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Induction of conditioned place preference and dopamine release by salsolinol in posterior VTA of rats: Involvement of μ-opioid receptors

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ABSTRACT

Salsolinol (Sal), locally administered into the posterior VTA (pVTA) of rats, produces psychomotor responses and reinforcing effects, probably, through the activation of μ-opioid receptors (MORs). The neurochemical correlates of these phenomena are, however, practically unknown. In this paper, we explore the neurochemical events and the mechanisms involved in these behaviors. To do that, we test the ability of Sal, directly microinjected into the pVTA, to induce conditioned place preference (CPP) and to increase dopamine levels in the nucleus accumbens shell. Bilateral injections of 30 pmol of Sal induced a strong CPP (rats spent around 70% of the total test time), a result that could be explained by the fact that Sal microinjected into the pVTA increased DA levels in the ipsilateral accumbens up to 141% of baseline. The local pretreatment with β-FNA, an antagonist of MORs, prevented this increase, supporting our hypothesis on the involvement of MORs in the Sal-derived effects.

1. Introduction

Salsolinol (Sal) is a tetrahydroisoquinoline formed in brain as a result of the condensation of dopamine (DA) and acetaldehyde, the main metabolite of ethanol (Naoi et al., 2004).

In 1970, Sal was postulated as an important factor contributing to alcohol addiction (Cohen and Collins, 1970). Several controversial results, mainly on the levels of Sal in brain after ethanol ingestion questioned its involvement in alcoholism, although this has not been totally discarded (Myers, 1996). Recent studies have unequivocally demonstrated that Sal levels increase in several brain areas, including nucleus accumbens (NAc) and midbrain (Starkey et al., 2006; Rojkovicova et al., 2008) after very different alcohol drinking procedures; thus providing fresh impetus to investigations on its contribution to alcoholism.

As a consequence, in recent years several laboratories have explored the effects of Sal in some addiction-related behaviors. So, very recently, it has been demonstrated that very low doses (30 and 300 pmol) of Sal directly applied into the posterior ventral tegmental area (pVTA) induce motor activation in rats (Hipolito et al., 2010), an effect that was prevented by the local pre-treatment with antagonists of the μ-opioid receptors (MORs).

Also very recently, it was shown that Sal has reinforcing effects. So, using the intracranial-self administration (ICSA) technique, Rodd et al. (2008) showed that rats readily learn to self-administer low doses (0.01 pmol/100 nL) of this compound into the pVTA. An earlier study showed that intraperitoneal injections of 10 mg/kg of Sal produced a slight conditioned place preference (CPP) (Matsuzawa et al., 2000), although the drug was three times more potent to develop CPP when assays were conducted under the conditioned fear stress paradigm. Importantly, the pre-treatment with antagonists of MORs significantly attenuated the preference under fear conditioned stress. Moreover, it was also showed that Sal combined with morphine at doses unable to produce CPP, induced a marked CPP that was almost completely blocked by antagonists of MORs. Globally, these findings strongly suggested the involvement of the opioidergic system in the effects of Sal.

The neurochemical basis underlying Sal behavioral effects, are practically unknown. To our knowledge, few authors have paid adequate attention to the neurochemical consequences of the central administration of this compound. Our group showed that Sal directly applied into the NAc by reverse dialysis was able to modify, in a dose and subregion-dependent manner, the DA levels in the NAc (Hipolito et al., 2009). So, when applied into the shell of the NAc, Sal decreased DA levels, whereas applications in the core caused an increase in the DA levels. These findings were very intriguing, since similar responses were obtained after intra-NAc applications of μ- and δ-opioid receptors agonists (Hipolito et al., 2008). Although it was not directly demonstrated, our data strongly suggested the possibility of a Sal-MORs interaction.

In the present paper, we perform two additional experiments in order to prolong our knowledge on the behavioral and neurochemical consequences of Sal central administration. We have firstly

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explored the ability of Sal directly microinjected into the pVTA to induce CPP. Second, we have performed an *in vivo* microdialysis study in order to evaluate if the reinforcing and motor activating effects of Sal could be explained by an increase in the activity of the mesolimbic DA system. To do that, we have microinjected this compound into the pVTA of rats and evaluated the changes in DA levels of the ipsilateral NAc shell. The involvement of the MORs located in this brain area in these effects has been also analyzed.

2. Experimental procedures

2.1. Animals

Male Wistar rats (300 g) were used for our experiments. Rats were housed in plastic cages ($27 \times 50 \times 14\ cm^3$) in groups of four with controlled humidity and temperature (22 °C), a 12:12-h light/dark cycle and had free access to regular food and water. Procedures were carried out in accordance with the EEC Council Directive 86/609, Spanish laws (RD 223/1998) and animal protection policies. Experiments were previously approved by the Animal Care Committee of the Facultat de Farmàcia, Universitat de València, Spain.

2.2. Drugs and chemicals

Sal was purchased from Sigma Chemical Co. (St. Louis, MO). β-Funaltrexamine (β-FNA, an irreversible antagonist of the μ-opioid receptors) was obtained from Tocris (Bristol, UK). Sal was freshly dissolved in artificial cerebrospinal fluid (aCSF) solution immediately before use (Hipolito et al., 2010). Stock solutions of β-FNA were prepared by dissolving the compound in the correct volume of distilled water and prior to use, were conveniently diluted with aCSF solution.

All the other reagents used were purchased from Sigma Chemical Co. (St. Louis, MO).

2.3. Surgical procedures

Rats were anaesthetized with isoflurane (1.5 MAC) and placed in a stereotaxic apparatus (Stoelting, USA). Animals for the CPP experiment were implanted bilaterally, with two 28-gauge guide cannula (Plastics One) aimed at 1.0 mm above the pVTA. On the other hand, animals for the microdialysis experiment were implanted with two guide cannulae, one aimed at 1.00 mm above the pVTA (28-gauge) for drug administration and the second one aimed at 2.8 mm above the shell of the NAc (20-gauge) for microdialysis probe implantation. The stereotaxic coordinates relative to bregma and skull surface for both brain areas were the same than in our previous published studies (Hipolito et al., 2010, 2009). Following surgery; rats were housed in individual rectangular plastic cages ($15 \times 30 \times 14\ cm^3$), with free access to food and water.

2.4. Experiments

2.4.1. Experiment 1: CPP

The CPP test was performed in a two-compartment box connected by a removal barrier with an open door in the middle. The two compartments differed by the wall color (black and white vertical stripes and black and white horizontal stripes) and the shape of the inner space.

Sixteen animals were used in this experiment. Prior and posterior to the surgery, animals were handled everyday for 4 days. The day prior to the start of the conditioning sessions, animals were exposed to the CPP box for 5 min in order to habituate them to the apparatus. During the conditioning phase, animals belonging to the experimental group ($n = 8$) were subjected to intra-VTA Sal bilateral administration (30 pmol/200 nL/hemisphere) in one of the two compartments of the box, on three occasions and, to intra-VTA aCSF microinjection in the other compartment, on other three occasions on the intervening days. The control group ($n = 8$) received six aCSF microinjections (3/compartment). Injections were always performed out of the CPP box, using the same microinjection procedure than in a previous study of our group (Hipolito et al., 2010). After the injection animals were immediately placed into the appropriate compartment for 30 min.

Animals were randomly assigned to the experimental or control group and the exposure to conditioning compartments was counterbalanced in both groups. The next day to the last conditioning session, each animal was tested for place preference; it was placed in the open door of the barrier, and the time spent in each compartment was recorded over 15 min. The time spent (seconds) in both compartments was quantified using Raddot program (Universitat de València, Spain). At the end of the test, rats were overdosed with chloral hydrate and brains were removed for histological validation of probe placements. Only data from animals in which cannulae were correctly placed were included in the data analysis.

2.4.2. Experiment 2: microdialysis experiment

Four groups ($n = 6$/group) were planned: (i) two experimental groups that received intra-pVTA either aCSF or β-FNA (2.5 nmol/300 nL), 24 h previous to the experimental day and Sal (30 pmol/200 nL)during the microdialysis experiment(Groups: ''aCSF+Sal'' and ''β-FNA+Sal'', respectively) and (ii) two control groups which received intra-pVTA either aCSF or β-FNA (2.5 nmol/300 nL) 24 h previous the experimental day and aCSF during the microdialysis experiment (Groups: ''aCSF+aCSF'' and ''β-FNA+aCSF'', respectively). All animals received intra-pVTA pre-treatments with aCSF or β-FNA in their respective home cages and treatments with Sal or aCSF also in their home cage while connected to the microdialysis setup.

Animals used in this experiment were handled every day for 5 min for 4 days, starting 2 days after the surgery. Two days before starting the microdialysis experiment the microdialysis probe (2 mm of active membrane, Hospal, AN69) was lowered into the shell of the NAc. At the time of starting, dialysis probes were perfused at 3.5 μL/min with aCSF. DA concentrations in the dialysates were on-line analyzed every 20 min by HPLC with electrochemical detection (Hipolito et al., 2009).

Treatments were performed after the establishment of a baseline (defined as three consecutive samples with less than 10% variation in DA content). Afterwards, DA in dialysates was monitored over 120 min.

At the end of the experiment, probe and injection cannulae locations were histologically evaluated and animals whose probe and/or cannulae were not simultaneously placed in the shell and the pVTA were discarded for the data analysis.

2.5. Statistical analysis

Results from experiment 1, were expressed in seconds spent in both compartments (mean ± SEM). Differences were analyzed through the Student's *t*-test for paired data.

DA baseline levels obtained in each group from experiment 2 were expressed in nanomolar. Differences among the mean baseline levels in the four groups were analyzed by using the one-way ANOVA.

DA content in dialysates was expressed as percent of the respective baseline. The effects of treatments on DA levels were analyzed by a mixed three-way ANOVA. Simple main effects analysis was performed when significant interactions were detected.

The level of significance was always set at $p < 0.05$. Statistical analysis were performed using SPSS v 17.