al., 1998; Funk et al., 2006; Le et al., 2000; Rassnick et al., 1993). In mammals, CRF-like signaling is transmitted by two receptors, termed CRF_1 and CRF_2 (Hauger et al., 2003). The two known CRF receptors may differentially contribute to opiate withdrawal signs and symptoms. In particular, CRF_1 receptor-deficient mice do not show any conditioned aversion for places paired with morphine withdrawal but display elevated somatic signs and impaired ability to cope with the stressful condition of opiate withdrawal, as compared to wild-type mice (Papaleo et al., 2008, 2007). In contrast, CRF_2 receptor-deficiency completely eliminates the somatic signs and the negative affective-like states of opiate withdrawal without impairing stress-coping abilities (Ingallinesi et al., 2012; Papaleo et al., 2008). However, the role for the CRF system in the motivational effects of opiate withdrawal is yet poorly understood.

Available CRF receptor antagonists do not provide definitive evidence in favor of the specific role for the CRF_2 (versus the CRF_1) receptor in behavior. Indeed, although some compounds show higher CRF_2 (versus CRF_1) *in vitro* receptor binding affinity (Grace et al., 2007; Ruhmann et al., 1998), to our knowledge neither behavioral nor CRF receptor activity studies yet exist to support receptor-selectivity. Moreover, based on the reported CRF_1/CRF_2 receptor binding affinity ratios (ranging from 100 to 500), it cannot be excluded that such compounds interact with both CRF receptors, at least at the behaviorally active doses usually employed. On the other hand, $CRF_2-/-$ mice show preserved CRF_1 receptor activity (Bale et al., 2000; Contarino et al., 2000; Coste et al., 2000; Papaleo et al., 2008), suggesting the possibility to investigate CRF_2 (versus CRF_1) receptor function. Thus, to address the role for the CRF_2 receptor in the motivational effects of opiate withdrawal, in the present study we use male and female wild-type and $CRF_2-/-$ mice (Bale et al., 2000). Notably, to mimic the clinical setting motivation for palatable food is assessed following body clearance of the drug, i.e., during "spontaneous" opiate withdrawal (Rouibi and Contarino, 2012).

2. Material and methods

2.1. Subjects

Group-housed littermate wild-type (25 males and 24 females) and $CRF_2-/-$ (25 males and 22 females) mice with a mixed C57BL/6Jx129 strain background are used throughout (Bale et al., 2000). The mice derive from mating $CRF_2+/-$ mice, are identified by PCR analysis of tail DNA and are 3–6 months old at the beginning of the experiment. They are housed in a colony room (22 ± 2 °C, relative humidity: 50–60%) on a 12-h light/dark cycle (lights on at 0800 h). Standard laboratory food (3.3 kcal/g; SAFE, Augy, France) and water are available *ad libitum*. All studies are conducted in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC) and are approved by the local Animal Care and Use Committee. All efforts are made to minimize animal suffering and to reduce the number of animals used.

2.2. Operant behavior apparatus

Each operant behavior apparatus (length: 22 cm, width: 14 cm, height: 20 cm) is equipped with dim light sources and with two nose-poke holes (1 cm in diameter, 8.5 cm apart, 2.5 cm from the grid floor) mounted at the opposite ends of the same wall, each equipped with infrared photo-beams connected to a computer (Imetronic, Pessac, France). Nose-poking into one of the two holes, i.e., the active hole, results in food pellet delivery whereas nose-poking into the other hole, i.e., the inactive hole, has no consequences. Centered between the nose-poke holes is a food trough situated 2 cm from the grid floor; food pellet delivery occurs when the photo-beam of the active nose-poke hole is interrupted for at least 500 msec. We use 20-mg palatable food pellets (5-TUL, 3.4 kcal/g; PMI Nutrition International, LLC, St. Louis, MO, USA), which are delivered by an automated dispenser situated outside the apparatus. Photo-beams allow monitoring of food trough visits and an additional food pellet is not delivered until a food trough visit (removal of the previously delivered food pellet), thereby allowing resolution of food-directed behavior at the unit of an individual food pellet. The wire grid floor of the cage allows the passage of uneaten food pellets to a sliding drawer, making storage impossible and allowing evaluation of food spillage. Each apparatus is also equipped with two series of photo-beams that serve to record horizontal and vertical ambulatory activity.

2.3. Experimental design

Prior to the beginning of the operant behavior experiment, on alternate days each mouse is handled for 1 min for a total of three times. Starting on the day after the last handling session, at 1600 h the mice are daily confined to the apparatus for a 2-h test. Within genotype and gender, half of the mice are assigned the left and the other half the right nose-poke hole as the active hole. A fixed ratio (FR)-1 reinforcement schedule is initially applied for 10 consecutive days, i.e., one nose-poke results in the delivery of one food pellet. Then, an FR-3 and an FR-6 reinforcement schedule are each applied for three consecutive days, during which 3 or 6 active nose-pokes produce the delivery of one food pellet, respectively. For male mice, food pellets obtained, food pellets spilt and extra-pokes (non-reinforced active nose-pokes) made during each FR phase are also calculated. The mice are then switched to a progressive ratio (PR)-2 reinforcement schedule for 19 days (3 baseline, 6 morphine and 10 opiate withdrawal days). In particular, the mice are daily confined to the apparatus for a 2-h test from 1600 h to 1800 h, i.e., 8–10 h after the first daily morphine injection during the morphine treatment period. Under the PR-2 reinforcement schedule, the number of active nose-pokes required to obtain each successive food pellet is progressively increased by 2. We calculate the breakpoint as the last PR level achieved during the 2-h test. For example, to earn the fourth food pellet a mouse has to nose-poke 2 + 4 + 6 + 8 times in the active hole, and thus is given a breakpoint value of 8. The breakpoint is a well-validated measure of the strength of the reinforcer and of the motivational state of the animal (Arnold and Roberts, 1997; Hodos, 1961). Learning criteria are also applied: mice that during the third PR-2 baseline day ingest less than 8 food pellets and/or show a discrimination index (active nose-pokes/total nose-pokes*100) lower than 75% are excluded from the study. Then, within genotype the mice are divided into two groups with similar breakpoint values on the third PR-2 day. Starting on the following day, at 0800 and 2000 h one group is intraperitoneally (i.p.) injected with physiological saline (10 ml/kg) and the other with morphine HCl (Francopia, Gentilly, France), as follows: day 1: 20 mg/kg, day 2: 40 mg/kg, day 3: 60 mg/kg, day 4: 80 mg/kg, day 5: 100 mg/kg, day 6: 100 mg/kg, only one injection at 0800 h. The latter drug regimen allows partial opiate withdrawal between drug injections (Papaleo and Contarino, 2006). The mice are weighed just before each injection and body weight changes calculated as percentage of the body weight recorded just prior to the first injection. Following cessation of saline or morphine injections, body weight is measured every 3 days for an additional 12-day period.

2.4. Statistical analysis

A 3-way analysis of variance (ANOVA) with genotype (wild-type, $CRF_2-/-$), gender (male, female) as between subjects factors and repeated measures (daily operant tests) as a within-subject factor is used to examine active nose-poke, discrimination index, food pellet intake, breakpoint, horizontal and vertical activity recorded during the FR and the PR phases preceding saline or morphine injections. A 2-way ANOVA with genotype as a between subjects factor and repeated measures as a within-subject factor is used to analyze the mean daily number of food pellets obtained, food pellets spilt and extra-pokes made by male mice during the FR-1, FR-3 and FR-6 phases. A 2-way ANOVA with genotype and gender as between subjects factors is used to analyze the mean daily number of food pellets eaten during the 3 baseline PR days. A 4-way ANOVA with genotype, gender and treatment (control, opiate-withdrawn) as between subjects factors and repeated measures (daily operant tests) as a within-subject factor is used to analyze breakpoint, food pellet intake, discrimination index, horizontal activity, vertical activity and body weight changes recorded prior to (third PR-2 baseline day), during the partial opiate withdrawal periods occurring between morphine injections and following drug discontinuation. The Student–Newman–Keuls *post-hoc* test is used for individual group comparisons. The accepted value for significance is $P < 0.05$.

3. Results

3.1. CRF_2 receptor-deficiency does not impair the acquisition of operant behavior

Wild-type and $CRF_2-/-$ mice do not differ in active nose-pokes performed during the FR reinforcement schedules (Fig. 1A). Increasing the FR requirement leads to a genotype-independent elevation in active nose poke (repeated measure effect: $F_{15,1275} = 36.21$, $P < 0.0001$). However, during the FR-3 and the FR-6 exercises female mice make less active nose-pokes than male mice (gender × repeated measures interaction effect: $F_{15,1275} = 3.02$, $P < 0.0001$; Fig. 1A). Male and female wild-type and $CRF_2-/-$ mice also show similar discrimination indices, which progressively