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IMPRAMINE, FLUOXETINE AND CLOZAPINE DIFFERENTLY AFFECTED REACTIVITY TO POSITIVE AND NEGATIVE STIMULI IN A MODEL OF MOTIVATIONAL ANHEDONIA IN RATS

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Abstract—Anhedonia is a relevant symptom in depression and schizophrenia. Chronic stress exposure induces in rats escape deficit, disrupts the dopaminergic response to palatable food and the competence to acquire sucrose self-administration (SA), thus configuring a possible model of motivational anhedonia. Repeated lithium administration reverts stress effects and brings back to control values the breaking point (BP) score, a measure of reward motivation. In this study, we tested on this model two antidepressants, imipramine and fluoxetine, and two antipsychotics, haloperidol and clozapine. The dopaminergic response to sucrose consumption was studied in non food-deprived rats in terms of dopamine D_1 receptor signaling in the nucleus accumbens shell (NAcS). More specifically, we studied the modifications in cAMP-regulated phosphoprotein of Mr 32,000 (DARPP-32) phosphorylation pattern following sucrose consumption. Fluoxetine reverted the escape deficit and showed no effects on dopaminergic response and sucrose SA. Imipramine reverted sucrose SA and dopamine response deficit in half of the rats and the escape deficit in all animals. Haloperidol did not affect stress-induced deficits. Clozapine-treated rats recovered the dopaminergic response to sucrose consumption and the competence to acquire sucrose SA, although they still showed the escape deficit, thus confirming that motivation toward reward may be dissociated from that to punishment escape. These results indicate that imipramine or fluoxetine are not endowed with a rapid onset antianhedonic effect. On the other hand, clozapine treatment showed a motivational antianhedonic activity similar to that observed after lithium treatment. © 2015 Published by Elsevier Ltd. on behalf of IBRO.

Key words: dopamine, dopamine and cAMP-regulated phosphoprotein of Mr 32,000 (DARPP-32), stress, sucrose, self-administration.

INTRODUCTION

Anhedonia is considered a core symptom of depression and schizophrenia, although it is a symptom as difficult to define as to treat (Treadway and Zald, 2011). The DSM-IV-TR and the DSM-V (DSM-IV-TR®, 2000; DSM-V-TR®, 2014) refer to anhedonia as diminished interest or pleasure in response to stimuli perceived as rewarding during a premorbid state. Thus, clinical diagnosis does not discriminate between a decrease in motivation and a reduction in experienced pleasure, although the neurobiological mechanisms underpinning the consummatory ("liking") and preparatory ("wanting") behaviors controlled by positive stimuli clearly distinguish pleasure from motivation (Treadway and Zald, 2011). In rodents, responses to palatable food are a validated index of hedonic responsiveness (Willner et al., 1987) and, although palatability is independent of dopaminergic transmission (Berridge and Robinson, 1998), palatable food consumption induces a phasic increase in extraneuronal dopamine levels in the mesolimbic areas that confer to it incentive salience (Berridge, 2007). Non food-deprived rats can be trained to self-administer sucrose and the breaking point (BP) score can be recorded. BP measures the effort animals are willing to exert in order to obtain the reinforcing stimulus (Salamone et al., 2012) and is considered an index of animal motivation.

The ingestion of a food of unexpected palatability induces in non food-deprived rats a consistent dopami nergic response in the shell portion of the nucleus accumbens shell (NAcS) in terms of increased extran euronal dopamine concentration and dopamine D_1 receptor-dependent signaling (Bassareo and Di Chiara, 1999; Gambarana et al., 2003; Rauggi et al., 2005). In particular, an increase in PKA-dependent phosphorylation of cAMP-regulated phosphoprotein Mr 32,000 (DARPP-32) in the Thr34 residue is observed and increases in both extraneuronal dopamine and phospho-Thr34-DARPP-32 levels are reduced after a second consumption of the same food, indicating that the actual hedonic value is dependent on novelty besides palatability (Danielli et al., 2010). Repeated exposure to unavoidable stress induces two distinct behavioral modifications in rats: reduced reactivity to aversive stimuli and reduced motivation to earn palatable food (Gambarana et al., 2001; Marchese et al., 2013).

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Abbreviations: ANOVA, analysis of variance; BP, breaking point; DARPP-32, dopamine and cAMP-regulated phosphoprotein of Mr 32,000; FR, fixed-ratio; NAcS, nucleus accumbens shell; PR, progressive ratio; SA, self-administration; SDS, sodium dodecyl sulfate; Thr, threonine.

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Moreover, unavoidable stress exposure disrupts the dopaminergic responses to palatable food consumption, and repeated lithium treatment reverts all these effects (Marchese et al., 2013). Thus, we proposed an experimental model that conforms to face validity for decreased appetitive motivation and is responsive to lithium treatment (Marchese et al., 2013), although clinical studies on lithium efficacy did not specifically address this issue. Modifications in signaling after palatable food consumption seem to match the modifications observed in extraneuronal dopamine levels (Danielli et al., 2010). Thus, we first verified whether modifications in dopamine D_1-dependent signaling represented a useful index of the NAcS dopaminergic response to the consumption of a natural reward. To this end, we studied whether these modifications following sucrose ingestion matched extraneuronal dopamine modifications in the NAcS of non food-deprived control rats and rats expressing chronic stress-induced decrease in appetitive motivation, treated or not with lithium. A pattern of changes consistent with the previously reported extraneuronal dopamine increase (Marchese et al., 2013) was observed after sucrose consumption in lithium-treated rats, exposed or not to chronic stress, confirming the efficacy of lithium to restore the dopaminergic response to palatable food in chronically stressed rats, as well as the validity of the proposed index. On these premises, using the same experimental protocol utilized with lithium (Marchese et al., 2013), we then studied the possible activity of some antidepressant and antipsychotic drugs in reinstating appetitive motivation in non food-deprived rats.

EXPERIMENTAL PROCEDURES

Animals

Experiments were carried out on male Sprague–Dawley rats (Charles River, Calco, Italy), weighing 200–225 g when the experimental procedures began, allowing 10 days of habituation to the animal colony. Animals were housed 4–5 per cage (bedding Lignocel® 3/4S, Harlan Laboratories, San Pietro al Natisone, Italy) in an environment maintained at a constant temperature and humidity with free access to food (4RF21, Mucedola, Settimo Milanese, Italy) and water. 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 all the experiments, body weight did not significantly differ between groups at the beginning and at the end of experimental procedures. The procedures used were in accordance with the European legislation on the use and care of laboratory animals (EU Directive 2010/63) and they were approved by the University of Siena Ethics Committee. All efforts were made to minimize the number of animals used and their suffering.

Immunoblotting

Rats were killed and the NAcS was excised using the rapid head-freeze dissection technique previously described (Danielli et al., 2010). Tissues were solubilized in boiling 1% sodium dodecyl sulfate (SDS) and 50 mM NaF. Small aliquots of the homogenate were used for protein determination by a modified Lowry protein assay method (DC protein assay, Bio-Rad Laboratories, Hercules, CA, USA). Western blot analysis was performed as previously described (Danielli et al., 2010). Briefly, proteins (30 μg) were loaded into 10% SDS–PAGE gels (Invitrogen, Carlsbad, CA, USA), transferred onto nitrocellulose membranes, and incubated with antibodies against phospho-Thr34 DARPP-32, phospho-Thr75 DARPP-32 and total DARPP-32 (Cell Signaling Technology, Beverly, MA, USA). Blots were developed using a chemiluminescence detection system (Pierce Biotechnology Inc., Rockford, IL, USA) and quantified with the Versa Doc 1000 Imaging System (Bio-Rad Laboratories). Samples containing the same amount of total proteins from rats in each experimental group were run on the same immunoblots and then analyzed together. To control for equal loading, blots were reprobed with the non-phosphorylation-state-specific antibody; when a greater than 10% difference in the levels of total DARPP-32 was detected, protein concentrations were determined again and a new immunoblotting experiment was performed. Thus, although levels of phosphorylated proteins were not normalized to the respective total protein levels, only the data obtained with equal protein loading were utilized. Stress exposure and lithium, imipramine, fluoxetine, haloperidol or clozapine 10-day administration *per sè* did not modify baseline expression levels of DARPP-32 and its Thr34 and Thr75 phosphorylated forms.

Chronic stress protocol

The experimental procedure, previously described (Gambarana et al., 2001), consisted in the induction of an escape deficit and its maintenance by exposure to minor unavoidable stressors. Briefly, rats were immobilized with a flexible wire-net and administered about 80 tail shocks (1 mA × 5 s, 1 every 30 s). Twenty-four hours later, rats were exposed to a shock-escape test. Rats were then exposed on alternate days to unavoidable stressors, beginning 48 h after the escape test. Rats were exposed to stress sessions in the afternoon, 3–4 h after the end of self-administration (SA) sessions. Control rats were manipulated daily by experimenters. Since rats exposed to chronic stress show scarce interest in sucrose pellets and a variable latency to approach and consume them, in order to study the dopaminergic response to sucrose consumption (immunoblotting experiments), rats were habituated for a week to be handled and 30 min before sacrifice the sucrose solution (10%) was administered orally.

SA procedure

Experiments were conducted in operant chambers (MED Associates Inc., St. Albans, VT, USA) as previously described (Marchese et al., 2013). Chambers were enclosed in ventilated, sound-attenuating boxes and they contained two response levers; during SA testing, a lever-press response at the active lever delivered a sucrose pellet (Bio-Serv, Frenchtown, NJ, USA) into the food

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