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Corticotropin-releasing factor 1 receptor mediates the activity of the reward system evoked by morphine-induced conditioned place preference

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ABSTRACT

Different neurotransmitter systems are involved in behavioural and molecular responses to morphine. The brain stress system is activated by acute administration of drugs of abuse, being CRF the main neuropeptide of this circuitry. In this study we have studied the role of CRF1R in the rewarding effects of morphine using the CPP paradigm. For that, animals were treated with a CRF1R antagonist (CP-154,526) or vehicle during 6 days. Thirty min after receiving the antagonist, mice were injected with morphine on the same days that CP-154,526 was administered; another group received saline on the same days that vehicle was administered, and both groups were immediately conditioned. Control animals received vehicle and saline every day. On day 7, animals were tested for morphine-induced CPP. c-Fos, TH and OXA immunohistochemistry, NA turnover (HPLC), and corticosterone plasma concentration (RIA) were evaluated. Administration of a CRF1R antagonist CP-154,526 blocked the morphine-induced CPP and the increased NA turnover in the NAc in morphine-paired mice. CP-154-526 antagonised the enhancement in c-Fos expression evoked by morphine-induced CPP in the VTA and NAc, and the activation of the orexinergic neurons in the LLH. Present work demonstrates that morphine-induced CPP activates different brain areas involved in reward, and points out a critical role of CRF1R in molecular changes involved in morphine-conducted behaviours. Thus, our study supports a therapeutic potential of CRF1R antagonists in addictive disorders.

1. Introduction

Opiate drugs are the most powerful agents used for treating intense acute and chronic pain. However, the nonmedical use, misuse and abuse of opiates, such as morphine and heroine, are increasing in the last years (Casati et al., 2012; Manchikanti et al., 2012). Repeated opiates administration has been long-term associated with adverse consequences, such as tolerance, withdrawal and addiction, which limit their clinical use. These processes have been extensively investigated during the last years (Kreek et al., 2012; Dacher and Nugent, 2011; Christie, 2008; Bailey and Connor, 2005; Williams et al., 2001), but it is still a challenge to reveal the neurobiological substrates involved in the behavioural changes induced by the consumption of opiates and other drugs of abuse.

Conditioned place preference (CPP) has been widely used to study the rewarding properties of addictive substances (Tzschentke, 2007). At present, it is known that the ventral tegmental area (VTA) and its dopaminergic projections to the nucleus accumbens (NAc) are one of the most important substrates for drug reward. Virtually, all drugs of abuse, directly or indirectly, promote the release of DA in the NAc. More recently, other neurotransmitter systems have been involved in the rewarding effects of drugs of abuse, such as noradrenaline (NA; Smith and Aston-Jones, 2008; Weinshenker and Schroeder, 2006)and

Abbreviations: BNST, bed nucleus of the stria terminalis; CeA, central nucleus of the amygdala; CPP, conditioned place preference; CP, CP-154,526; CRF, corticotrophin releasing factor; DMH, dorsomedial hypothalamus; GC, glucocorticoids; HPA axis, hypothalamus pituitary adrenal axis; HPLC, high performance liquid chromatography; LC, locus coeruleus; LH, lateral hypothalamus; LLH, lateral region of the lateral hypothalamus; NAc, nucleus accumbens; NTS-A2, nucleus of the solitary tract-A2 cell group; OXA, orexin A; PFA, perifornical area; PVN, paraventricular nucleus; RIA, radioimmunoassay; Tween, tween-80 (10%); VTA, ventral tegmental area.

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orexins (OX; Tabaeizadeh et al., 2013; Richardson and Aston-Jones, 2012). Noradrenergic neurons from the locus coeruleus (LC) and the nucleus of the solitary tract-A_2 noradrenergic cell group (NTS-A_2) innervate the reward system and provide excitatory drive to dopaminergic and dopaminoceptive neurons (Weinshenker and Schroeder, 2006; Brogg et al., 1993). Additionally, it has been reported that OXA increases the firing frequency of VTA dopaminergic neurons in rat brain slices as well as the presence of OX receptors in the VTA (Korotkova et al., 2003).

As previously stated, acute exposure to drugs of abuse promotes an increase of activity in the reward circuit. Furthermore, an activation of the brain stress system has also been described (Koob and Le Moal, 2001). Corticotropin-releasing factor (CRF), the main neuropeptide regulating the stress system activity, is widely distributed throughout the brain and plays a key role in coordinating behavioural and autonomic responses to stress (Owens and Nemeroff, 1991). CRF binds to two G protein-coupled receptors: CRF receptor type 1 (CRF1R) and CRF receptor type 2 (CRF2R), both of them expressed in the VTA and NAc (Sauvage and Steckler, 2001; Van Pett et al., 2000; Lemos et al., 2012). The VTA and NAc receives CRF innervations from multiple sources, including those from the central nucleus of the amygdala (CeA), the nucleus of the bed nucleus of the stria terminalis (BNST) and the hypothalamic paraventricular nucleus (PVN; Swanson et al., 1983; Rodaros et al., 2007; Lemos et al., 2012). These nuclei also send CRF projections to the LC and NTS-A_2. Additionally, recent studies provide evidence for projections from CRF neurons to orexinergic cells in the lateral hypothalamus (LH) and vice versa (Winsky-Sommerer et al., 2004; Harris and Aston-Jones, 2006).

Recently, we have shown that morphine-induced CPP evokes an increase in DA and NA turnover in the NAc, accompanied by an enhancement of tyrosine hydroxylase (TH; the rate-limiting enzyme of catecholamine biosynthesis) enzymatic activity, as shown by its phosphorylation state (González-Cuello et al., 2010). Given the possible relevance of the CRF inputs to the neurocircuitry involved in the rewarding effects of drugs of abuse, in the present work we evaluated the role of CRF1R in (i) mediating somatic and behavioural states produced by morphine administration; (ii) the activation (as shown by c-Fos expression) of different brain nuclei involved in drug reward in morphine-paired mice; (iii) the responses of both the mesolimbic dopaminergic system and its noradrenergic innervations to morphine-induced CPP; and (iv) the activity of the hypothalamus-pituitary-adrenal (HHA) axis elicited by morphine associated stimuli. We have used CP-154,526 as a selective CRF1R antagonist. c-Fos expression was measured as an index of neuronal activation.

2. Material and methods

2.1. Animals

All surgical and experimental procedures were performed in accordance with the European Communities Council Directive of 22 September 2010 (2010/63/UE), and were approved by the local Committees for animal research (CEEA; RD 53/2013). Adult male Swiss mice (25–35 g at the beginning of the experiments) were housed in cages sized 36 × 15 × 20 cm, and in groups of six animals per cage under a 12-h light/dark cycle (light: 8:00–20:00 h) in a room with controlled temperature (22 ± 2 °C). Food and water were available *ad libitum*. Animals were conditioned and tested during the light phase of the cycle. They were handled daily during the first week after arrival to minimize stress.

2.2. Materials

Morphine hydrochloride was obtained from Alcaliber Laboratories (Madrid, Spain). NA bitartrate, MHPG hemipiperazinium salt, DA HCl and DOPAC (used as HPLC standards) were purchased from Sigma–Aldrich (Sigma, St Louis, MO, USA). CP-154,526, [N-butyl-N-ethyl-2,5-dimethyl-7-(2,4,6-trimethylphenyl)-pyrrolo-[3,2-e]-pyrimidin-4-amine] (selective CRF1R antagonist), was kindly provided by Pfizer (New York, NY) and dissolved in Twen-80 (10%; Sigma–Aldrich). Reagents included goat serum (Vector Laboratories, Burlingame, CA, USA) and avidine–biotin complex (Vectastain ABC, Elite Kit).

The place conditioning apparatus is based on that used by Valverde et al. (1996) with some modifications and consisted in two rectangular polycarbonate compartments (20 × 18 × 25 cm) spaced at 4 cm from each other, both accessible from a rectangular polyvinyl chloride exterior area (20 × 7 × 25 cm). In order to distinguish the three compartments, visual and sensory texture cues were used; one compartment was grey striped wall with black smooth floor and the other was black spotted wall with grey rough floor. The neutral area providing access the compartments had transparent wall and floor. One compartment of the place preference apparatus was randomly chosen to be paired to drug administration and the other to saline. The time spent in each chamber was recorded for each mouse by a computer program (CPP Win 2.0. Panlab, Barcelona, Spain).

2.3. Place preference paradigm

The rewarding effects of morphine were evaluated using the CPP paradigm, as previously described (Maldonado, 1997). The place preference conditioning schedule consisted of three phases:

Preconditioning phase, during which the mice were placed in the middle of the neutral area, and their localization was recorded for the following 15 min. After the session, animals were randomized to be paired to drug (morphine-paired group) or saline (saline-paired group) administration and to be assigned to a compartment. Animals that spent less than 6 min in either the white or black compartment were considered not to be neutral in preference for either side and were excluded from further study (less than 5% of mice).

Conditioning phase, during which the mice were treated on 6 consecutive days with an injection of the CRF1R antagonist (30 mg/kg, i.p.) on days 1, 3 and 5, or vehicle (Tween 80 10%, i.p.) on days 2, 4 and 6. Peripherally administered CP-154,526 crosses the blood brain barrier and reaches maximal brain concentration after 20 min (Keller et al., 2002). Thus, 30 min after receiving the antagonist, mice received a dose of morphine hydrochloride (6 mg/kg, s.c.). Given that saline does not evoke spatial conditioning and that CRF can modulate learning and memory consolidation (Abiri et al., 2014), a dose of vehicle instead of CRF1R antagonist was given to animals on the same days that saline (10 mL/kg, s.c.) was administered. Doors matching the walls of the compartment allowed confinement of the mice for 20 min immediately after morphine or saline injections. Mice received morphine on days 1, 3 and 5 and saline on days 2, 4 and 6. Control animals received Tween 80 (10%) and saline every single day.

Testing phase was conducted exactly as the preconditioning phase (free access to both compartments for 18 min, 24 h after the final conditioning session).

A score was calculated for each mouse as the difference between post-conditioning and the pre-conditioning time spent in the drug-paired compartment, in order to measure this parameter.

2.4. Perfusion and immunohistochemical detection of c-Fos

Immediately after the testing phase, mice were deeply anesthetized with a subletal dose of pentobarbital and quickly perfused through the ascending aorta with saline followed by ice-cold fixative (paraformaldehyde 4%). Brains were post-fixed in the fixative with sucrose (30%) for 3 h and then placed in PBS containing 30% sucrose overnight. Series of 30 μm frontal sections were cut on freezing microtome, collected in cryoprotectant and stored at −20 °C until processing. After blocking with H_2O_2 and normal goat serum (Sigma, St Louis, MO, USA), tissue sections were incubated in the primary antibody rabbit anti-c-Fos (sc-52, 1:10,000, Santa Cruz Biotechnology, Santa Cruz, CA, USA). This was followed by application of a biotinylated anti-rabbit IgG (1:500, Vector Laboratories), and then with the avidin–biotin complex. Visualization of the antigen–antibody reaction sites was performed using 3, 3′-diaminobenzidine (DAB, Sigma) nickel intensification. Sections were mounted onto chrome-alumn gelatine coated slides, dehydrated through graded alcohols, cleared in xylene and coverslipped with dibutylphtalate (DPX).

2.5. Double-labelling immunohistochemistry of c-Fos immunoreactive nuclei and TH- or OXA-positive neurons

For double-labelling, tissue sections from each mouse in each treatment group were processed for c-Fos immunoreactivity using DAB nickel intensification and then TH was revealed using DAB chromogen only. Briefly, c-Fos immunostaining was performed as described above. Following the c-Fos staining, sections were rinsed in PBS, treated with bovine albumin serum and incubated with the rabbit polyclonal anti-TH antibody (AB152, Chemicon, USA; 1:6000). The same immunohistochemistry procedures described above were followed. The TH- antibody–peroxidase complex was developed in DAB. The sections were mounted onto chrome-alumn gelatine coated slides and coverslipped.

For the c-Fos/OXA double labelling, the process was the same as described before for c-Fos/TH. The primary anti OXA antibody was purchased from Santa Cruz Biotechnology (sc-8070) and was diluted 1:2000.

2.6. Quantification of c-Fos immunoreactivity

Images were captured by means of DM4000B Leica microscope (Leica, Madrid, Spain) equipped with a video camera (DFC290; Leica). The distribution of c-Fos-positive cell nuclei was plotted using a computer assisted image analysis system

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