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Journal of Neuroimmunology 178 (2006) 62–75

Journal of Neuroimmunology

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Amphetamine triggers an increase in met-enkephalin simultaneously in brain areas and immune cells

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Received 21 October 2005; received in revised form 16 May 2006; accepted 17 May 2006

Abstract

We analyzed effects of amphetamine on proenkephalin-derived peptides in brain areas and immune cells in rats. Acute, as well as a repeated amphetamine treatment, decreased the concanavalin-A-induced lymphocyte proliferation, concomitantly with an increase of free met-enkephalin in nucleus accumbens, prefrontal cortex, spleen, thymus and splenic macrophages. Proenkephalin protein increased in prefrontal cortex, thymus (32 kDa isoform), nucleus accumbens and spleen (44 kDa isoform), while proenkephalin mRNA levels decreased in brain stem. The influence of met-ENK in key brain areas for sensitization and in immune organs is consistent with the idea that changes on met-ENK could underlie amphetamine's effects on brain and IS.

Keywords: Psychostimulants; Proenkephaline; Sensitization; T-cell proliferation; Rat

1. Introduction

Drug addiction is a devastating illness with deleterious consequences not only on behavior, but also on immune system (IS) functions, rendering the individuals more vulnerable to infectious diseases. It is currently known that drugs of abuse clearly perturb immune functions as do stress, mood and emotion (Woiciechowsky et al., 1999; Pruett, 2001; Galinowski et al., 1992; Baldwing et al., 1998; Padgett and Glaser, 2003). Furthermore, the interchangeability between psychostimulant drugs and stress observed at behavioral level, showing long-lasting changes in dopaminergic neurons and in the pituitary functions (Diaz-Otañez et al., 1997; Knych and Eisenberg, 1979; Saal et al., 2003), was also found in the IS (Basso et al., 1999).

Sensitization is a well known adaptive process which has been associated to the long-lasting behavioral consequences following exposure to psychostimulants (Robinson and Berridge, 2000; Vezina et al., 2002). Several of the changes

Abbreviations: IS, immune system; DA, dopamine; NAc, nucleus accumbens; PfC, prefrontal cortex; ENK, enkephalin; AMPH, amphetamine; Con A, concanavalin-A; VEH, vehicle; ZT, zeitgeber; RIA, radioimmunoassay; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; FITC, fluorescein isothiocyanate; MoAb, monoclonal antibody; PE, R-phycoerythrin; CREB, cAMP-response element binding protein; AP-1, activator protein-1.

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[1] Fellowship from CONICET.

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doi:10.1016/j.jneuroim.2006.05.009

that underlie this process occur in the mesocorticolimbic dopamine (DA) systems, of which the ventral tegmental area, nucleus accumbens (NAc) and prefrontal cortex (PfC) are the main components. However, enhanced dopaminergic transmission is only one component of the motive circuit, which mediates behavioral sensitization to psychostimulants. Other neurotransmitter systems, such as enkephalins (ENKs) and glutamate, are also among the most important neurotransmitters in the mesocorticolimbic systems associated with the development and/or expression of sensitization (Kalivas and Stewart, 1991; Pierce and Kalivas, 1997a). Notwithstanding, no evidence has so far been provided regarding possible psychostimulant-induced long-lasting changes in the ENK levels in these brain areas.

It has been shown that the administration of central nervous system (CNS) acting drugs into the lateral ventricle of the brain, induced a similar effect on the IS as that observed following the systemic infusion of the drug (Pellegrino and Bayer, 1998a, 2000) suggesting that a central component could be involved. Related to this, it was found that brain DA and opioid systems have been involved in the impairment of IS functions following psychostimulants and/or stress (Basso et al., 1999; Saravia et al., 1998; Kubera et al., 2002; Jankovic and Radulovic, 1992). However, it is also possible that autocrine and paracrine actions of opioid peptides and DA produced by immune cells may be responsible for the effects of drug abuse on IS (Gordon and Barnes, 2003; Pellegrino and Bayer, 1998b).

ProENK mRNA and proENK derived peptides were detected in immune cells of bone marrow, thymus, spleen and alveolar macrophages (Vindrola et al., 1990; Saravia et al., 1993; Linner et al., 1995, 1991; Padrós et al., 1989; Kuis et al., 1991; Roth et al., 1989; Rittner et al., 2001). However, the myeloid lineage cells are the only immune cells that contain the prohormone converting enzymes PC1 and/or PC2, which are involved in the post-translational processing of proENK to produce the opioid peptides (Roth et al., 1989; Vindrola and Lindberg, 1992; Mathis and Lindberg, 1992). Because of this, only macrophages and neutrophils are able to synthesize opioid peptides derived from proENK (i.e. met-ENK) (Saravia et al., 1998; Kuis et al., 1991). In agreement with this, it was shown that T-lymphocytes release cryptic met-ENK-containing peptides but they do not have opioid activity (Roth et al., 1989; Padrós et al., 1995). On the other hand, several physiological studies have demonstrated the immunosuppressive effect of opioid peptides (Jankovic and Radulovic, 1992). Interestingly, a stressful stimulus inducing immunosuppression modified proENK-derived peptide levels and their release from cells of peripheral immune tissues (Saravia et al., 1998) as it did in CNS (Borsook et al., 1994; Lightman and Young, 1987). In addition to these neuropeptides, the catecholamines, including DA, are also present in lymphocytes, macrophages and neutrophils, which also express the transporters, receptors and synthesis enzymes for these neurotransmitters (Bergquist et al., 1994; Amenta et al., 2001; McKenna et al., 2002; Cosentino et al., 2002).

It has been shown that long-lasting behavioral sensitization is induced following either a single dose or a chronic amphetamine (AMPH) treatment (Vanderschuren and Kalivas, 2000). In this work, we showed that a single dose of AMPH is also able to induce effects on IS, which were observable after 4 days, as in the case of a repeated regime with the drug. At the same time of observation, it could be seen that both acute and repeated AMPH treatments had induced a simultaneous increase of met-ENK in IS, as well as in CNS. We also found that AMPH was a sufficient stimulus for splenic T-lymphocytes to produce almost as many cryptic proENK-derived peptides as concanavalin-A (Con A). Interestingly, it was shown that splenic macrophages were the cells that synthesized elevated amounts of met-ENK following the drug. All these data suggest that the activation of enkephalinergic systems in CNS and IS may be involved in the AMPH's effects on both systems. Furthermore, changes in the proENK-derived peptide levels from peripheral immune cells might be an index reflecting an opioid central effect of AMPH.

2. Materials and 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. All procedures were conducted in accordance with the NIH Guide for the Care and Use of Laboratory Animals as approved by Animal Care and Use Committee of the Facultad de Ciencias Químicas, Universidad Nacional de Córdoba.

2.2. Drugs

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

2.3. Experimental procedures

2.3.1. Repeated AMPH treatment

Rats were randomly assigned to one of three treatments: VEH group, AMPH 5 × 1 (1 mg/kg/day i.p.) and AMPH 5 × 2