Brain, Behavior, and Immunity 23 (2009) 464–473

Contents lists available at ScienceDirect

Brain, Behavior, and Immunity

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

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

María Amparo Assis [a], Cristian Hansen [a], Victoria Lux-Lantos [b], Liliana Marina Cancela [a,*]

[a] National University of Córdoba, School of Chemical Sciences, Department of Pharmacology, Instituto de Farmacología Experimental de Córdoba - CONICET, Medina Allende y Haya de la Torre, Ciudad Universitaria, X5000HUA Córdoba, Argentina
[b] Instituto de Biología y Medicina Experimental, IBYME-CONICET, Vuelta de Obligado 2490, C1428ADN, Ciudad Autónoma de Buenos Aires, Argentina

ARTICLE INFO

Article history:
Received 2 September 2008
Received in revised form 6 January 2009
Accepted 6 January 2009
Available online 14 January 2009

Keywords:
Amphetamine
Met-enkephalin
Glutamate
NMDA receptors
Lymphoproliferative response
Sensitization

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,

* Corresponding author. Fax: +54 351 4334420.
E-mail address: lcancela@fcq.unc.edu.ar (L.M. Cancela).

0889-1591/$ - see front matter © 2009 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbi.2009.01.003

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.

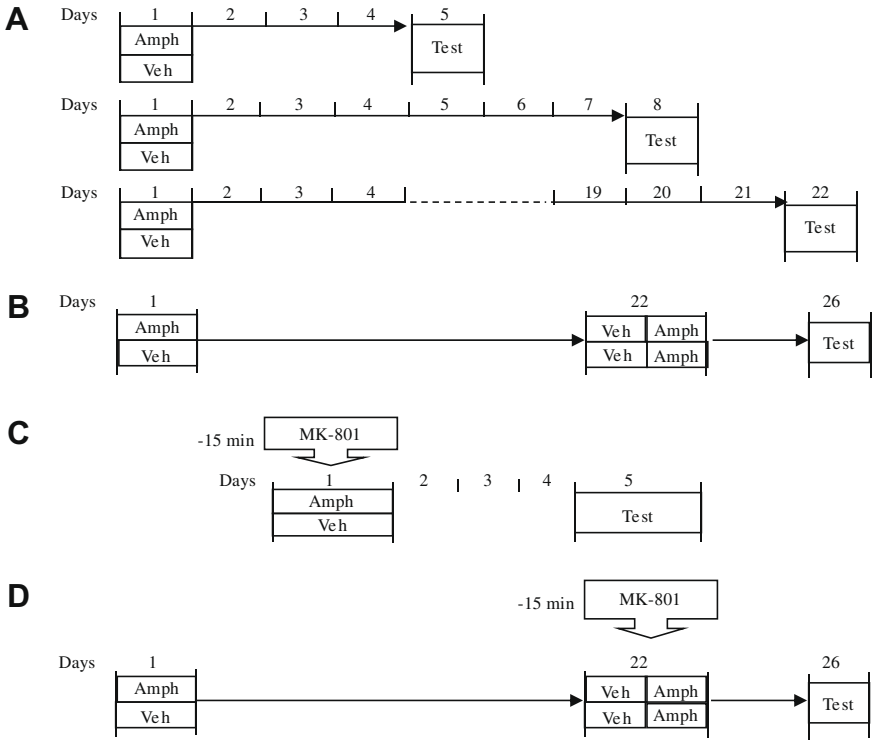

Fig. 1. Schematic diagrams of drug treatments. (A) Acute amphetamine (Amph) (5 mg/kg, i.p.) or vehicle (Veh) was administered on day 1 and the tests were carried out 4, 7 or 21 days after drug exposure. (B) Amphetamine treatment to induce sensitization: acute amphetamine (5 mg/kg, i.p.) or vehicle was administered on day 1, then 21 days later the animals were re-exposed to a challenge amphetamine dose (0.5 or 1 mg/kg, i.p.) or vehicle, and on day 26 tests were carried out. (C) MK-801 pre-treatment prior acute amphetamine: 15 min before the acute amphetamine (5 mg/kg, i.p.) or vehicle injection, the animals were pre-treated with MK-801 (0.1 mg/kg, i.p.) or vehicle, and on day 5 the tests were carried out. (D) MK-801 pre-treatment prior expression of amphetamine-induced sensitization: amphetamine (5 mg/kg, i.p.) or vehicle was administered on day 1, and 3 weeks later the animals were pre-treated with MK-801 (0.1 mg/kg, i.p.) or vehicle fifteen minutes prior to a challenge amphetamine dose (1 mg/kg, i.p.) or vehicle re-exposure. On day 26, the tests were carried out.

2.4. Isolation of mononuclear cells

Spleen cell suspensions were obtained by gently grinding tissue into RPMI 1640 culture medium (Sigma-Aldrich, Steinheim, Germany) under sterile conditions. Mononuclear cells were separated by Ficoll–Hypaque density gradient (1.083 g/ml) centrifugation. The mononuclear cells concentrated at the surface were collected and washed twice in RPMI 1640 medium. After cell counting, splenocytes were suspended at a final concentration of 2×10^6 cells/ml in RPMI 1640 medium supplemented with 10% of inactivated fetal bovine serum, 2 mM glutamine, 10 mM sodium bicarbonate, 100 UI/ml penicillin and 100 μg/ml streptomycin (denominated complete RPMI 1640 medium).

2.5. Mitogenic assay

The mitogen Con A (Sigma-Aldrich, Steinheim, Germany) was used to evaluate the splenic T-cell response. We chose a functional parameter of the immune system, as this would be able to provide information regarding the immunecompetence of T-cell populations. The Con A-induced lymphoproliferative response was utilized as it is the most widely used test to reflect the *in vitro* T-cell functional response (Coligan et al., 1999). Thus, splenocytes were added in quadruplicate to each well of 96-well flatbottom tissue culture plates (TPP, Switzerland) in the presence of 5 μg/ml of Con A. These plates were incubated at 37 °C in a humidified atmosphere with 5% CO_2 for 48 h. Then, 18 h before harvesting, cells were pulsed with 1 μCi [^{3}H]-thymidine (PerkinElmer Life Sciences, Wellesley, MA). Finally, quadruplicates were collected onto glass fiber filter paper (Wattman, UK) using a Scatron micro cell harvester (SIEM, Córdoba, Arg.). A 1205 Betaplate liquid scintillation counter (PerkinElmer Life Sciences, Wellesley, MA) was used to detect the incorporation of radioactive thymidine in a Packard, Tri-Carb Liquid Scintillation Analyzer. The radioactivity was expressed as c.p.m. and the percentage of responses was calculated relative to VEH-treated animals.

2.6. Free met-ENK radioimmunoassay

Frozen immune tissues and brain areas were suspended in 1 M acetic acid containing 50 mM HCl, then boiled for 15 min, homogenized with a Polytron, and centrifuged at 50,000*g* for 1 h. An aliquot of the supernatant was lyophilized and reconstituted in 50 mM Tris–HCl buffer, pH 8.4, and 2 mM $CaCl_2$. Met-ENK and policlonal met-ENK antibody were provided by Peninsula Lab-Bachem (San Carlos, USA). Free immunoreactive met-ENK was determined by RIA as described in Assis et al. (2006).

2.7. Statistical analysis

Cell proliferation was measured by the [^{3}H]-thymidine incorporation assay and results were plotted as the percentage of changes between amphetamine- and VEH-treated rats. Data represent means ± SD, and correspond to quadruplicate values of an average of five different rats. The Student's *t*-test was used to evaluate the statistical significance. Data from the amphetamine treatments of sensitization and from pre-treatments were analyzed with a two-way ANOVA (drug treatment × drug re-exposure) (drug pre-treatment × drug treatment). There were two levels for the drug treatment factor (amphetamine or VEH), two levels for the drug re-exposure factor (amphetamine or VEH), and two levels for the drug pre-treatment factor (MK-801 or

VEH). Following significance in the two-way ANOVA, post-hoc comparisons among means were performed with the Fisher test. Data from the experiments with pre-treatments before the expression of sensitization were analyzed with a three-way ANOVA (drug treatment × drug pre-re-exposure treatment × drug re-exposure), with two levels for the drug treatment factor (amphetamine or VEH), two levels for the drug pre-re-exposure treatment factor (MK-801 or VEH) and two levels for the drug re-exposure factor (amphetamine or VEH). Following significance in the three-way ANOVA, post-hoc comparisons among means were performed with the Tukey test (the level of significance was set at $p < 0.05$). Data represented means ± SD, and corresponded to values of 4–6 different rats. For all the statistical tests, the level of significance was set at $p < 0.05$. All the statistical information is provided in Table 1.

3. Results

3.1. Long-term effect of a single dose of amphetamine

3.1.1. Experiment 1. Time-dependent effect of a single dose of amphetamine on the lymphoproliferative response

Exposure of rats to a single dose amphetamine treatment resulted in a time-dependent decrease in the splenic proliferative response to Con A, relative to VEH-treated controls. Significant decreases were observed in the ^{3}H-thymidine incorporation of 68% ($p < 0.01$) and 27% ($p < 0.05$) in Con A-stimulated splenocytes from rats treated with 5 mg/kg of amphetamine, 4 or 7 days previously, respectively (Fig. 2). No differences were detected in rats treated with amphetamine 3 weeks before. The proliferative response to Con A in animals treated with vehicle represent the maximum percentage of proliferation.

3.1.2. Experiment 2. Time-dependent effect of a single dose of amphetamine on limbic (NAc and PfC) and immune (spleen and thymus) met-ENK levels

As previously demonstrated (Assis et al., 2006), a single dose of amphetamine, administered 4 days previously, increased the met-ENK content in the limbic brain areas: 65% ($p < 0.05$) in NAc and 43% ($p < 0.05$) in PfC (Fig. 3A); and in immune organs: 76% ($p < 0.05$) in spleen and 36% ($p < 0.05$) in thymus (Fig. 3B). These effects completely disappeared one and three weeks following amphetamine.

3.2. Amphetamine-induced sensitization

3.2.1. Experiment 3. Amphetamine-induced sensitization on lymphoproliferative response: amphetamine challenge exposure 3 weeks after a previous single dose of amphetamine

A challenge amphetamine dose (1 mg/kg, i.p.) decreased by 54% ($p < 0.01$) the splenic proliferative response to Con A, in rats that had received a single dose of amphetamine (5 mg/kg, i.p.) 3 weeks before, respect to VEH-treated controls. This value was also statistically different from those observed in the remaining groups ($p < 0.01$). A single exposure to the challenge dose of amphetamine induced a decrease of 24% ($p < 0.01$) in the proliferative response respect to control animals (Fig. 4A).

3.2.2. Experiment 4. Amphetamine-induced sensitization on limbic (NAc and PfC) and immune (spleen and thymus) met-ENK levels: amphetamine challenge exposure 3 weeks after a previous single dose of amphetamine

A challenge amphetamine dose (1 mg/kg, i.p.) increased the met-ENK levels in NAc (101%, $p < 0.01$), spleen (65%, $p < 0.01$) and thymus (36%, $p < 0.01$), in rats that had received a single dose of

Table 1
Statistical analysis: two- and three-way ANOVA results.

Experiment	ANOVA	Parameter evaluated	Effect	*F*	*p*
3	Two-way	Lymphoproliferative response	Drug re-exposure	(1,20) = 10.72	<0.01
			Drug treatment	(1,20) = 43.06	<0.01
			Drug treatment × drug re-exposure	(1,20) = 4.81	<0.05
4	Two-way	Met-ENK levels in NAc	Drug re-exposure	(1,12) = 4.98	<0.05
			Drug treatment × drug re-exposure	(1,12) = 5.40	<0.05
		Met-ENK levels in spleen	drug re-exposure	(1,12) = 6.78	<0.05
			Drug treatment × drug re-exposure	(1,12) = 7.60	<0.05
		Met-ENK levels in thymus	Drug re-exposure	(1,12) = 35.48	<0.01
			Drug treatment × drug re-exposure	(1,12) = 9.31	<0.01
5	Two-way	Lymphoproliferative response	Drug pre-treatment	(1,31) = 19.29	<0.01
			Drug treatment	(1,31) = 12.82	<0.01
6	Two-way	Met-ENK levels in NAc	Drug pre-treatment	(1,12) = 9.49	<0.01
			Drug treatment	(1,12) = 6.10	<0.05
			Drug pre-treatment × drug treatment	(1,12) = 13.61	<0.01
		Met-ENK levels in spleen	Drug pre-treatment	(1,12) = 22.54	<0.01
			Drug treatment	(1,12) = 15.78	<0.01
			Drug pre-treatment × drug treatment	(1,12) = 19.57	<0.01
7	Three-way	Lymphoproliferative response	Drug treatment	(1,61) = 7.56	<0.01
			Drug pre-re-exposure	(1,61) = 20.71	<0.01
			Drug re-exposure	(1,61) = 16.81	<0.01
			Drug pre-re-exposure × drug re-exposure	(1,61) = 6.36	<0.05
			Drug treatment × pre-re-exposure × drug re-exposure	(1,61) = 7.28	<0.01
8	Three-way	Met-ENK levels in NAc	Drug treatment	(1,25) = 4.33	<0.05
			Drug re-exposure	(1,25) = 5.12	<0.05
			Drug treatment × drug re-exposure	(1,25) = 5.81	<0.05
		Met-ENK levels in spleen	Drug pre-re-exposure	(1,24) = 6.40	<0.05
			Drug re-exposure	(1,24) = 4.49	<0.05
			Drug treatment × drug pre-re-exposure	(1,24) = 6.02	<0.05
			Drug treatment × drug re-exposure	(1,24) = 5.56	<0.05
			Drug pre-re-exposure × drug re-exposure	(1,24) = 5.49	<0.05
			Drug treatment × drug pre-re-exposure × drug re-exposure	(1,24) = 5.61	<0.05

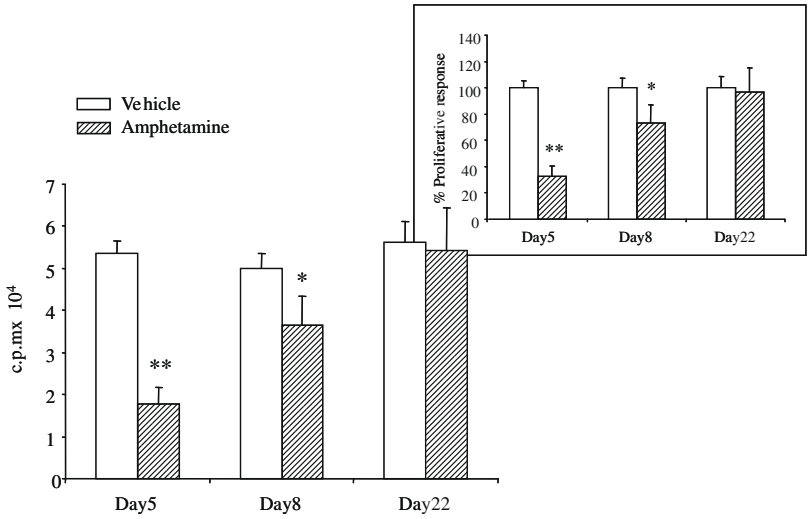

Fig. 2. Time-dependent effect of acute amphetamine treatment on the lymphoproliferative response. Animals were treated with amphetamine (5 mg/kg, i.p.) or vehicle on day 1, and on day 5, 8 or 22 the Con A-induced lymphoproliferative response was evaluated. Data are expressed as c.p.m. (main panel) or as a percentage of proliferative responses related to the control group (right panel) and show the means ± SD of 4–6 rats per group. $^{*}p < 0.05$, $^{**}p < 0.01$, Student's *t*-test, different from the corresponding control group.

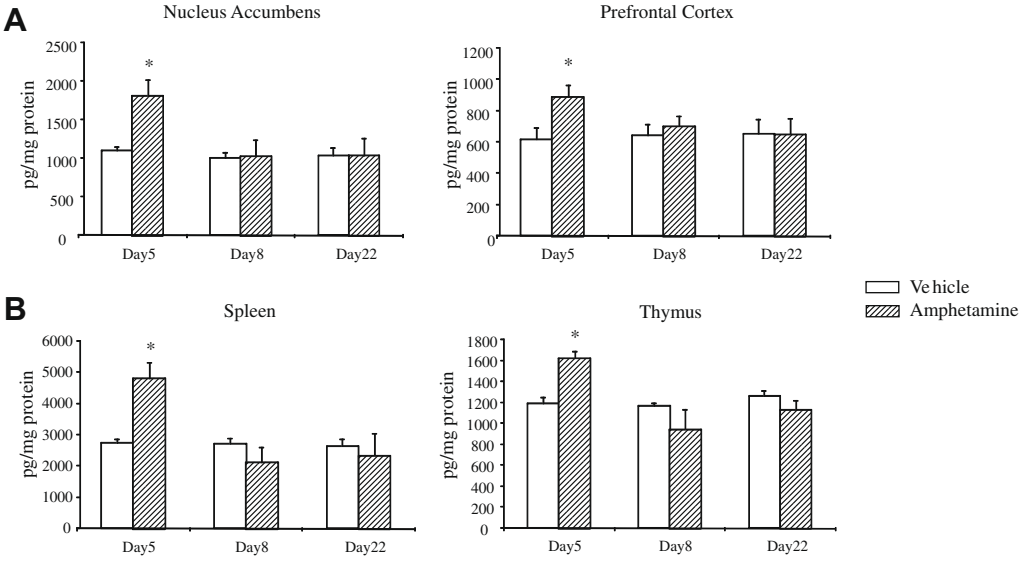

Fig. 3. Time-dependent effects of acute amphetamine treatment on limbic (A) and immune (B) met-ENK levels (pg/mg protein). Animals were treated with amphetamine (5 mg/kg, i.p.) or vehicle (Veh) on day 1, and on day 5, 8 or 22 the met-ENK content in NAc, PfC, spleen and thymus was evaluated. Data show the means ± SD of 4–6 rats per group. $^{*}p < 0.05$, Student's *t*-test, different from the corresponding control group.

amphetamine (5 mg/kg, i.p.) 3 weeks before, relative to the remaining experimental groups. In PfC, no differences were observed in the met-ENK content of rats submitted to any of the experimental treatments (Fig. 4B). The absolute values (pg/mg protein) of met-ENK levels for the control group were: 1281 ± 93 for NAc, 466 ± 32 for PfC, 2411 ± 195 for spleen and 1576 ± 172 for thymus.

3.3. MK-801 pretreatment: influence on a single dose of amphetamine

3.3.1. Experiment 5. MK-801 previous to a single dose of amphetamine: effects on lymphoproliferative response

As described above, a single amphetamine dose decreased the Con A-stimulated lymphoproliferative response 4 days after the drug exposure (63%, $p < 0.05$). This effect was not still evident in rats administered with MK-801 prior to amphetamine. No effect was observed following MK-801 in VEH-treated animals (Fig. 5A).

3.3.2. Experiment 6. MK-801 previous to a single dose of amphetamine: effects on limbic (NAc) and immune (spleen) met-ENK levels

MK-801 administration abrogated the amphetamine induced increase in met-ENK levels in NAc (91%, $p < 0.01$) and spleen (47%, $p < 0.01$), 4 days after the drug exposure. MK-801 had no effect on its own on the met-ENK level in the NAc and spleen (Fig. 5B). The absolute values (pg/mg protein) of met-ENK levels for the control group in this experiment were: 1777 ± 517 for NAc and 2523 ± 321 for spleen.

3.3.3. Experiment 7. MK-801 previous to amphetamine re-exposure (expression of sensitization): effects on lymphoproliferative response

MK-801 blocked the amphetamine-induced sensitization to the drug effects on the splenic proliferative response to Con A. In this experiment, and as described previously, the animals treated with a single dose of amphetamine (5 mg/kg, i.p.) and then re-exposed to a challenge amphetamine dose (1 mg/kg, i.p.) showed a decrease

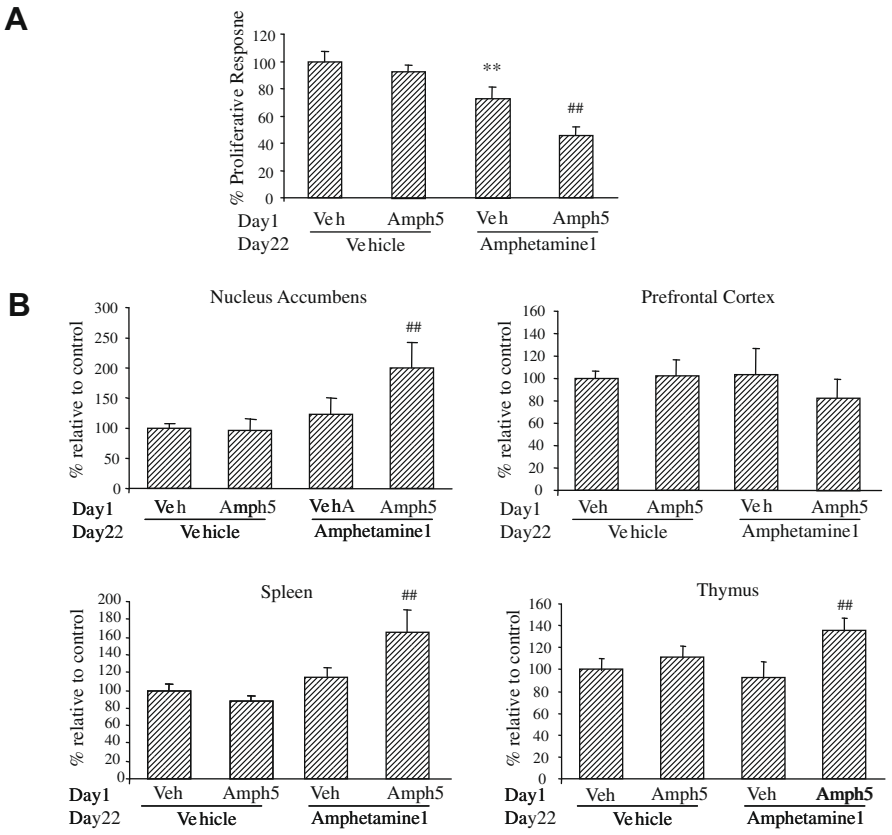

Fig. 4. Amphetamine-induced sensitization on the lymphoproliferative response (A) and on met-ENK levels (B). Animals were treated with amphetamine (5 mg/kg, i.p.) (Amph 5) or vehicle (Veh) on day 1, then 21 days later the animals were re-exposed to a challenge amphetamine dose (1 mg/kg, i.p., referred to as Amphetamine 1) or Veh, and on day 26 the Con A-induced lymphoproliferative response and the met-ENK levels were evaluated. Data are expressed as a percentage related to the control group and show the means ± SD of 4–6 rats per group. $^{**}p < 0.01$, Student's *t*-test, different from the corresponding control group. $^{\#\#}p < 0.01$, Fisher's test, different from all the remaining groups.

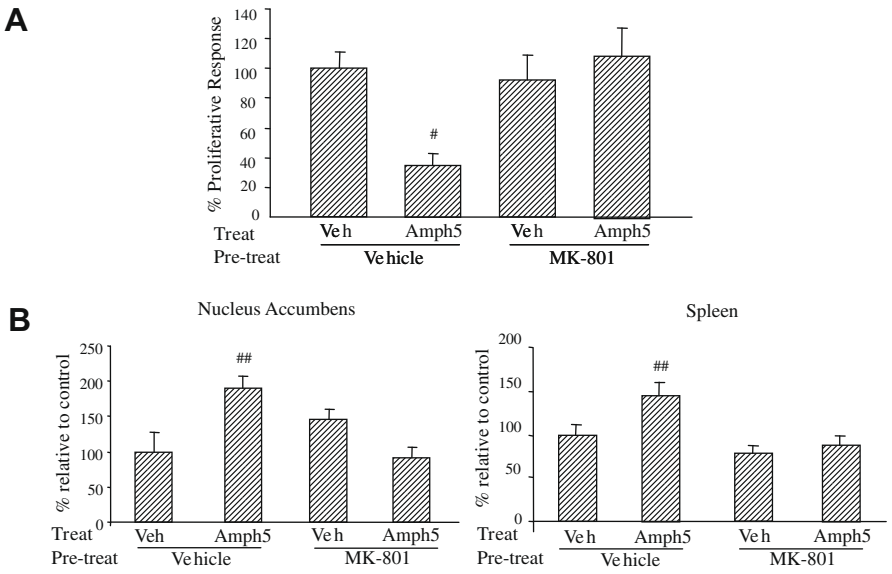

Fig. 5. Influence of MK-801 pre-treatment on the acute amphetamine-induced effects on lymphoproliferative response (A) and on met-ENK levels (B). Animals were pre-treated (Pre-treat) with MK-801 (0.1 mg/kg, i.p.) or Veh fifteen minutes before a treatment (Treat) with amphetamine (5 mg/kg, i.p.) (Amph 5) or vehicle (Veh) on day 1. Then, on day 5, the Con A-induced lymphoproliferative response (A) and the met-ENK levels in NAc and spleen (B) were evaluated. Data are expressed as a percentage related to the control group and show the means ± SD of 4–6 rats per group. $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, Fisher's test, different from all the remaining groups.

(55%, $p < 0.05$) in the splenic proliferative response to Con A, respect to VEH-treated controls. This value was also statistically different from those observed in the remaining groups ($p < 0.05$). Only animals exposed to the challenge dose showed a decrease (37%, $p < 0.01$) in the proliferative response, respect to the VEH-control group. Neither effect was evident in rats that received an MK-801 injection prior to the amphetamine challenge on day 22 (Fig. 6 A).

3.3.4. Experiment 8. MK-801 previous to amphetamine re-exposure (expression of sensitization): effects on limbic (NAc) and immune (spleen) met-ENK levels

Animals treated with a single dose of amphetamine (5 mg/kg, i.p.) and then re-exposed to a challenge amphetamine dose (1 mg/kg, i.p.) showed an increase in the met-ENK levels in NAc (93%, $p < 0.05$) and spleen (49%, $p < 0.05$), respect to VEH-treated controls. In this experiment, it was shown that MK-801 prior to amphetamine re-exposure abrogated the increase in the met-ENK levels for both NAc and spleen, when observed after the amphetamine challenge on day 22. An MK-801 injection alone did not induce any changes in the met-ENK levels in animals treated with VEH (Fig. 6 B and C). The absolute values (pg/mg protein) of met-ENK levels for the control group were 1508 ± 113 for NAc and 2712 ± 212 for spleen.

4. Discussion

In a previous study (Assis et al., 2006), we demonstrated that a single dose treatment with amphetamine (5 mg/kg, i.p.) was able to induce, 4 days after the drug injection, a decrease in the lymphoproliferative response together with an increase in the met-ENK content from the NAc, PfC, spleen and thymus. In the present study, we demonstrated that all these effects completely disappeared 3 weeks following amphetamine administration. At this time, the re-exposure to a challenge dose of amphetamine (1 mg/kg, i.p.) induced the expression of sensitization to the drug-induced effects on the lymphoproliferative response and on the met-ENK levels in the NAc, spleen and thymus, but not in the PfC. Pre-treatment with MK-801 (0.1 mg/kg, i.p.) blocked the effects of amphetamine on the lymphoproliferative response and the met-ENK in NAc and spleen, both before the first dose of amphetamine (i.e. development of sensitization) and following the re-exposure to a second challenge dose (expression of sensitization).

In the current work, the amphetamine-induced decrease in the lymphoproliferative response observed simultaneously with an increase in met-ENK (from NAc and spleen) were both sensitized and reversed by MK-801 pre-treatment, which leads us to suggest a possible association of both amphetamine effects. Related to this, met-ENK has been consistently associated with a decrease in the adaptive immune response, particularly in the T-cell functionality (Fulford et al., 2000; Saravia et al., 1998). However, a biphasic mod-

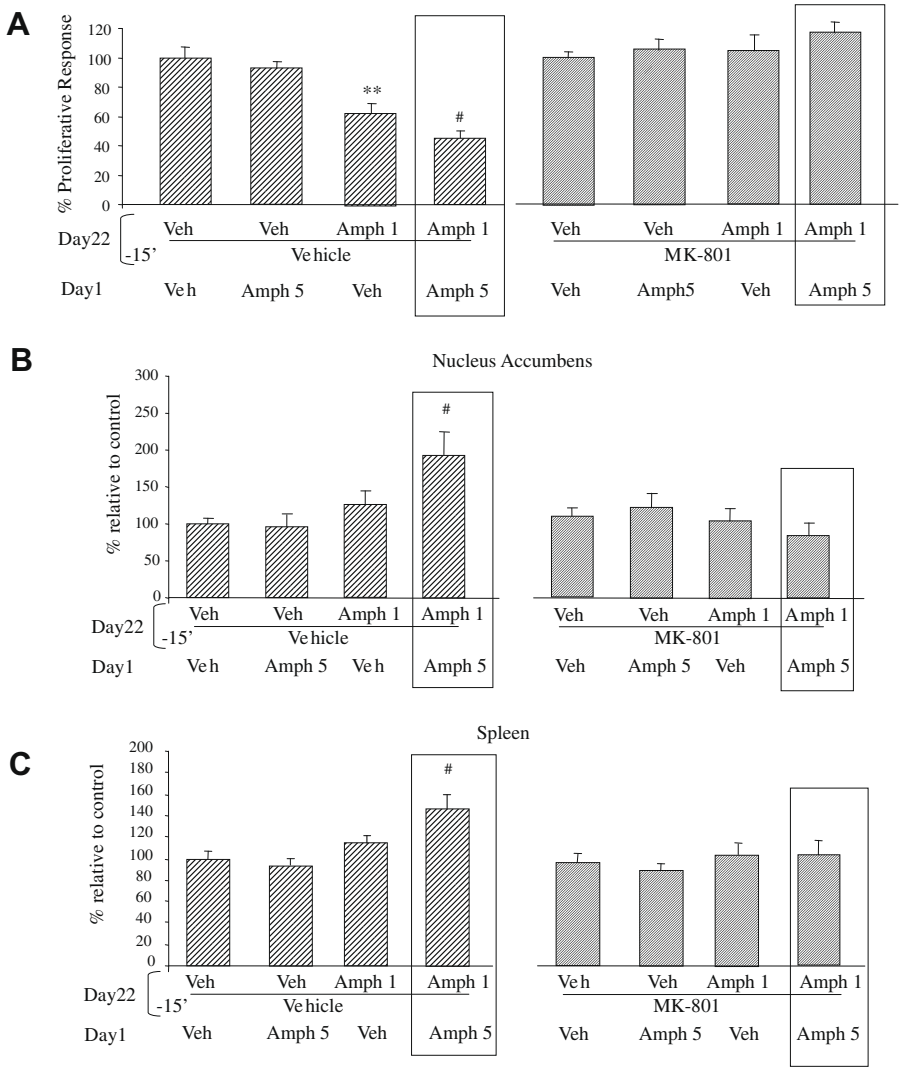

Fig. 6. Influence of MK-801 pre-treatment on the expression of amphetamine-induced sensitization on the lymphoproliferative response (A) and on met-ENK levels (B and C). Amphetamine (5 mg/kg, i.p.) (Amph 5) or Veh was administered on day 1, and on day 22 the animals were pre-treated with MK-801 (0.1 mg/kg, i.p.) or Veh fifteen minutes prior to a challenge amphetamine dose (1 mg/kg, i.p.) (Amph 1) or Veh re-exposure. On day 26, the Con A-induced lymphoproliferative response was evaluated. Data are expressed as a percentage of the proliferative responses related to the control group and show the means ± SD of 4–6 rats per group. $^{**}p < 0.01$, Student's *t*-test, different from the corresponding control group; $^{\#}p < 0.05$, Tukey's test, different from all the remaining groups.

ulation that depends on met-ENK levels has also been suggested, and demonstrated when Sizemore et al. (2004) showed that low doses of met-ENK (YGGFM peptide) and two derived peptides (YGG and YG) increase and accelerate the delayed hypersensitive response, whereas higher doses of these peptides suppress this reaction. Piva et al. (2005) showed that met-ENK and their derived peptides at either low or high doses *in vitro* are able to induce or inhibit, respectively, the IFN-γ production. High concentrations of met-ENK and YG also suppress the IL-2 and IL-4 production, but the previous administration of naloxone (a μ/δ-opioid receptor antagonist) blocks the met-ENK effect only on IFN-γ production. Thus, it is reasonable to assume that the enhancement of splenic met-ENK could modulate the cytokine profile and/or other immune mechanisms responsible for the long-lasting decrease in the lymphoproliferative response which remains even after changes in the met-ENK levels. This could explain why, either 8 days following 5 mg/kg of amphetamine or 4 days after 1 mg/kg of amphetamine, a decrease in the lymphoproliferative response was observed although no significant change in met-ENK could be seen.

4.1. Amphetamine-induced sensitization in CNS and immune system

The mechanisms underlying the amphetamine-induced sensitization on the CNS are well understood and are usually divided into two periods: development and expression. The development is characterized by increased extracellular dopamine levels (Kalivas and Duffy, 1991) and short-term molecular changes, such as increased expression of AMPA and NMDA receptor subunits (Carlezon and Nestler, 2002; Fitzgerald et al., 1996) and subsensitivity of D2 receptors in VTA (White and Wang, 1984). The expression can be elicited by a drug re-exposure and is characterized by dopamine release sensitization in NAc (Pierce and Kalivas, 1995; Wolf et al., 1993b). At the immune level, the psychostimulant-induced sensitization of the lymphoproliferative response was previously (Kubera et al., 2004) demonstrated by using a repeated treatment of five daily drug injections, with the challenge dose being administered 4 days following the last drug exposure. Thus, our findings are the first evidence of a long-lasting (3 weeks) sensitization being induced by a single amphetamine exposure on this immune parameter. As there is no dopamine in the peripheral nervous system, the dopamine receptor expression in several immune cell lines (McKenna et al., 2002) indicates that plasma dopamine (Van Loon, 1983), DOPA (Kvetnansky et al., 1992) or dopamine synthesized by immune cells could potentially activate these receptors (Bencsics et al., 1997). Splenic noradrenergic terminals are capable to re-uptaking dopamine from circulation, partially transforming it into noradrenaline and releasing both dopamine and noradrenaline in response to neural activity. Thus, the central neurochemical evidence of dopamine release sensitization could be associated, at least in part, with the effects of amphetamine on the immune function, bearing in mind the immunomodulatory role previously described for this catecholamine (Cosentino et al., 2002; Saha et al., 2001). Evidence of the development of amphetamine-induced sensitization on ACTH and corticosterone hypersecretion 3 weeks after amphetamine treatment has been described (Schmidt et al., 1999). Moreover, long-term amphetamine-induced sensitization on the ACTH and corticosterone responses has been proposed to participate in the autonomic and affective consequences induced by amphetamine exposure (Prasad et al., 1996; Schmidt et al., 1999), which may be extendible at immune level. Regarding this, it was previously shown that *in vivo* amphetamine affects blood cellularity, at least in part, as a consequence of drug effects on the HPA system (Ligeiro de Oliveira et al., 2004, 2008). However, Pacheco-López et al. (2003) demonstrated that the influence of central catecholaminergic transmission on the lymphoproliferative response is mediated by the sympathetic nervous system, without any changes being observed in the plasmatic corticosterone levels. Notwithstanding this, since the HPA axis might have been activated without apparent changes in corticosterone serum level, an influence due to this axis on the amphetamine-induced effects at immune level can not be discarded. Furthermore, other mechanisms might have affected the results from our laboratory, i.e. a suppressive effect occurring on the immunoenhancing prolactin system (Bernton et al., 1988).

In addition to the effects observed on the lymphoproliferative response, our current data constitute the first specific evidence regarding a sensitized response being induced by amphetamine on the met-ENK levels not only in the brain area relevant for addiction (NAc), but also in immune organs (in particular, the spleen) as implied by the T-cell response. By using a pharmacological approach and KO μ-opioid receptor mice, a role for the opioid system was previously demonstrated in the expression of the sensitization to amphetamine (Magendzo and Bustos, 2003) and in the modulation of the dopaminergic transmission in the NAc (Mathon et al., 2006), respectively. Due to the fact that met-ENK in PfC was not sensitized by amphetamine, this constitutes more evidence about molecular changes occurring in the NAc and not in the PfC during the expression of psychostimulant sensitization (i.e. a hypersensitivity of D1 receptors (Wolf et al., 1993a)). Moreover, since a sensitized response on the met-ENK was also observed following amphetamine in immune organs, it is possible to suggest that this could have been mediated by a similar biological mechanism being triggered by the drug (i.e. sensitization on dopamine release).

4.2. NMDA receptors in amphetamine effects in CNS and immune system

In this study, we have demonstrated for the first time the involvement of NMDA glutamatergic receptors in the effect of a single dose of amphetamine as well as in the expression of amphetamine-induced sensitization on the lymphoproliferative response and on met-ENK in the NAc and spleen. NMDA-mediated effects of amphetamine on the CNS (Wolf et al., 1994; Wolf, 1998; Pacchioni et al., 2007), but not on immune system, have been previously demonstrated. Glutamate has recently been considered to be an immunotransmitter (Boldyrev et al., 2005). It is released by the dendritic cell and can modulate T-cell proliferation via metabotropic glutamatergic receptors (mGlu1R and mGlu5R) (Pacheco et al., 2006). The first study describing the presence of ionotropic glutamate receptors (iGluR) in human resting lymphocytes was reported by Lombardi et al. (2001). These authors also demonstrated a functional role for iGluR as modulators of immune cell activation by mediating the rise in intracellular calcium induced by PHA- or anti-CD3 antibody. In addition, Boldyrev et al. (2004) showed the expression of NMDA receptors in rodent lymphocytes, with their activation elevating intracellular calcium and ROS levels (Pacheco et al., 2007). As mentioned before, the NMDA receptors could underlie the amphetamine effect on the splenic lymphoproliferative response, either directly by the immune cells and/or by an indirect message from the CNS. Although a study on the proENK gene transcription was not carried out in the current work, the involvement of the NMDA receptor in the increase of met-ENK after amphetamine is consistent with previous evidence that showed a contribution of these receptors to the amphetamine-induced proENK expression in this brain area (Mao and Wang, 2003).

Another novel finding of this work is to show the involvement of NMDA receptors in the long-lasting amphetamine-induced sensitization on the lymphoproliferative response and on met-ENK in the NAc and spleen. There is strong evidence of long-lasting changes being induced by psychostimulants on the glutamatergic transmission, which could help to interpret these current findings.

At CNS level, cellular changes in the glutamatergic inputs from PfC to VTA and NAc have been shown to underlie psychostimulant-induced sensitization (Cador et al., 1999; Wolf, 1998). The plasticity of the glutamatergic synapses in VTA constitutes the main psychostimulant-induced mechanism involved in the long-lasting sensitization of the mesocorticolimbic pathway (Ungless et al., 2001). Our present findings with MK-801 extend at immune level previous evidence regarding the involvement of NMDA glutamatergic transmission in the long-lasting effects of amphetamine. In this *in vivo* study, it is not possible to distinguish the effects that the drug could exert on the CNS from those which could affect the immune cells directly. Thus, the effects observed could be attributed to: (1) a central message, which involves the NMDA glutamatergic activation, reaching the immune organs through the sympathetic nervous system and/or the HPA axis and (2) the direct influence of amphetamine increasing the autocrine and paracrine release of glutamate on the immune cells, and leading to the subsequent activation of proENK expression as previously noted. Furthermore, since a previous study demonstrated a central μ-opioid mechanism mediating the morphine-induced immunomodulation (Mellon and Bayer, 1998), it is conceivable that the increase of met-ENK in NAc could participate in the message towards the immune system.

4.3. General conclusions

Summing up, we can conclude that a single amphetamine exposure is sufficient to induce long-lasting sensitization, not only at behavioral, neurochemical, and endocrine levels (Vanderschuren et al., 1999), but also on the immune system. These findings, together with the glutamate-ENK interaction observed on the amphetamine-induced effects, are consistent with the idea that similar biological mechanisms on the brain and immune organs could be triggered by psychostimulants. Furthermore, the amphetamine effects on the brain and immune met-ENK are proposed to play a role in the modulation of the immune response following the administration of a psychostimulant drug. Since drugs of abuse not only modify the behavior of individuals, but also compromise their immune functions (Baldwin et al., 1998; Friedman and Eisenstein, 2004), it is highly relevant to follow up the changes in CNS at a peripheral level (Assis et al., 2006; Blandini et al., 2004). Finally, considering the comorbidity of drug abuse with numerous infectious diseases (Howard et al., 2002; Nath et al., 2002; Rich et al., 2006), MK-801 and other similarly acting agents may open new possibilities for the treatment of immunological disorders in drug abusers.

Acknowledgments

This study was supported by grants from FONCyT, CONICET and SeCyT (Argentina). The authors wish to express their sincere gratitude to Ms. Estela Salde, Ms. Paula Icely and Ms. Elsa R. Pereyra for their excellent technical assistance, and also to Dr. Claudia Sotomayor, CIBICI-CONICET and Dr. Carlos Libertun, IBYME-CONICET, for their kind suggestions concerning technical issues of this work. We are grateful to Dr. Paul Hobson, native speaker, for revision of the manuscript.

References

Abarca, C., Albrecht, U., Spanagel, R., 2002. Cocaine sensitization and reward are under the influence of circadian genes and rhythm. Proc. Natl. Acad. Sci. USA 99, 9026–9030.

Amenta, F., Bronzetti, E., Cantalamessa, F., El-Assouad, D., Felici, L., Ricci, A., Tayebati, S.K., 2001. Identification of dopamine plasma membrane and vesicular transporters in human peripheral blood lymphocytes. J. Neuroimmunol. 117, 133–142.

Assis, M.A., Collino, C., Figuerola Mde, L., Sotomayor, C., Cancela, L.M., 2006. Amphetamine triggers an increase in met-enkephalin simultaneously in brain areas and immune cells. J. Neuroimmunol. 178, 62–75.

Assis, M.A., Pacchioni, A.M., Collino, C., Paz, M.C., Sotomayor, C., Basso, A.M., Cancela, L.M., 2008. A dopamine mechanism is implied in the acquisition and expression of amphetamine and stress-induced effects observed in the lymphocyte subpopulations. Eur. J. Pharmacol. 584, 405–414.

Baldwin, G.C., Roth, M.D., Tashkin, D.P., 1998. Acute and chronic effects of cocaine on the immune system and the possible link to AIDS. J. Neuroimmunol. 83, 133–138.

Bencsics, A., Sershen, H., Baranyi, M., Hashim, A., Lajtha, A., Vizi, E.S., 1997. Dopamine, as well as norepinephrine, is a link between noradrenergic nerve terminals and splenocytes. Brain Res. 761, 236–243.

Bergquist, J., Tarkowski, A., Ekman, R., Ewing, A., 1994. Discovery of endogenous catecholamines in lymphocytes and evidence for catecholamine regulation of lymphocyte function via an autocrine loop. Proc. Natl. Acad. Sci. USA 91, 12912–12916.

Bernton, E.W., Meltzer, M.S., Holaday, J.W., 1988. Suppression of macrophage activation and T-lymphocyte function in hypoprolactinemic mice. Science 239 (4838), 401–404.

Blandini, F., Cosentino, M., Mangiagalli, A., Marino, F., Samuele, A., Rasini, E., Fancellu, R., Tassorelli, C., Pacchetti, C., Martignoni, E., Riboldazzi, G., Calandrella, D., Lecchini, S., Frigo, G., Nappi, G., 2004. Modifications of apoptosis-related protein levels in lymphocytes of patients with Parkinson's disease. The effect of dopaminergic treatment. J. Neural. Transm. 111, 1017–1030.

Boldyrev, A.A., Carpenter, D.O., Johnson, P., 2005. Emerging evidence for a similar role of glutamate receptors in the nervous and immune systems. J. Neurochem. 95, 913–918.

Boldyrev, A.A., Kazey, V.I., Leinsoo, T.A., Mashkina, A.P., Tyulina, O.V., Johnson, P., Tuneva, J.O., Chittur, S., Carpenter, D.O., 2004. Rodent lymphocytes express functionally active glutamate receptors. Biochem. Biophys. Res. Commun. 324, 133–139.

Cador, M., Bjijou, Y., Cailhol, S., Stinus, L., 1999. D-Amphetamine-induced behavioral sensitization: implication of a glutamatergic medial prefrontal cortex-ventral tegmental area innervation. Neuroscience 94, 705–721.

Carlezon Jr., W.A., Nestler, E.J., 2002. Elevated levels of GluR1 in the midbrain: a trigger for sensitization to drugs of abuse? Trends Neurosci. 25, 610–615.

Coligan, J.E., Kruisbeek, A.M., Margulies, D.H., Shevach, E.M., Strober, W., 1999. Measurement of proliferative response of cultured lymphocytes in immunologic studies in humans. In: Coico, R. (Ed.), Currents Protocols in Immunology. John Wiley and Son Inc., p. 7.10.1. Chapter 7.

Cosentino, M., Zaffaroni, M., Marino, F., Bombelli, R., Ferrari, M., Rasini, E., Lecchini, S., Ghezzi, A., Frigo, G., 2002. Catecholamine production and tyrosine hydroxylase expression in peripheral blood mononuclear cells from multiple sclerosis patients: effect of cell stimulation and possible relevance for activation-induced apoptosis. J. Neuroimmunol. 133, 233–240.

Dudman, J.T., Eaton, M.E., Rajadhyaksha, A., Macias, W., Taher, M., Barczak, A., Kameyama, K., Huganir, R., Konradi, C., 2003. Dopamine D1 receptors mediate CREB phosphorylation via phosphorylation of the NMDA receptor at Ser897-NR1. J. Neurochem. 87, 922–934.

Fitzgerald, L.W., Ortiz, J., Hamedani, A.G., Nestler, E.J., 1996. Drugs of abuse and stress increase the expression of GluR1 and NMDAR1 glutamate receptor subunits in the rat ventral tegmental area: common adaptations among cross-sensitizing agents. J. Neurosci. 16, 274–282.

Friedman, H., Eisenstein, T.K., 2004. Neurological basis of drug dependence and its effects on the immune system. J. Neuroimmunol. 147, 106–108.

Fulford, A.J., Harbuz, M.S., Jessop, D.S., 2000. Antisense inhibition of pro-opiomelanocortin and proenkephalin A messenger RNA translation alters rat immune cell function in vitro. J. Neuroimmunol. 106, 6–13.

Gordon, J., Barnes, N.M., 2003. Lymphocytes transport serotonin and dopamine: agony or ecstasy? Trends Immunol. 24, 438–443.

Haas, H.S., Schauenstein, K., 1997. Neuroimmunomodulation via limbic structures: the neuroanatomy of psychoimmunology. Prog. Neurobiol. 51, 195–222.

Haus, E., Smolensky, M.H., 1999. Biologic rhythms in the immune system. Chronobiol. Int. 16, 581–622.

Howard, A.A., Klein, R.S., Schoenbaum, E.E., Gourevitch, M.N., 2002. Crack cocaine use and other risk factors for tuberculin positivity in drug users. Clin. Infect. Dis. 35, 1183–1190.

Kalivas, P.W., 1985. Sensitization to repeated enkephalin administration into the ventral tegmental area of the rat. II. Involvement of the mesolimbic dopamine system. J. Pharmacol. Exp. Ther. 235, 544–550.

Kalivas, P.W., 2007. Cocaine and amphetamine-like psychostimulants: neurocircuitry and glutamate neuroplasticity. Dialogues Clin. Neurosci. 9, 389–397.

Kalivas, P.W., Duffy, P., 1991. A comparison of axonal and somatodendritic dopamine release using in vivo dialysis. J. Neurochem. 56, 961–967.

Kalivas, P.W., Stewart, J., 1991. Dopamine transmission in the initiation and expression of drug- and stress-induced sensitization of motor activity. Brain Res. Brain Res. Rev. 16, 223–244.

Kauer, J.A., Malenka, R.C., 2007. Synaptic plasticity and addiction. Nat. Rev. Neurosci. 8, 844–858.

Kavelaars, A., Cobelens, P.M., Teunis, M.A., Heijnen, C.J., 2005. Changes in innate and acquired immune responses in mice with targeted deletion of the dopamine transporter gene. J. Neuroimmunol. 161, 162–168.

Kubera, M., Filip, M., Basta-Kaim, A., Nowak, E., Budziszewska, B., Tetich, M., Holan, V., Korzeniak, B., Przegalinski, E., 2002. The effect of amphetamine sensitization on mouse immunoreactivity. J. Physiol. Pharmacol. 53, 233–242.

Kubera, M., Filip, M., Basta-Kaim, A., Nowak, E., Siwanowicz, J., Zajicova, A., Holan, V., Maes, M., Lason, W., 2004. The effect of cocaine sensitization on mouse immunoreactivity. Eur. J. Pharmacol. 483, 309–315.

Kvetnansky, R., Armando, I., Weise, V.K., Holmes, C., Fukuhara, K., Deka-Starosta, A., Kopin, I.J., Goldstein, D.S., 1992. Plasma dopa responses during stress: dependence on sympathoneural activity and tyrosine hydroxylation. J. Pharmacol. Exp. Ther. 261, 899–909.

Ligeiro de Oliveira, A.P., Fialho de Araujo, A.M., Lazarini, R., Silva, Z.L., De Nucci, G., Muscará, M.N., 2004. Effect of amphetamine on immune-mediated lung inflammatory response in rats. Neuroimmunomodulation 11, 181–190.

Ligeiro de Oliveira, A.P., Lazzarini, R., Cavriani, G., Quinteiro-Filho, W.M., Tavares de Lima, W., Palermo-Neto, J., 2008. Effects of single or repeated amphetamine treatment and withdrawal on lung allergic inflammation in rats. Int. Immunopharmacol. 8, 1164–1171.

Liste, I., Munoz, A., Guerra, M.J., Labandeira-Garcia, J.L., 2000. Fenfluramine-induced increase in preproenkephalin mRNA levels in the striatum: interaction between the serotonergic, glutamatergic, and dopaminergic systems. Synapse 35, 182–191.

Lombardi, G., Dianzani, C., Miglio, G., Canonico, P.L., Fantozzi, R., 2001. Characterization of ionotropic glutamate receptors in human lymphocytes. Br. J. Pharmacol. 133, 936–944.

Magendzo, K., Bustos, G., 2003. Expression of amphetamine-induced behavioral sensitization after short- and long-term withdrawal periods: participation of mu- and delta-opioid receptors. Neuropsychopharmacology 28, 468–477.

Mao, L., Wang, J.Q., 2003. Contribution of ionotropic glutamate receptors to acute amphetamine-stimulated preproenkephalin mRNA expression in the rat striatum in vivo. Neurosci. Lett. 346, 17–20.

Mathon, D.S., Vanderschuren, L.J., Ramakers, G.M., 2006. Reduced psychostimulant effects on dopamine dynamics in the nucleus accumbens of mu-opioid receptor knockout mice. Neuroscience 141, 1679–1684.

McKenna, F., McLaughlin, P.J., Lewis, B.J., Sibbring, G.C., Cummerson, J.A., Bowen-Jones, D., Moots, R.J., 2002. Dopamine receptor expression on human T- and B-lymphocytes, monocytes, neutrophils, eosinophils and NK cells: a flow cytometric study. J. Neuroimmunol. 132, 34–40.

Mellon, R.D., Bayer, B.M., 1998. Evidence for central opioid receptors in the immunomodulatory effects of morphine: review of potential mechanism(s) of action. J. Neuroimmunol. 83, 19–28.

Nath, A., Hauser, K.F., Wojna, V., Booze, R.M., Maragos, W., Prendergast, M., Cass, W., Turchan, J.T., 2002. Molecular basis for interactions of HIV and drugs of abuse. J. Acquir. Immune Defic. Syndr. 31 (Suppl. 2), S62–S69.

Pacchioni, A.M., Cador, M., Bregonzio, C., Cancela, L.M., 2007. A glutamate-dopamine interaction in the persistent enhanced response to amphetamine in nucleus accumbens core but not shell following a single restraint stress. Neuropsychopharmacology 32, 682–692.

Pacheco-López, G., Niemi, M.B., Kou, W., Bildhauser, A., Gross, C.M., Goebel, M.U., del Rey, A., Besedovsky, H.O., Schedlowski, M., 2003. Central catecholamine depletion inhibits peripheral lymphocyte responsiveness in spleen and blood. J. Neurochem. 86, 1024–1031.

Pacheco, R., Gallart, T., Lluis, C., Franco, R., 2007. Role of glutamate on T-cell mediated immunity. J. Neuroimmunol. 185, 9–19.

Pacheco, R., Oliva, H., Martinez-Navio, J.M., Climent, N., Ciruela, F., Gatell, J.M., Gallart, T., Mallol, J., Lluis, C., Franco, R., 2006. Glutamate released by dendritic cells as a novel modulator of T cell activation. J. Immunol. 177, 6695–6704.

Pellegrino, T., Bayer, B.M., 1998. In vivo effects of cocaine on immune cell function. J. Neuroimmunol. 83, 139–147.

Pierce, R.C., Kalivas, P.W., 1995. Amphetamine produces sensitized increases in locomotion and extracellular dopamine preferentially in the nucleus accumbens shell of rats administered repeated cocaine. J. Pharmacol. Exp. Ther. 275, 1019–1029.

Pierce, R.C., Kalivas, P.W., 1997. Repeated cocaine modifies the mechanism by which amphetamine releases dopamine. J. Neurosci. 17, 3254–3261.

Piva, M., Moreno, J.I., Jenkins, F.S., Smith, J.K., Thomas, J.L., Montgomery, C., Wilson, C.B., Sizemore, R.C., 2005. In vitro modulation of cytokine expression by enkephalin-derived peptides. Neuroimmunomodulation 12, 339–347.

Prasad, B.M., Ulibarri, C., Kalivas, P.W., Sorg, B.A., 1996. Effect of adrenalectomy on the initiation and expression of cocaine-induced sensitization. Psychopharmacology (Berl.) 125, 265–273.

Rawls, S.M., McGinty, J.F., 2000. Delta opioid receptors regulate calcium-dependent, amphetamine-evoked glutamate levels in the rat striatum: an in vivo microdialysis study. Brain Res. 861, 296–304.

Rich, J.D., Anderson, B.J., Schwartzapfel, B., Stein, M.D., 2006. Sexual risk for hepatitis B virus infection among hepatitis C virus-negative heroin and cocaine users. Epidemiol. Infect. 134, 478–484.

Robinson, T.E., Berridge, K.C., 2000. The psychology and neurobiology of addiction: an incentive-sensitization view. Addiction 95 (Suppl. 2), S91–S117.

Saha, B., Mondal, A.C., Majumder, J., Basu, S., Dasgupta, P.S., 2001. Physiological concentrations of dopamine inhibit the proliferation and cytotoxicity of human CD4+ and CD8+ T cells in vitro: a receptor-mediated mechanism. Neuroimmunomodulation 9, 23–33.

Saravia, F., Padros, M.R., Ase, A., Aloyz, R., Duran, S., Vindrola, O., 1998. Differential response to a stress stimulus of proenkephalin peptide content in immune cells of naive and chronically stressed rats. Neuropeptides 32, 351–359.

Schmidt, E.D., Tilders, F.J., Binnekade, R., Schoffelmeer, A.N., De Vries, T.J., 1999. Stressor- or drug-induced sensitization of the corticosterone response is not critically involved in the long-term expression of behavioural sensitization to amphetamine. Neuroscience 92, 343–352.

Sizemore, R.C., Piva, M., Moore, L., Gordonov, N., Heilman, E., Godfrey, H.P., 2004. Modulation of delayed-type hypersensitivity responses in hairless guinea pigs by peptides derived from enkephalin. Neuroimmunomodulation 11, 141–148.

Stanojevic, S., Mitic, K., Vujic, V., Kovacevic-Jovanovic, V., Dimitrijevic, M., 2007. The influence of stress and methionine-enkephalin on macrophage functions in two inbred rat strains. Life Sci. 80, 901–909.

Ungless, M.A., Whistler, J.L., Malenka, R.C., Bonci, A., 2001. Single cocaine exposure in vivo induces long-term potentiation in dopamine neurons. Nature 411, 583–587.

Van Loon, G.R., 1983. Plasma dopamine: regulation and significance. Fed. Proc. 42, 3012–3018.

Vanderschuren, L.J., Kalivas, P.W., 2000. Alterations in dopaminergic and glutamatergic transmission in the induction and expression of behavioral sensitization: a critical review of preclinical studies. Psychopharmacology (Berl.) 151, 99–120.

Vanderschuren, L.J., Schmidt, E.D., De Vries, T.J., Van Moorsel, C.A., Tilders, F.J., Schoffelmeer, A.N., 1999. A single exposure to amphetamine is sufficient to induce long-term behavioral, neuroendocrine, and neurochemical sensitization in rats. J. Neurosci. 19, 9579–9586.

Vindrola, O., Mayer, A.M., Citera, G., Spitzer, J.A., Espinoza, L.R., 1994. Prohormone convertases PC2 and PC3 in rat neutrophils and macrophages. Parallel changes with proenkephalin-derived peptides induced by LPS in vivo. Neuropeptides 27, 235–244.

Wang, J.Q., McGinty, J.F., 1996. D1 and D2 receptor regulation of preproenkephalin and preprodynorphin mRNA in rat striatum following acute injection of amphetamine or methamphetamine. Synapse 22, 114–122.

White, F.J., Wang, R.Y., 1984. Electrophysiological evidence for A10 dopamine autoreceptor subsensitivity following chronic D-amphetamine treatment. Brain Res. 309, 283–292.

Wolf, M.E., 1998. The role of excitatory amino acids in behavioral sensitization to psychomotor stimulants. Prog. Neurobiol. 54, 679–720.

Wolf, M.E., White, F.J., Hu, X.T., 1993a. Behavioral sensitization to MK-801 (dizocilpine): neurochemical and electrophysiological correlates in the mesoaccumbens dopamine system. Behav. Pharmacol. 4, 429–442.

Wolf, M.E., White, F.J., Hu, X.T., 1994. MK-801 prevents alterations in the mesoaccumbens dopamine system associated with behavioral sensitization to amphetamine. J. Neurosci. 14, 1735–1745.

Wolf, M.E., White, F.J., Nassar, R., Brooderson, R.J., Khansa, M.R., 1993b. Differential development of autoreceptor subsensitivity and enhanced dopamine release during amphetamine sensitization. J. Pharmacol. Exp. Ther. 264, 249–255.