Psychoneuroendocrinology 63 (2016) 94–101

Contents lists available at ScienceDirect

Psychoneuroendocrinology

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

The neuroendocrine response to stress under the effect of drugs: Negative synergy between amphetamine and stressors

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ARTICLE INFO

Article history:
Received 30 April 2015
Received in revised form 7 September 2015
Accepted 7 September 2015

Keywords:
Hypothalamic-pituitary-adrenal axis
CRH
Plasma glucose
Immobilization
Forced swim
Interleukin-1β

ABSTRACT

There have been numerous studies into the interaction between stress and addictive drugs, yet few have specifically addressed how the organism responds to stress when under the influence of psychostimulants. Thus, we studied the effects of different acute stressors (immobilization, interleukin-1β and forced swimming) in young adult male rats simultaneously exposed to amphetamine (AMPH, 4 mg/kg SC), evaluating classic biological markers. AMPH administration itself augmented the plasma hypothalamic-pituitary-adrenal (HPA) hormones, adrenocorticotropin (ACTH) and corticosterone, without affecting plasma glucose levels. By contrast, this drug dampened the peripheral HPA axis, as well as the response of glucose to the three stressors. We also found that AMPH administration completely blocked the forced swim-induced expression of the corticotropin-releasing hormone (hnCRH) and it partially reduced c-fos expression in the paraventricular nucleus of the hypothalamus (PVN). Indeed, this negative synergy in the forced swim test could even be observed with a lower dose of AMPH (1 mg/kg, SC), a dose that is usually received in self-administration experiments. In conclusion, when rats that receive AMPH are subjected to stress, a negative synergy occurs that dampens the prototypic peripheral physiological response to stress and activation of the PVN.

1. Introduction

Over the past decades a wide range of interactions between stress and addictive drugs have been demonstrated, such as cross-sensitization between addictive drugs and chronic stress (Kalivas and Stewart, 1991), facilitation in the acquisition of drug self-administration by prior chronic stress (Piazza and Le Moal, 1998), and stress-induced drug-seeking after extinction (Shaham et al., 2000). The interactions between addictive drugs and stress appear to involve some of the main components of the hypothalamic-pituitary-adrenal (HPA) axis, one of the key systems involved in the stress response. HPA activation is a common feature of all addictive drugs (Armario, 2010), and glucocorticoids have been implicated in the cross-sensitization and enhanced vulnerability to psychostimulant self-administration (Marinelli and Piazza, 2002). Moreover, the corticotropin-releasing hormone (CRH), the main hypothalamic factor regulating the HPA axis, is strongly involved in different aspects of addiction (Zorrilla et al., 2014).

Psychostimulant drugs like amphetamine (AMPH) and stressors activate a range of common brain areas (e.g. Cullinan et al., 1995; Rotllant et al., 2010), paving the way for certain functional interactions between these inputs. In fact, there are important antecedents in the literature regarding the interaction between psychostimulant drugs (mainly AMPH) and stress. Psychostimulant administration provokes greater locomotor activity in a novel environment than in their home cages, in conjunction with enhanced activation (c-fos expression) in the caudate-putamen (Badiani et al., 1998). Moreover, AMPH administration in the home cage selectively increases dopamine D1 receptor positive striatal neurons, while D2 receptor positive neurons were also activated by administration of this drug in a novel environment (Badiani et al., 1999).

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http://dx.doi.org/10.1016/j.psyneuen.2015.09.006
0306-4530/

However, the response to the interaction between AMPH and the novel environment is specific to the particular brain area (Day et al., 2001; Uslaner et al., 2001). Interestingly, a negative synergy between various stressors and AMPH was observed in the central amygdala (CeA) and the oval bed nucleus of the stria terminalis (BSTov) after simultaneous exposure to these two stimuli (Day et al., 2001, 2005, 2008). Therefore, it appears that some responses to simultaneous stress and psychostimulant exposure might be positively modulated and others negatively so, although the reasons underlying such brain specific interactions are unclear. In brain areas containing monoaminergic neurons, AMPH might inhibit stress induced activation by enhancing the somatodendritic release of monoamines and the ensuing activation of autoreceptors (Rotllant et al., 2010). In areas innervated by monoaminergic axons, such response could be more complex in function of the contribution of non-monoaminergic terminals to the stress-induced activation observed, and of the balance between the depression of monoaminergic neuron activity and the activity-independent release of monoamines by AMPH.

Hence, we set out to study on a particular aspect of the possible AMPH-stress interaction, specifically how acute exposure to AMPH influences the response of the HPA axis to stress. To achieve this, we chose not follow the more classical approach of injecting the drug and then later studying the response to stress for three main reasons. First, the neuronal population activated by each stimulus is better characterized after simultaneous exposure, avoiding the possible modifications to the system that prior AMPH treatment might produce and that might alter the response to subsequent stress. Second, any reduction in the HPA response to stressors could be interpreted in terms of enhanced negative feedback caused by prior corticosterone release caused by the drug. Finally, while AMPH and severe stressors (such as immobilization on boards — IMO) induce similar c-fos expression in the paraventricular nucleus of the hypothalamus (PVN), the critical brain area that controls the HPA axis, IMO activates more CRH positive neurons than AMPH (Rotllant et al., 2007). Although these data might suggest that IMO and AMPH partially activate different PVN neuronal populations, simultaneous exposure to AMPH and IMO did not produce an additive effect in terms of the number of neurons expressing Fos (Rotllant et al., 2007), indirectly suggesting that one stimulus may inhibit the other. Thus, in the present work we tested the hypothesis that simultaneous exposure to stress and AMPH may dampen the activation of the HPA axis, as well as other physiological responses to stress.

2. Materials and methods

2.1. Animals and general procedures

Two-month-old male Sprague–Dawley rats from the Animal Facility of the Universitat Autònoma de Barcelona were used in this study and they were housed in pairs under standard conditions: temperature (21 °C), a 12 h light–dark schedule (lights on at 8:00), food and water ad libitum. All experimental treatments were delivered in the morning. The experimental protocol was approved by the Committee of Ethics of the Universitat Autònoma de Barcelona and the Generalitat de Catalunya, and it was carried out in compliance with the European Council Directive (2010/63/EU) and Spanish legislation (RD 53/2013).

2.2. Techniques

2.2.1. Radioimmunoassays

Plasma adrenocorticotropin (ACTH) and corticosterone levels were analyzed by radioimmunoassay, and glucose was assayed in an enzymatic procedure, as described previously (Rabasa et al., 2011).

2.2.2. In situ hybridization (ISH) assays

Immediately after exposure to the stressor terminated, the rats were anesthetized with isoflurane and then perfused transcardially with saline solution (0.9% NaCl, 4 °C) for 2 min. The rats were then perfused for a further 12 min with 4% paraformaldehyde (PFA) and sodium tetraborate 3.8% (borax, 4 °C), after which their brain was removed, submerged in PFA and stored at 4 °C for 24 h. The brains were then cryoprotected in a 30% sucrose solution diluted in potassium phosphate-buffered saline (PBS), and they were maintained for 48 h at 4 °C. Subsequently, the brains were frozen in isopentane chilled to −50 °C on dry ice and they were conserved at −80 °C. Coronal cryostat sections (14 μm, Leica) of the PVN were obtained and preserved at −20 °C in a cryoprotectant solution (sodium phosphate 25 mM [pH 7.3], 30% ethylene glycol and 20% glycerol).

The antisense probes to detect CRH hnRNA were generated by in vitro transcription (SP6/T7 Transcription Kit, Roche) and the protocol used for radioactive ISH of CRH hnRNA was as reported previously (Vallès et al., 2003). To measure c-fos mRNA in the PVN, non-radioactive fluorescent ISH (FISH) was performed using a digoxigenin-11-UTP labeled riboprobe (Roche Applied Science). After hybridization, the slides were washed, and the endogenous peroxidase activity was quenched for 30 min at room temperature (RT) with 3% H_2O_2 in Tris-buffered saline (TBS, pH 7.5) and gentle agitation. To detect the labeled DIG-probe, the slides were washed in TBS containing 0.05% Tween 20 (T-TBS, pH 7.5) and incubated for 1 h at 37 °C in blocking buffer (2% bovine serum albumin -BSA- in T-TBS). The slides were then incubated overnight at 4 °C in a humidified chamber with an anti-digoxigenin-peroxidase antibody (diluted 1:500 in blocking buffer, 2% fetal calf serum -FCS-, 0.1% acetylated BSA and 0.1% Tween 20: 175 μl/slide; Roche Applied Science) and with gentle agitation. The next day, the coverslips were removed and the slides were washed with T-TBS, and the digoxigenin-UTP-fos probe was visualized with TSA-Plus-Cyanine 3 kit (1:50; 100 μl/slide, PerkinElmer) for 7 min and 30 s at RT in a humid chamber, producing a red signal. Finally, the slides were washed in T-TBS, counterstained for 5 min at RT with Hoescht (1:10,000), washed in T-TBS, rinsed with TBS to remove any remaining detergent, washed in milliQ H_2O, air dried and coverslipped with mounting medium (Fluoromount, Sigma). Control slides of the same tissue were processed without adding the probe to check the non-specific binding of the anti-digoxigenin-peroxidase, and they produced no detectable fluorescence.

2.2.3. Image analysis

Densitometric analysis of radioactive ISH was performed on autoradiographic films using bright field microscopy, paying particular attention to control the light intensity during the analysis. Images were taken from the medial dorsal parvocellular subdivision of the PVN (mpdPVN) according to the stereotaxic atlas of Paxinos and Watson (2007). Data from 4 to 8 images per animal and area (considering both sides) were obtained with a digital camera (Nikon, DMX 1200) coupled to a microscope with a 10× objective (Nikon, Eclipse E400), and they were averaged. The RNA levels were determined semi-quantitatively from the optical density using the ImageJ 1.47 software and based on the number of pixels in a given area. RNA values were expressed as arbitrary units (A.U., number of pixels × optical density) and the background signal was obtained from the area adjacent to the PVN. For FISH, 4-6 confocal microscopy (Zeiss LSM 700, Barcelona, Spain) images per animal and area were analyzed. The fluorescent signal for c-*fos* mRNA was quantified in individual cells using ImageJ 1.47 software.