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Sensitization to amphetamine occurs simultaneously at immune level and in met-enkephalin of the nucleus accumbens and spleen: An involved NMDA glutamatergic mechanism

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ABSTRACT

Administration of psychostimulants can elicit a sensitized response to the stimulating and reinforcing properties of the drugs, although there is scarce information regarding their effects at immune level. We previously demonstrated that an acute exposure to amphetamine (5 mg/kg, i.p.) induced an inhibitory effect on the splenic T-cell proliferative response, along with an increase in met-enkephalin at limbic and immune levels, 4 days following drug administration. In this study, we evaluated the amphetamine-induced effects at weeks one and three after the same single dose treatment (5 mg/kg, i.p.) on the lymphoproliferative response and on the met-enkephalin in the nucleus accumbens (NAc), prefrontal cortex (PfC), spleen and thymus. It was demonstrated that these effects disappeared completely after three weeks, although re-exposure to an amphetamine challenge induced the expression of sensitization to the effects of amphetamine on the lymphoproliferative response and on the met-enkephalin from NAc, spleen and thymus, but not in the PfC. Pre-treatment with MK-801 (0.1 mg/kg, i.p.), an *N*-methyl-D-aspartate (NMDA) glutamatergic receptor antagonist, blocked the effects of a single amphetamine exposure on the lymphoproliferative response and on met-enkephalin in the NAc and spleen. Furthermore, the NMDA receptor antagonist administered prior to amphetamine challenge also blocked the expression of sensitization in both parameters evaluated. These findings show a long-lasting amphetamine-induced sensitization phenomenon at the immune level in a parallel way to that occurring in the limbic and immune enkephalineric system. A glutamate mechanism is implied in the long-term amphetamine-induced effects at immune level and in the met-enkephalin from NAc and spleen.

1. Introduction

Acute and repeated amphetamine administration leads to a progressive and long-lasting enhancement of its behavioral effects. This phenomenon, called behavioral sensitization, is a useful model for drug-induced neuroplasticity in neuronal circuits pivotal for addiction (Kalivas and Stewart, 1991; Robinson and Berridge, 2000; Kauer and Malenka, 2007). Although at immune level there is also evidence of amphetamine sensitization on immunoreactivity in mice repeatedly treated with this drug (Kubera et al., 2002), the long-lasting effects of a single amphetamine exposure are still unknown.

Dopamine and glutamate are among the main neurotransmitters associated with behavioral sensitization, but enkephalin (ENK) has also been investigated (Pierce and Kalivas, 1997; Wolf, 1998). There is ample evidence that psychostimulants modulate dopaminergic and glutamatergic transmission (Vanderschuren and Kalivas, 2000; Kalivas, 2007), while their modulation on the enkephalinergic system has been frequently less studied (Mao and Wang, 2003; Wang and McGinty, 1996). Glutamatergic and enkephalinergic transmissions have been shown be mutually influenced after amphetamine administration (Liste et al., 2000; Rawls and McGinty, 2000), with glutamate as well as dopamine being able to regulate the synthesis of ENK (Dudman et al., 2003; Mao and Wang, 2003). Behavioral data has shown that daily microinjections with an ENK analog into the ventral tegmental area (VTA) result in a progressive increase of the spontaneous motor activity and response to amphetamine (Kalivas, 1985), and also that the opioid system is involved in the expression of amphetamine sensitization (Magendzo and Bustos, 2003). Related to this, we have recently provided the first demonstration of an increase in met-ENK levels in key mesocorticolimbic areas related to sensitization, such as the nucleus accumbens (NAc) and prefrontal cortex (PfC), 4 days after a single amphetamine exposure (Assis et al., 2006). However,

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there is as yet no evidence concerning psychostimulant-induced sensitization on central met-ENK.

Other results have shown dopamine, glutamate and met-ENK to influence the immune response (Kavelaars et al., 2005; Pacheco et al., 2006; Stanojevic et al., 2007). Previous evidence from our laboratory demonstrated that dopamine has a modulatory role on the chronic amphetamine-induced effects on the peripheral lymphocyte subpopulation levels (Assis et al., 2008), with a single dose treatment of amphetamine inducing increased met-ENK levels in the spleen and thymus together with a decreased lymphoproliferative response (Assis et al., 2006). Specifically, we found that the increased splenic met-ENK was produced by macrophages (Assis et al., 2006) which contain the PC1 and/or PC2 enzymes involved in the post-translational processing of proENK to produce opioid peptides (Vindrola et al., 1994). It is important to bear in mind the presence of dopamine, ENK and glutamate in the immune cells, which also express the transporters, receptors and synthesis enzymes (Amenta et al., 2001; Bergquist et al., 1994). Therefore, the psychostimulant-effects on the immune function could be mediated not only by the activation of specific receptors on the central nervous system (CNS) (Haas and Schauenstein, 1997), but also by the direct effect of these neurotransmitters on the immune cells (Gordon and Barnes, 2003; Pellegrino and Bayer, 1998). With respect to glutamate, it is conceivable that it could also modulate the psychostimulant-induced effects on the immune system, due to the fact that both, ionotropic and metabotropic glutamate receptors expressed on immune cells, have been previously functionally identified as modulators of cellular activation (Lombardi et al., 2001; Pacheco et al., 2006).

Since a single dose treatment with amphetamine (5 mg/kg, i.p.) induces a decrease in the lymphoproliferative response concomitantly with an increase in the met-ENK levels in limbic (NAc and PfC) and immune organs (spleen and thymus) (Assis et al., 2006), the main goal of this study was to determine the time dependence of these effects and the development of sensitization to amphetamine by administering a challenge dose of amphetamine (1 mg/kg, i.p.) (Vanderschuren et al., 1999). Another aim was to investigate the influence of MK-801, an NMDA glutamatergic receptor antagonist, on the effects of a single dose of amphetamine or a re-exposure to this drug (expression of sensitization) by evaluating the lymphoproliferative response and the met-ENK levels of the NAc and spleen. We demonstrate long-lasting sensitization, following a single amphetamine exposure, to both the decrease in the lymphoproliferative response and the increase in the met-ENK levels in CNS and immune system, with all these effects being reverted by MK-801.

2. Methods

2.1. Animals

Adult male Wistar rats (250–330 g) from the Facultad de Ciencias Veterinarias of the Universidad Nacional de La Plata (Buenos Aires, Argentina) were maintained at 20–24 °C under a 12 h light–dark cycle (lights on at 07:00 a.m.) with free access to food and water. Rats were collectively housed in cages in the experimental room for at least 7 days before starting the experiments, with an average of five rats per group being used in the experiments. Every attempt was made to minimize the pain and discomfort of the experimental animals, with all procedures being conducted in accordance with the NIH Guide for the Care and Use of Laboratory Animals as approved by the Animal Care and Use Committee of the Facultad de Ciencias Químicas, Universidad Nacional de Córdoba.

2.2. Drugs

For all experiments, D-amphetamine sulfate and (+)-MK-801 hydrogen maleate (Sigma Co., St. Louis, MO) were dissolved in an isotonic saline solution (0.9% NaCl) which was also used for vehicle (VEH) control injections. All injections were administered intraperitoneally (i.p.) at a volume of 1 ml/kg and the treatments were performed at 11 a.m. (ZT 4) to avoid the influence of the circadian rhythm on the immune response (Haus and Smolensky, 1999) or on the behavioral sensitization to psychostimulants (Abarca et al., 2002).

2.3. Drug treatments

The following treatments were performed. For each one, an independent control group was used.

2.3.1. Single dose treatment with amphetamine

Rats were randomly assigned to one of two treatments: the VEH group or amphetamine (5 mg/kg, i.p.) group. These treatments were administered during day 1, and on days 5, 8 and 22, animals were killed by decapitation (Fig. 1A). Then, the brain, spleen and thymus were removed. The NAc and PfC of both hemispheres were dissected and splenic mononuclear cells were isolated as mentioned below.

2.3.2. Amphetamine treatment to induce sensitization

According to the drug administration schedule used by Vanderschuren et al. (1999), who demonstrated amphetamine sensitization at behavioral, neurochemical and endocrine levels, rats were randomly assigned to one of two treatments: VEH group or amphetamine (5 mg/kg, i.p.) group. These treatments were administered during day 1, with animals being re-exposed to a challenge dose of amphetamine (1 mg/kg, i.p.) or VEH on day 22. Four days following the last injection, on day 26, the animals were killed by decapitation (Fig. 1B). Then, the brain, spleen and thymus were removed. The NAc and PfC of both hemispheres were dissected, and splenic mononuclear cells were isolated as detailed below.

2.3.3. MK-801 pre-treatments

In order to assess the participation of the glutamatergic mechanisms in the effects of a single dose of amphetamine and in the amphetamine-induced sensitization, we used a selective NMDA glutamate receptor antagonist pre-treatment to block NMDA receptors during the presence of amphetamine. In this group of experiments, we focused the investigation of met-ENK levels on the spleen and NAc due to the results obtained regarding the amphetamine-induced sensitization on limbic met-ENK levels and because the spleen is the source of the lymphocytes used to evaluate the lymphoproliferative response (see below).

Single dose treatment: Fifteen minutes before the amphetamine (5 mg/kg, i.p.) or VEH injection, the animals were pre-treated with MK-801 (0.1 mg/kg, i.p.) or VEH. On day 5 (4 days following the drug injection), the animals were killed by decapitation and the spleen and brains were removed (Fig. 1C). The NAc of both hemispheres were dissected and splenic mononuclear cells were isolated.

Expression of sensitization: Fifteen minutes before the challenge dose of amphetamine (1 mg/kg, i.p.) or VEH injection, the animals were pre-treated with MK-801 (0.1 mg/kg, i.p.) or VEH. Four days following the last drug injection, the animals were killed by decapitation and spleen and brains were removed (Fig. 1D). The NAc of both hemispheres were dissected and splenic mononuclear cells were isolated.