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Maintenance of amphetamine-induced place preference does not correlate with astrocytosis

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ABSTRACT

Astrocytosis, a process in which astrocytes undergo proliferation and enhancement of glial fibrillary acidic protein (GFAP) expression, has been suggested to play important roles in the maintenance of dependence to amphetamine and its derivatives. It was previously shown that mice with genetic deletion of pleiotrophin (PTN), a neurotrophic factor upregulated in different brain areas after administration of amphetamine, show a longer lasting amphetamine-induced conditioned place preference (CPP) when compared to wild type mice. In this work, we aimed to pursue the possibility of a different astrocytic response induced by amphetamine in PTN−/− and PTN+/+ mice, which could underlie the higher vulnerability of PTN−/− mice to maintain amphetamine CPP. In confirmation of previous studies, we found that PTN−/− mice significantly maintained amphetamine (3 mg/kg)-induced CPP 5 days after the last drug administration compared to PTN+/+ mice. Interestingly, the number of astrocytes in nucleus accumbens (NAcc), cingulate cortex (CG) and caudate putamen (CPu) did not differ between mice that maintained and did not maintain amphetamine-induced CPP independently of the genotype considered. However, we found that PTN−/− mice showed significantly decreased numbers of astrocytes in CG and CPu compared to PTN+/+ mice independently of whether they maintained amphetamine-induced CPP 5 days after the last drug administration or not. The data demonstrate that maintenance of amphetamine-induced CPP depends on the endogenous expression of PTN. The data tend to discard a correlation between activated astrocytes and maintenance of amphetamine conditioning effects and suggest PTN as a potential modulator of activation of astrocytes after amphetamine treatment.

1. Introduction

Alterations in the number, morphology and physiology of glial cells in critical areas for development of addictive behaviours such as nucleus accumbens (NAcc), caudate putamen (CPu) and cingulate cortex (CG) after exposure to drugs of abuse, may contribute to the vulnerability in maintaining drug seeking behaviours (Miguel-Hidalgo, 2009). These alterations include astrocytosis (Raivich et al., 1999), a process in which astrocytes undergo proliferation, morphological changes and enhancement of glial fibrillary acidic protein (GFAP) expression, which has been suggested as playing an important role in the development of drug dependence (Narita et al., 2008). Administration of amphetamine and its derivatives and most psychostimulants induces astrocytosis (Itzhak and Achat-Mendes, 2004; Fattore et al., 2002; Hebert and O'Callaghan, 2000). For instance, the levels of GFAP in the mouse NAcc and CG were found to be increased after chronic treatment with methamphetamine (Narita et al., 2005). Interestingly, the neurotrophic activity of these astrocytes seems to be important for the development and maintenance of drug addictive behaviours (Miguel-Hidalgo, 2009). For instance, it has been demonstrated that increased expression of glial-derived neurotrophic factor (GDNF) efficiently blocks methamphetamine-induced conditioned place preference (Niwa et al., 2007).

Another neurotrophin known to be expressed in astrocytes, pleiotrophin (PTN), has been recently confirmed as an important regulator of drug of abuse-induced neurotoxic and rewarding effects (see reviews by Herradon et al., 2009; Gramage and Herradon, 2011). Endogenous expression of PTN has been recently found to be key in the modulation of amphetamine-induced cognitive, addictive and neurotoxic effects (Herradon and Ezquerra, 2009; Gramage et al., 2010a, b, in press). In those reports, PTN knockout (PTN−/−) mice exhibited longer lasting amphetamine-induced conditioning effects (Gramage et al., 2010a) and exacerbated neurotoxic effects induced by amphetamine in the striatum and substantia nigra (Gramage et al., 2010b). In these studies, the astrocytosis observed 4 days after a neurotoxic

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regime of amphetamine injections (10 mg/kg, four administrations, one every 2 h) was significantly increased in the CPu and substantia nigra of PTN−/− mice compared to wild type (PTN+/+) mice. Whether or not this differential astrocytic response generated by PTN−/− mice in response to the neurotoxic regime of amphetamine injections could be observed after administration of amphetamine rewarding regimes remains to be studied.

In the present study, we have pursued the possibility of a different astrocytic response induced by amphetamine in both mouse genotypes, PTN−/− and PTN+/+, which could underlie the higher vulnerability of mice lacking endogenous PTN to maintain amphetamine (3 mg/kg)-induced place preference 5 days after the last administration of the drug (Gramage et al., 2010a). We have tested the numbers of GFAP+ cells in NAcc, CG and CPu of mice from both genotypes that differ in the maintenance of amphetamine-induced place preference. These brain areas were chosen because a correlation was previously shown between development of astrocytosis in NAcc, CPu and CG and the rewarding effects of amphetamine (Narita et al., 2005, 2008).

2. Materials and methods

2.1. Pleiotrophin genetically deficient (PTN−/−) mice

PTN−/− mice, generated as previously described (Amet et al., 2001; Herradon et al., 2004; Gramage et al., 2012), were kindly provided by Dr. Thomas F. Deuel (The Scripps Research Institute, La Jolla, CA). The PTN gene consists of five exons encoding an 18-kDa protein with a 32 amino acid signal peptide. The replacement targeting vector generated a PTN null allele (PTN $^{2-4neo}$) by deleting exons 2 to 4. In both behavioural and immunohistochemical studies, we used male PTN−/− and wild type (PTN+/+) animals of 9–10 weeks (20–25 g). It has to be noted that the lack of gross alterations of basic behavior was previously described in PTN−/− mice (Pavlov et al., 2002; Gramage et al., in press).

All the animals used in this study were maintained in accordance with European Union Laboratory Animal Care Rules (86/609/ECC directive) and the protocols were approved by the Animal Research Committee of USP-CEU. Mice were housed at 22 ±1 °C with a 12 h light/12 h dark cycle (lights on at 7 am) with standard mouse chow (Harlan, Barcelona, Spain) and water provided ad libitum.

2.2. Conditioned place preference (CPP)

2.2.1. Apparatus

The apparatus used consisted of two Plexiglas square compartments of the same size (20 cm long × 14 cm high × 27 cm wide). One compartment had black plexiglas floor and walls and the other had black plexiglas floor and white walls. During the amphetamine- and saline-paired sessions, the compartments were closed by a removable black guillotine door.

2.2.2. CPP procedure

The procedure selected for this study was based on a modification of the method previously used in our laboratory (Morales et al., 2007). The procedure to evaluate amphetamine-induced conditioning consisted of a 5-day schedule with three phases: preconditioning (day 1), conditioning (days 2–4) and testing (day 5). During preconditioning, PTN+/+ mice and PTN−/− mice were free to explore the two compartments for a 30-min period; their behaviour was monitored by a videotracking system (San Diego, California, USA) to calculate the time spent in each compartment. Placement was counterbalanced within each treatment group such that half the animals started in one chamber and half started in the other. As expected, and previously shown (Gramage et al., 2010a, 2011), the compartment with white walls was the less-preferred compartment by both mouse genotypes (~30% stay of total time in the preconditioning phase).

In experiments to evaluate amphetamine-induced CPP, the conditioning phase consisted of a 3-day schedule of double conditioning sessions. The first one involved a morning session starting at 8 am, in which animals received a single injection of saline i.p. (10 ml/kg) and were immediately confined to the initially preferred compartment for 30 min. In the evening session starting at 3 pm, the animals were injected (i.p.) with 3 mg/kg amphetamine (PTN+/+, $n=12$; PTN−/−, $n=18$) and confined to the initially less-preferred chamber for 30 min. During the following 2 days, the procedure used was the same but the order of the treatments (morning/evening) was changed to avoid the influence of circadian variability. In control experiments, PTN+/+ and PTN−/− mice received injections of saline i.p. (10 ml/kg) at both times (morning/evening) during the 3 days of the conditioning phase.

The testing phase was carried out on day 5 of the schedule. In this phase the animals freely moved throughout the apparatus for 30 min, exactly as in the preconditioning phase (day 1). The time spent in each compartment was also registered. The percentage of time-spent (stay) in the less-preferred compartment was then calculated in all cases and the difference between the time spent in the drug-paired (or saline-paired in the case of controls) compartment in this phase and the time spent in the same compartment in the preconditioning was considered as indicative of the degree of conditioning induced by the drug.

In previous studies, we already showed that amphetamine (3 mg/kg) exerts a similar CPP in PTN−/− and PTN+/+ mice as assessed in the testing phase (day 5) of this procedure (Gramage et al., 2010a). In those studies, it was also shown that amphetamine-induced CPP was only significantly maintained in PTN−/− mice 5 days after the last administration of the drug. In the present studies we aimed to test the possibility of a different astrocytic response in both genotypes correlating with the genotypic differences found in the maintenance of amphetamine CPP. For this purpose, after the testing phase (day 5), animals were returned to their cages and were neither injected nor re-exposed to the CPP apparatus for a 4-day period. After that period of time (5 days after last drug administration; day 9 of the procedure), place conditioning was re-examined by conducting a new 30-min free choice test.

2.3. Immunohistochemistry studies

As indicated above, the purpose of the immunohistochemical analysis was to study the astrocytic response in different brain areas of mice from both genotypes that maintained and did not maintain amphetamine-induced CPP on day 9 of the procedure. The selection criteria of mice for immunohistochemical analysis according to maintenance of amphetamine-induced CPP was as follows. Mice that increased their stay in the drug-paired compartment by 15% or more in the testing phase (day 5) compared to the preconditioning phase were considered to be efficiently conditioned by the drug. After the test on day 9 of the CPP procedure, these mice were separated in two different groups per genotype: The ones that did not maintain (NM) drug-induced CPP, whose percentages of stay in the drug-paired compartment were reduced by more than 10% compared to the testing phase (day 5), and the ones that maintained (M) drug-induced CPP, whose percentages of stay in the amphetamine-paired compartment were similar or even greater to those in the testing phase (day 5).

Mice from both M and NM groups in every genotype were euthanized and brains rapidly removed and conserved in *p*-formaldehyde for 7 days and transferred to a solution of 0.1 M phosphate buffer containing 0.02% sodium azide for storage at 4 °C. Following