Neuroscience 352 (2017) 19–29

FASTING BIASES μ-OPIOID RECEPTORS TOWARD β–ARRESTIN2-DEPENDENT SIGNALING IN THE ACCUMBENS SHELL

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Abstract—The μ-opioid receptor (MOR) and dopamine D_1 receptor are co-expressed in the medium spiny neurons of striatal areas and the signaling pathways activated by these two receptors are in functional competition. However, in certain conditions an integrated response mediated by the dopamine D_1 receptor transduction system is observed. In mice, morphine administration induces hypermotility and this response has been described in terms of a β-arrestin2-dependent mechanism that favors prevalent dopamine D_1 receptor activation. In rats, acute morphine administration induces hypermotility only when the animals are food-deprived (FD). We aimed to further investigate the functional interaction between the MOR and dopamine D_1 receptors in striatal areas and we studied the effects of acute pharmacological MOR stimulation on motility and nucleus accumbens shell (NAcS) dopamine D_1 receptor signaling in control rats and rats with reduced β-arrestin2 expression in the NAcS, either non food-deprived (NFD) or FD. Motility and dopamine D_1 receptor signaling increased only in FD rats in a β-arrestin2-dependent way. Moreover, FD rats showed a β-arrestin2-dependent increase in the levels of MOR-dopamine D_1 receptor heteromeric complexes in the NAcS. Sucrose consumption is accompanied by release of endogenous opioids and dopamine in the NAcS. We then examined MOR-dopamine D_1 receptor interactions after sucrose consumption. Sucrose increased NAcS dopamine D_1 receptor signaling in NFD and FD rats, and a reduction in β-arrestin2 expression prevented this effect selectively in FD rats. These results show the β-arrestin2-dependent prevalence of dopamine D_1 receptor signaling in response to acute morphine or sucrose consumption elicited by food deprivation in rats.

Key words: dopamine D_1 receptors, dopamine and cAMP-regulated phosphoprotein of Mr 32000 (DARPP-32), morphine, rat, sucrose.

INTRODUCTION

In striatal areas, both in the dorsal (caudate-putamen, CPu) and ventral (nucleus accumbens, NAc) components, μ-opioid receptors (MORs) are predominantly expressed in the medium spiny neurons that also express dopamine D_1 receptors (Noble and Cox, 1995; Georges, 1999; Lindskog et al., 1999; Chartoff and Connery, 2014). Neurons expressing both receptors have been observed in the cortex and striatum where MOR can form a stable heteromeric complex with the dopamine D_1 receptor with no intermediary protein (Juhasz et al., 2008). In the basal ganglia MORs are coupled with a $G_{i/o}$ protein and inhibit adenylyl cyclase activity (Childers, 1991), while the dopamine D_1 receptor effect is mediated by the activation of the adenylyl cyclase-cAMP-dependent protein kinase (PKA) signaling cascade through a G_s protein. Thus, the transduction systems of the two receptors appear to be in functional competition in the neurons where they are co-expressed. In mice acute morphine administration induces an increase in locomotor activity (Stevens et al., 1986; Kieffer and Gavériaux-Ruff, 2002; Patti et al., 2005) that is dopamine D_1 receptor-dependent (Longoni et al., 1987; Bohn et al., 2003; Borgkvist et al., 2007). This effect is mediated by the formation of a β-arrestin2/phospho-ERK signaling complex elicited by MOR stimulation that results in activation of dopamine D_1 receptor signaling in the NAc (Urs et al., 2011); that is, the two receptors may also concur to elicit a common effect. β-Arrestins are multifunctional scaffold proteins involved in desensitization of G protein-coupled receptors signaling and G protein–independent signal transduction (Reiter et al., 2012). We were intrigued by the fact that in rats acute morphine administration induces hypermotility only when they are food-deprived (FD) (Deroche et al., 1993), and hypothesized that this effect, in a condition of acute food deprivation, could be sustained by a β-arrestin2-mediated interaction between the MOR and dopamine D_1 receptor, as observed in mice independently of their feeding state (Urs et al., 2011). Thus, the first aim of this study was to investigate the influence of a 18-h food deprivation on the MOR-dopamine D_1 receptor interaction in the shell portion of the NAc (NAcS) of rats. In order to assess this hypothesis, we studied the effects of MOR acute pharmacological stimulation induced by morphine administration on motility and dopamine D_1 receptor signaling in the NAcS of control rats

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Abbreviations: ANOVA, analysis of variance; CPu, caudate-putamen; DARPP-32, dopamine and cAMP-regulated phosphoprotein of Mr 32,000; FD, food-deprived; GPCRs, G protein-coupled receptors; MOR, μ-opioid receptor; NAcC, nucleus accumbens core; NAcS, nucleus accumbens shell; NFD, non food-deprived; SDS, sodium dodecyl sulfate; shRNA, short hairpin RNA; Thr, threonine.

http://dx.doi.org/10.1016/j.neuroscience.2017.03.056
0306-4522/

and rats with regionally reduced β-arrestin2 expression, either non food-deprived (NFD) or FD.

The second aim of the study was to assess whether β-arrestin2 in the NAcS, besides its role in the response to a pharmacological stimulation of MOR, also played a role in the dopamine D_1 receptor-mediated signaling modifications in response to a natural stimulus such as palatable food consumption, and whether food deprivation affected the possible β-arrestin2-mediated interaction. Sucrose is commonly used to study behavioral and neurochemical responses to food consumption and is endowed with two distinct hedonic components, taste (palatability) and post-ingestive caloric value (Elizalde and Sclafani, 1990; Drucker et al., 1994). The sweet taste of sucrose evokes a series of 'liking' reactions that seem to be mediate by MOR stimulation at different central nervous system levels, such as the nucleus of the solitary tract (Kotz et al., 1997), the parabrachial nucleus (Chaijale et al., 2013) and the NAcS (Peciña and Berridge, 2005). Moreover, sucrose consumption induces in rats a transient increase in extraneuronal dopamine levels in the NAcS associated with dopamine D_1 receptor-sustained modifications in the phosphorylation pattern of some PKA substrates such as dopamine and cAMP-regulated phosphoprotein of Mr 32,000 (DARPP-32) (Rauggi et al., 2005). The consumption of a sweet caloric meal elicits slightly distinct yet consistent behavioral and neurochemical responses in FD and NFD rats (Danielli et al., 2010; Scheggi et al., 2013). We therefore studied these dopamine D_1 receptor-sustained modifications in the DARPP-32 phosphorylation pattern in response to sucrose consumption in control rats and rats with regionally reduced β-arrestin2 expression, either NFD or FD. We report that the locomotor activity and dopamine D_1 receptor signaling in response to acute morphine administration increased only in FD rats, and the reduced expression of β-arrestin2 in the NAcS prevented the occurrence of these effects. Moreover, in FD rats we observed a β-arrestin2-dependent increase in the levels of immunoprecipitates of MOR-dopamine D_1 receptor complexes in the NAcS. In agreement with previous observations (Danielli et al., 2010; Scheggi et al., 2013), sucrose consumption induced an increase in NAcS dopamine D_1 receptor signaling in NFD and FD rats, yet this increase was dependent on β-arrestin2 expression selectively in FD rats. Thus, this study suggests that a condition of mild food deprivation induces a β-arrestin2-mediated increase in dopamine D_1 receptor signaling upon MOR stimulation by either pharmacological (morphine) or endogenous (opioid peptides released in response to sucrose) agonists.

EXPERIMENTAL PROCEDURES

Animals

Experiments were carried out on male Sprague–Dawley rats (Charles River, Calco, Italy), weighing 275–300 g when the experimental procedures began, allowing 10 days of habituation to the animal colony. Rats were group-housed (4–5 animals per cage) with a Lignocel® 3/4S bedding (Envigo, Bresso, Italy) in an environment maintained at a constant temperature and humidity with free access to food (4RF21, Mucedola, Settimo Milanese, Italy) and water, unless differently specified. A 12-h reverse light/dark cycle (7:00 a.m. lights off, 7:00 p.m. lights on) was used. Experiments were carried out from 9:00 a. m. to 5:00 p.m. under a red light and controlled noise conditions.

In order to study the modifications in DARPP-32 phosphorylation in response to sucrose consumption, rats were habituated for a week to be handled and 500 μl of water or sucrose solution (10% wt/vol) were administered orally 30 min before sacrifice. Rats were randomly assigned to the different experimental groups. Sample sizes (number of animals) were not predetermined by a statistical method and rats were randomly assigned to experimental or control groups. Experimental or control groups were blinded for measurement of locomotor activity, and for immunoblotting and immunoprecipitation quantification. No predefined exclusion criteria were set. The procedures used were in accordance with the European legislation on the use and care of laboratory animals (EU Directive 2010/63) and were approved by the Ethics Committee of the University of Siena. All efforts were made to minimize the number of animals used and their suffering.

Locomotor activity

Locomotor activity was evaluated in motility cages that detected horizontal activity (Imetronic, Pessac, France) as previously described (Grappi et al., 2011). Rats were individually placed in the locomotor activity cages to habituate to the new environment and after 15 min they received saline (1 ml/kg, s.c.) or morphine (5 mg/kg, s.c.) and locomotor activity was recorded for 35 min (the first 5 min were not taken into account) by an experimenter blinded to the experimental conditions. Total motility counts in 30 min are represented as the mean ± S.E.M.

Immunoblotting

At the end of behavioral observation, rats were sacrificed by decapitation and the NAcS, identified using the Atlas of Rat Brain corresponding to plates 10–12 (Paxinos and Watson, 1997), was excised using the rapid head-freeze dissection technique as previously described (Danielli et al., 2010; Scheggi et al., 2010). For immunoblotting of DARPP-32, tissues were solubilized in boiling 1% sodium dodecyl sulfate (SDS) and 50 mM NaF. For immunoblotting of MOR and dopamine D_1 receptor, tissues were solubilized in cell lysis buffer (50 mM TRIS, pH 7.4, 250 mM NaCl, 5 mM EDTA, 50 mM NaF, 1 mM sodium orthovanadate, 1% Triton X-100, 0.02% NaN_3) containing 1 mM phenylmethylsulfonyl fluoride and protease inhibitor cocktail. Small aliquots of the homogenate were used for protein determination by a modified Lowry protein assay method (DC protein assay, Bio-Rad