Progress in Neuro-Psychopharmacology & Biological Psychiatry 47 (2013) 77–84

Contents lists available at ScienceDirect

Progress in Neuro-Psychopharmacology & Biological Psychiatry

journal homepage: www.elsevier.com/locate/pnp

Assessment of the abuse potential of MDMA in the conditioned place preference paradigm: Role of CB1 receptors ☆,☆☆

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ARTICLE INFO

Article history:
Received 23 May 2013
Received in revised form 18 July 2013
Accepted 19 July 2013
Available online 17 August 2013

Keywords:
CB1 receptor
Conditioned place preference
Knockout mice
MDMA

ABSTRACT

Numerous reports have highlighted the role of the endocannabinoid system in the addictive potential of MDMA (3,4-methylenedioxy-methamphetamine). A previous report showed that CB1 knockout (KOCB1) mice do not acquire MDMA self-administration, despite developing conditioned place preference (CPP). This contradiction could be due to the particular procedure of place conditioning used. The present work compares MDMA-induced CPP in KOCB1 mice using *unbiased* and *biased* procedures of place conditioning. In the *unbiased* procedure, MDMA induced CPP and reinstatement of the extinguished preference in wild type (WT) mice, but not in KOCB1 mice. In contrast, in a *biased* protocol of CPP, MDMA produced preference in both types of mice. The anxiolytic response induced by MDMA in the elevated plus maze (EPM) was observed only in KOCB1 mice and may have been responsible, at least partially, for the CPP in the *biased* procedure. A neurochemical analysis revealed that KOCB1 mice presented higher striatal DA and DOPAC levels in response to MDMA, but no alterations in their levels of monoamine transporters. In line with previous self-administration studies, our data suggest that CB1 receptors play an important role in the reinforcing effects of MDMA, and that the experimental procedure of CPP employed should be taken into account when drawing conclusions.

1. Introduction

Use of the illicit drug MDMA (3,4-methylenedioxy-methamphetamine), known in popular terms as "ecstasy", has spread over time, with many users consuming the drug frequently (George et al., 2010). MDMA acts as a reinforcer in both CPP (Daza-Losada et al, 2007; Robledo et al., 2004) and self-administration paradigms (Ratzenboeck et al., 2001; Schenk et al., 2003; Trigo et al., 2006). Recent studies have shown that a priming injection of MDMA can reinstate a previously extinguished MDMA-induced CPP in rodents (Daza-Losada et al., 2009, 2011; Manzanedo et al., 2010; Ribeiro do Couto et al., 2012; Rodriguez-Arias et al., 2010). Additionally, extinguished MDMA self-administration can be reinstated by MDMA-conditioned cues (Orejarena et al., 2011; Schenk et al., 2011) or priming doses (Schenk et al., 2008).

Recent reports indicate that almost 90% of ecstasy users (with an age range of 15–64 years old) also consume cannabis, which make it the drug most widely consumed with MDMA, followed by alcohol, tobacco and cocaine (UNODC World Drug Report, 2010). The endocannabinoid system is the primary site of action of the rewarding and pharmacological responses induced by cannabinoids (Ledent et al., 1999), but it also exerts a general modulatory effect on the reward circuitry and is involved in the rewarding and addictive properties of some drugs of abuse as opioids (Manzanedo et al., 2004; Solinas et al., 2005), nicotine (Valjent et al., 2002), alcohol (Colombo et al., 2002) and cocaine (Fattore et al., 1999). Several physiological responses mediated by MDMA administration are also modulated by the endocannabinoid system (Giuffrida et al., 1999; Piomelli, 2005).

MDMA and cannabinoid agonists such as WIN 55212-2 produce reinforcing effects in mice and rats when administered alone (Daza-Losada et al., 2007; Manzanedo et al., 2004, 2010; Zarrindast et al., 2007). Previous studies suggest that cannabinoid agonists potentiate the rewarding effects of MDMA (Braida and Sala, 2002)

Abbreviations: 5-HT, Serotonin; 5-HIAA, 5-Hydroxyindoleacetic acid; CPP, Conditioned Place Preference; DA, Dopamine; DAT, Dopamine Transporter; DOPAC, 3,4-Dihydroxyphenylacetic acid; DTT, Dithiothreitol; EDTA, Ethylenediaminetetraacetic Acid Disodium Salt Dehydrate; EPM, Elevated Plus Maze; EtOH, Ethanol; HPLC, High-performance liquid chromatography; HVA, Homovanillic Acid; KOCB1, CB1 knockout; MDMA, 3,4-methylenedioxy-methamphetamine; N. Acc, Nucleus Accumbens; PFC, Prefrontal Cortex; Pre-C, Pre-conditioning phase; Post-C, Post-conditioning phase; SERT, Serotonin Transporter; THC, Delta-9-tetrahidrocannabinol; WT, Wildtype.

☆ Conflict of interest statement: None of the authors have any relevant financial interests to disclose, or a conflict of interest of any kind.

☆☆ Source of funding: This work was supported by the following grants: Ministerio de Economía y Competitividad, Dirección General de Investigación (PSI2008-00101, PSI2011-24762 to J.M. and SAF2010-15793 to O.V.); Instituto de Salud "Carlos III" (FIS), RETICS, Red de Trastornos Adictivos (RD06/001/0016; RD12/0028/005 to J.M. and RD06/001/1001; RD12/0028/0024 to O.V.); Generalitat Valenciana, Conselleria de Educación (PROMETEO/2009/072); and Generalitat de Catalunya (2009SGR684), Spain.

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http://dx.doi.org/10.1016/j.pnpbp.2013.07.013

and that cannabinoid antagonists exert the opposite action (Braida et al., 2005). However, Robledo et al. (2007) demonstrated that THC modifies sensitivity to the behavioral effects of MDMA in different ways (increase/decrease) depending on the dose employed. A series of studies carried out in our laboratory revealed that WIN 55212-2 increases the rewarding effects of MDMA (1.25 mg/kg), but only at low doses (Manzanedo et al., 2010). In addition, we have seen how the reinforcing effects of MDMA are enhanced in mice exposed to the cannabinoid agonist WIN 55,212-2 during adolescence (Rodríguez-Arias et al., 2010), and potentiate the effects of sub-threshold priming doses of MDMA (Daza-Losada et al., 2011). These results highlight the key role of the CB1 receptor in enhancing the reinstatement of drug-seeking behavior, and point to the importance of the endocannabinoid system in the addictive potential of MDMA.

The use of knockout CB1 (KOCB1) mice has helped to throw light on this complex interaction. Touriño et al. (2008) reported that CB1 knockout mice did not acquire self-administration behavior at any of the doses of MDMA administered, although the drug did induce CPP and enhanced extracellular levels of DA in the N Acc. of mutant mice. The CPP paradigm has been widely used to study the conditioned rewarding effects of addictive drugs (Tzschentke, 2007). The neutral pre-conditioning phase (Pre-C test) of the CPP can be designed in such a way that naive animals do not show a significant preference for any one of the conditioning compartments upon initial exposure (*unbiased* design), or it can be designed so that they show an unconditioned preference for one side rather than the other (*biased* design). In general, studies tend to employ *unbiased* experimental designs, as a *biased* design can yield false-positive results in place conditioning experiments. For example, if a drug has a strong anxiolytic component, it can override the initial aversion for the non-preferred compartment, thus increasing the preference scores for that particular compartment.

The discrepant data reported regarding the role of CB1 receptors in the reinforcing effects of MDMA in self-administration and CPP paradigms could be a result of employing different procedures of place conditioning. The present study was designed with the aim of exploring in depth the capacity of MDMA to induce CPP in KOCB1 mice. With this aim, CPP was carried out following a strictly *unbiased* procedure in which animals showing strong preference or aversion for any of the compartments were excluded from the rest of the procedure. In addition, all the mice performed a counterbalance procedure independently of their initial preference. In order to evaluate the influence of anxiety, the animals were placed in the elevated plus maze (EPM) under standard conditions and after being treated with MDMA. Changes in the concentration of brain monoamine in response to MDMA, and striatal concentrations of dopamine and serotonin transporters (DAT and SERT) were also determined.

2. Material and methods

2.1. Subjects

The experiments were carried out with male KOCB1 mice (n = 32) and wild-type littermates (n = 38) weighing 35 to 40 g on initiation of the experiments. Mice lacking the CB1 cannabinoid receptor were generated as described previously (Ledent et al., 1999). In order to homogenize the genetic background of the mice, the first heterozygous generation was bred for 30 generations on a CD1 background, with selection for the mutant CB1 gene at each generation. After the 30th generation of backcrossing, heterozygote–heterozygote mating of CB1 knockout mice produced wild-type (WT) and knockout littermates for subsequent experiments. These animals were housed in groups of four in plastic cages (25 × 25 × 14.5 cm) for the 10 days prior to initiation of experiments, under the following conditions: constant temperature (21 ± 2 °C), a reversed light schedule (white lights on: 19.30–07.30 h), and food and water available *ad libitum*, except during behavioral tests. Procedures involving mice and their care were conducted in compliance with national, regional and local laws and regulations, which are in accordance with the European Communities Council Directives (86/609/EEC, 24 November 1986).

2.2. Drugs

Animals were injected i.p. with 5 or 10 mg/kg of MDMA (3,4-methylenedioxymetamphetamine hydrochloride, Laboratorios Sigma–Aldrich, Spain) in a volume of 0.01 ml/g. Control groups were injected with the physiological saline (NaCl 0.9%) used to dissolve the drugs.

2.3. Experimental design

The experimental procedure is shown in Table 1.

2.3.1. Elevated plus maze

The apparatus consisted of two open arms (30 × 5 × 0.25 cm) and two enclosed arms (30 × 5 × 15 cm). The junction of the four arms formed a central platform (5 × 5 cm). The floor of the maze was made of black Plexiglas and the walls of the enclosed arms of clear Plexiglas. The open arms had a narrow edge (0.25 cm) to provide additional grip for the animals. The entire apparatus was elevated 45 cm above floor level. In order to facilitate adaptation, mice were transported to the dimly illuminated laboratory 1 h prior to testing. At the beginning of each trial, subjects were placed on the central platform so that they were facing an open arm, and were allowed to explore for 5 min. The maze was thoroughly cleaned with a damp cloth after each trial. The mice's behavior was video recorded and later analyzed by a "blind" observer using a computerized method. The measurements recorded during the test period were frequency of entries and the time and percentage of time spent in each section of the apparatus (open arms, closed arms, central platform). An arm was considered to have been entered when the animal placed all four paws on it. The numbers of open arm entries, time spent in open arms and percentage of open arm entries are generally used to characterize the anxiolytic effects of drugs (Pellow and File, 1986; Rodgers, 1997).

All the mice performed the EPM test as soon as the acclimatization period had concluded (day 11 of the experimental procedure) and underwent the CPP procedure a further 10 days later. Fifteen days after the last reinstatement test (day 85th of the experimental procedure), mice performed the EPM test after being treated with the same MDMA dose administered previously for the CPP procedure (n = 15 for WT conditioned with 5 mg/kg MDMA; n = 14 for WT conditioned with 10 mg/kg MDMA; n = 10 for KOCB1 conditioned with 5 mg/kg

Table 1
Experimental procedure.

Days since mice arrived at the laboratory	Day 1 to day 10	Day 11	Day 21 to Day 70	Day 85	Day 100
Behavioral test	Acclimatization period	Elevated plus maze	Conditioned place preference	Elevated plus maze after MDMA	Obtention of brain samples for monoamine and Western blot analyses