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Accumbal dopamine, noradrenaline and serotonin activity after naloxone-conditioned place aversion in morphine-dependent mice

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ABSTRACT

Dopamine (DA) neurons not only show a pattern signaling the magnitude, delay and probability of rewards but also code negative motivation and aversive events. Beside DA, other systems such as noradrenaline (NA) and serotonin (5-HT) may also be implicated in naloxone-induced conditioned place aversion (CPA; an index of the aversive consequences of withdrawal). The purpose of the present study was to evaluate: (i) the turnover of DA, NA and 5-HT in the nucleus accumbens (NAc), one of the most important substrates for aversive states, (ii) the changes in tyrosine hydroxylase (TH) gene expression in the ventral tegmental area, and (iii) total TH protein levels and TH phosphorylation in the NAc after naloxone-induced morphine withdrawal. DA, NA and 5-HT turnover was evaluated by high-performance liquid chromatography (HPLC). TH gene expression was determined by real time quantitative PCR (RT-PCR) and total TH and TH phosphorylated at Ser31 and Ser40 were analyzed by Western blot. Present results show that the aversion for environmental cues paired with opioid withdrawal was higher than that observed in the saline group treated with naloxone, which indicates that morphine pretreatment potentiated the ability of naloxone to produce place aversion. In addition, present data show that naloxone-induced CPA positively correlated with an increase of DA and NA turnover in the NAc, which paralleled an increase in TH gene expression in the VTA and TH phosphorylation and enhanced TH protein levels in the NAc. Thus, the present study indicates that naloxone-induced aversion in morphine-dependent mice enhances DA and NA activity in the NAc and suggests that transcriptional and post-transcriptional regulation of TH could be involved in the hyperactivity of mesolimbic dopaminergic system observed in morphine-withdrawn mice.

1. Introduction

Chronic exposure to opioids can lead to dependence, a phenomenon manifested by withdrawal-induced somatic, autonomic, and aversive symptoms (Badiani et al., 2011). These negative emotional states resulting from withdrawal may contribute to escalation to compulsive use, maintenance of use, and relapse after periods of abstinence (Aston-Jones and Harris, 2004; Koob and Le Moal, 2008; Self, 1998).

Many studies in the drug addiction field have identified the nucleus accumbens (NAc) and its dopaminergic inputs from the ventral tegmental area (VTA) of midbrain as one of the most important anatomical substrates for drug reward and aversion (Carlezon and Thomas, 2009; Koob and Le Moal, 2001). Previous studies have shown that the mesolimbic dopaminergic system that projects from the VTA to NAc is critical for initiation of opioid reinforcement and for the reward-related effects of drugs of abuse (Di Chiara and Bassareo, 2007; Koob, 1992).

Research indicates that midbrain DA neurons not only show a pattern signaling the magnitude, delay and probability of rewards but also code negative motivation and aversive events (Matsumoto and Hikosaka, 2009). In addition to DA, other systems such as noradrenaline (NA) and serotonin (5-HT) may play a large role in the aversive states (Kranz et al., 2010; Smith and Aston-Jones, 2008). Recently, the role of NA in addiction has received interest stemming from its importance in stress-induced reinstatement, drug-induced locomotion and opioid conditioned place preference (CPP). Direct noradrenergic inputs from the nucleus tractus

Abbreviations: CPA, conditioned place aversion; DA, dopamine; NA, noradrenaline; 5-HT, serotonin; NAc, nucleus accumbens; VTA, ventral tegmental area; TH, tyrosine hydroxylase; NTS-A2, nucleus of the solitary tract, noradrenergic cell group; 5-HIAA, 5-hydroxyindolacetic acid; DOPAC, dihydroxyphenyl acetic acid; MHPG, 3-metoxy-4-hydroxyphenylglycol.

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solitarius (NTS)-A2 to the VTA and NAc have been described (Weinshenker and Schroeder, 2006).

On the other hand, several studies found that stimulation of the dorsal and median raphe nucleus, the major sites of serotoninergic neurons that project to the entire brain, are effective reinforcers equal to stimulation of VTA and medial forebrain bundle (Van Der Kooy et al., 1978). There is a large body of research using different kind of reward paradigms that underpin the substantial account of 5-HT in reward (for Review see Kranz et al., 2010). However, the role of 5-HT in aversive processing remains unclear.

Conditioned place aversion (CPA) is a highly sensitive index of the aversive motivational consequences of withdrawal from a chronic state of opioid dependence (Stinus et al., 1990). Given the importance of the monoaminergic pathways in drug addiction, the aim of the present study was to investigate the possible mechanisms implicated in the negative aversive states of opioid withdrawal. For that, we have studied the changes in the content of DA and its metabolite dihydroxy phenyl acetic acid (DOPAC), NA and its metabolite 3-metoxy-4-hydroxyphenylglycol (MHPG) and 5-TH and its metabolite 5-hydroxyindolacetic acid (5-HIAA) in the NAc to determine DA, NA and 5-HT turnover after conditioned place aversion to morphine.

A variety of physiological stressors stimulate catecholamine synthesis through regulatory effects on TH. Long-term regulation of catecholamine synthesis is achieved through changes in the level of tyrosine hydroxylase (TH) protein (the rate-limiting enzyme in catecholamine synthesis), whereas short-term catecholamine synthesis regulation occurs through phosphorylation of TH, which enhances enzymatic activity (Dunkley et al., 2004; Kumer and Vrana, 1996). Changes in the state of phosphorylation of TH are critically involved in the regulation of catecholamine synthesis. In particular, increases in the phosphorylation of Ser31 and Ser40 accelerate TH activity, thereby stimulating production of neurotransmitter.

We have recently shown that naloxone-induced morphine withdrawal stimulates TH activity and NA turnover in the hypothalamic paraventricular nucleus (PVN) (Núñez et al., 2007b), which correlated positively with an increase in TH mRNA expression in the NTS-A_2 noradrenergic cell group, which is the main noradrenergic nucleus innervating the PVN. So, in the present study, we have checked whether the increase in DA turnover in the NAc is correlated with TH mRNA expression in the VTA and/or with TH phosphorylation in the NAc.

2. Methods

2.1. Animals

All surgical and experimental procedures were performed in accordance with the European Communities Council Directive 24 November 1986 (86/609EEC) and the local Animal Ethics Committee. Adult male Swiss mice (25–35 g at the beginning of the experiments) were housed seven 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 week preceding the experiments start to minimize stress.

2.2. Conditioned place aversion (CPA)

Negative affective states associated with opiate withdrawal were examined by using conditioned CPA test, a behavioral technique commonly used to evaluate the affective-like consequences of drug withdrawal in rodents. The place conditioning apparatus is based on that used by (Valverde et al., 1996) with some modifications and it consisted in two rectangular polycarbonate compartments (length, 20 cm; width, 18 cm; height, 25 cm) spaced at 4 cm from each other, both accessible from a rectangular polyvinyl chloride exterior area (length, 20 cm; width, 7 cm; height, 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 whereas the other compartment was black spotted wall with grey rough floor. The neutral area providing access to the compartments had transparent wall and floor. The guillotine doors, which were made with the color corresponding to the respective wall color, were inserted during the conditioning sessions and were removed during the preconditioning and postconditioning tests. The CPA schedule during 6 days consisted of three phases: preconditioning test, conditioning phase and postconditioning test.

2.2.1. Preconditioning

On day 0 (preconditioning test), each mouse was allowed to explore freely the CPA apparatus for 18 min, and time spent in each of the two compartments was recorded for each mouse by a computer program (CPP Win 2.0. Panlab, Barcelona, Spain). Animals than spent less than 8 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).

2.2.2. Conditioning

On conditioning phase, one group was assigned to receive saline and the other was assigned to receive morphine. Mice were rendered dependent on morphine by intraperitoneally injection of increasing doses of morphine (20–70 mg/kg, i.p.). The control group received saline instead of morphine. Starting on day 1, every 12 h (at 8 a.m. and 8 p.m.) mice were treated with saline or morphine according to the following protocol: day 1, 20 mg/kg; day 2, 40 mg/kg; day 3, 60 mg/kg; day 4, 70 mg/kg (only one injection in the morning). One hour after last morphine injection, one compartment of the place aversion apparatus was randomly chosen to be paired to naloxone (morphine-treated group and saline-treated group) or saline (saline-treated group and morphine-treated group) administration, and then doors matching the walls of the compartment allowed confinement of the mice for 20 min immediately after naloxone or saline injections.

2.2.3. Postconditioning Test

Twenty-four hours after the end of conditioning, the postconditioning test (day 5) allowed free exploration of the CPA apparatus for 18 min and it was conducted exactly as the preconditioning test. For each mouse, a place aversion score was calculated as the difference between postconditioning and preconditioning time spent in the conditioning compartment of the CPA apparatus.

The animals undergoing morphine withdrawal displayed characteristic withdrawal symptoms: Wet-dog shakes, teeth chattering, ptosis, tremor, piloerection, lacrimation, rhinorrhea, chromodiacryorrhea, spontaneous jumping, and diarrhea. In addition, we have quantified body weight loss after naloxone (1 mg/kg s.c.). Loss of body weight was calculated as the difference between the body weight determined immediately before saline or naloxone injection and a second determination made 20 min later, immediately after the conditioning.

2.3. Tissue preparation

Mice were decapitated immediately after the CPA test for HPLC, gene expression and Western blot assays. The brain regions of interest were micropunched from frozen sections (500 μm) prepared in a cryostat, according to the mice brain atlas of Frankin