Neuropharmacology 130 (2018) 10–17

Contents lists available at ScienceDirect

Neuropharmacology

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

Sucrose withdrawal induces depression and anxiety-like behavior by Kir2.1 upregulation in the nucleus accumbens

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ARTICLE INFO

Article history:
Received 22 August 2017
Received in revised form
24 November 2017
Accepted 25 November 2017
Available online 27 November 2017

Keywords:
Kir2.1
Depression
Anxiety
Sucrose withdrawal
Nucleus accumbens
Dopamine

ABSTRACT

Dieting induces depression and anxiety among other emotional symptoms. Animal models indicate that repeated access to palatable foods such as sugar induces depression and anxiety-like behavior when the food is no longer available. However, the neurobiological mechanisms of how dietary restriction influences mood have not been fully understood. We used the two-bottle sucrose choice paradigm as an overeating and withdrawal model. Withdrawal after lengthy sucrose overeating elicited depression and anxiety-like behavior, which was reversed by sucrose reinstatement. In the nucleus accumbens (NAc) of sucrose withdrawal animals, dopamine levels and cAMP response element binding protein (CREB) activity were significantly reduced, while the inwardly rectifying K^+ channel, Kir2.1, was significantly elevated. In addition, overexpression of Kir2.1 selectively in neurons expressing dopamine D1 receptors was sufficient to induce negative mood-linked behavior in the absence of sucrose overeating experience. As elevated K^+ channels reduce neuronal excitability, a sucrose withdrawal-induced increase in Kir2.1 expression is able to decrease NAc activity, which provides a cellular basis for depression and anxiety-like behavior in animals.

1. Introduction

The nucleus accumbens (NAc) is a major component of the brain reward system and has long been implicated in numerous neurological and psychiatric disorders, including mood disorders, neurodegenerative diseases, obesity, and addiction (Francis and Lobo, 2017; Kenny, 2011a; Nestler and Carlezon, 2006). Increased activity in the NAc is observed in association with reward-seeking behaviors. Conversely, decreased activity and dysfunction are seen in patients with mood and eating disorders (Shirayama and Chaki, 2006). Moreover, chronic stress or withdrawal from long-term exposure to drugs causes anhedonia (a lack of ability to experience pleasure) and depression and anxiety-like behavior in both human and rodents (Barr et al., 2002; Willner et al., 1992). Significantly, dopamine directly activates the NAc and affects both mood and food intake (Kaye et al., 2009). Palatable foods are a natural reward that activates the dopamine system in the NAc (Rada et al., 2005). Indeed, food high in sugar increases dopamine levels within the NAc (Avena et al., 2012). In addition, sugar bingeing or high-fat diet leads to sensitized behaviors, and withdrawal induces anxiety-like behavior and craving, which can be mediated by the altered dopamine signaling (Avena et al., 2012; Iemolo et al., 2012; Sharma et al., 2013; Teegarden et al., 2008). A disruption of dopaminergic function within the NAc induces anxiety and depression in humans as well as in rodents (Avena et al., 2008; Nestler and Carlezon, 2006; Shirayama and Chaki, 2006). These studies suggest that diminished dopamine signaling leads to decreased activity of the NAc, which could result in withdrawal-induced depression and anxiety-like behavior.

Medium spiny neurons (MSNs) are GABAergic inhibitory cells and represent the major neuron type within the NAc. There are two classes of MSNs, which can be differentiated by their output connectivity and their expression of dopamine receptors, either D1 or D2 receptors (Surmeier et al., 2007). These two subpopulations are homogenously distributed throughout the NAc and are known to have opposite behavioral effects, as they are coupled to output pathways with opposing properties (Alexander et al., 1986; Kauer and Malenka, 2007). D1 receptors in MSNs are positively coupled

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https://doi.org/10.1016/j.neuropharm.2017.11.041
0028-3908/

to adenylyl cyclase, while D2 receptors lead to inhibition of adenylyl cyclase (Gerfen et al., 1990; Greengard et al., 1999). In MSNs expressing D1 receptors (D1-MSNs), dopamine increases cytosolic cAMP levels, leading to the activation of protein kinase A (PKA) and phosphorylation of various intracellular targets, such as dopamine- and cAMP-regulated phosphoprotein-32 (DARP-32) and cAMP response element binding (CREB) protein, altering cellular function (Greengard et al., 1999). Significantly, CREB inactivation is strongly correlated with expression of an inwardly rectifying K^+ channel, Kir2.1 (Dong et al., 2006; Wallace et al., 2009). In the NAc, an increase in K^+ conductance induces hyperpolarization of membrane potentials and reduction of the firing of action potentials, resulting in decreased intrinsic membrane excitability and anxiety-like behavior in rats (Quintero, 2013; Wallace et al., 2009). Therefore, reduction of neuronal activity in the NAc could be a cellular basis for anxiety and depression-like behavior. Indeed, this is supported by studies showing that deep brain stimulation of the NAc may be employed as a therapy for mood disorders (Schlaepfer et al., 2008). Nonetheless, the neurobiological role of MSN excitability in depression and anxiety has not been fully addressed.

Several studies suggest that activity of D1 neurons mainly influences both eating and mood. 1) Activation of D1-MSNs in the NAc increases food intake, and inhibition suppresses food consumption, while the level of D2 receptors has no effect on eating behavior (Zhu et al., 2016). 2) Pharmacological inhibition or genetic deletion of the D1 receptors disrupts normal feeding and induces anxiety (Lutz et al., 2001; Smith et al., 1998; Wall et al., 2011). 3) Chronic restraint stress in mice that induces anorexia leading to weight loss and anhedonia, is linked to altered excitatory transmission selectively onto D1-MSNs (Lim et al., 2012). 4) Human brain imaging studies show that decreased striatal D1 receptor binding is found in patients with major depressive disorder (Dougherty et al., 2006). Conversely, other studies suggest that reduction of D2 receptor signaling is implicated in addiction-like reward deficits and compulsive overeating in animals during the development of obesity (Johnson and Kenny, 2010). This is consistent with the findings that a decrease in striatal D2 receptors is an important neuroadaptive response to weight gain in humans (Barnard et al., 2009; Stice et al., 2008; Wang et al., 2001). Moreover, allelic variants of D2 receptors gene are associated with eating disorders (Kenny, 2011b). This suggests that persistent overeating of palatable foods may alleviate defects in striatal D2 receptor signaling and associated reward impairment in individuals with obesity (Kenny, 2011b). Taken together, the exact degree of segregation between D1-and D2-MSNs has been much disputed. Extensive studies using pharmacology have yielded inconsistent data on the role of these neurons in food intake and mood-related behaviors, so that there remains active debate about the contribution of D1 or D2-MSNs to these behaviors.

Here, we show that withdrawal from sucrose after lengthy sucrose consumption reduces dopamine levels in the NAc, resulting in inactivation of CREB. This leads to upregulation of the expression of K^+ channels in D1-MSNs of the NAc, which was sufficient to induce negative mood-like behaviors in mice.

2. Methods and materials

2.1. Animal

C57BL/6 mice obtained from Taconic were used for the two-bottle sucrose diet. Drd1a-tdTomato mice (a transgenic mouse line that expresses red fluorescent protein, tdTomato, under the D1 receptor promoter) and Drd1BAC-rtTA animals (a transgenic mouse line that expresses reverse tetracycline transactivator under the D1 receptor promoter) were obtained from the Jackson Laboratory. We crossed Drd1BAC-rtTA animals with TetO-Kir2.1 mice (transgenic animals containing a tetracycline-responsive promoter element driving Kir2.1 expression, provided by Dr. C. Ron Yu in Stowers Institute, Kansas City, MO) (Yu et al., 2004) to selectively overexpress Kir2.1 in D1 neurons. All transgenic mice were genotyped by a standard PCR protocol provided from the Jackson Laboratory and the Yu laboratory. For rapid induction of Kir2.1 expression, 2 mg of doxycycline (Dox, Sigma) in 0.5 ml of 0.9% aqueous NaCl were injected intraperitoneally. The injections were repeated twice at intervals of 24 h, and saline was given to control animals as reported previously (Schonig et al., 2002). Animals were housed under 12:12 h light/dark cycle. All behavioral assays were conducted on male adult mice at the ages of 10–12 weeks. Animal experiments were conducted in compliance with the Institutional Animal Care and Use Committees at New York University Langone Medical Center and Colorado State University.

2.2. Two-bottle sucrose diet

We used the two-bottle sucrose choice paradigm modified from a previous study (Wallace et al., 2008) (Fig. 1a). 10% sucrose animals were given unlimited access to one bottle each of water (bottle 1) and 10% sucrose (bottle 2) for four weeks while control water animals were given two bottles of water (Fig. 1a). Withdrawal animals received one bottle each of water (bottle 1) and 10% sucrose for four weeks replaced by water for one week (bottle 2) (Fig. 1a). Reinstatement animals were reinstated to sucrose by providing one bottle each of water (bottle 1) and 2% sucrose (bottle 2) for two days after withdrawal, while control 2% sucrose animals received two bottles of water for five weeks, followed by one bottle each of water (bottle 1) and 2% sucrose (bottle 2) (Fig. 1a). The bottle preference, sucrose consumption and body weight of water and sucrose animals were determined at the day 1, 3, 5, 9, 14, 21, and 28 of the training.

2.3. Behavioral assays

The tail suspension test was performed as described previously (Cryan et al., 2005; Lim et al., 2012). In brief, male mice were suspended by their tails from a rod suspended 20 cm above the tabletop surface with adhesive tape placed 1 cm from the tip of the tail. Animals were considered to be immobile when they exhibited no body movement and hung passively for >10 s. The time during which mice remained immobile was quantified over a period of 6 min. Mice that successfully climbed their tails to escape were excluded from the analysis.

The elevated plus maze was used to determine anxiety-like behavior as shown previously (Wallace et al., 2009). Mice from each group were tested for the time spent in the open and closed arms of the maze over 5 min. Mice that fell off from the maze were excluded from the analysis.

Anhedonia was determined by using the sucrose preference test as described previously (Lim et al., 2012). Male mice from each group were given the choice between water and a sucrose solution after initial habituation to two bottles of water for 24 h. The following day, one bottle was filled with water containing 2% sucrose, and the total volume of liquid consumed from each bottle over the ensuing 24 h was measured. The sucrose preference was calculated as the fraction of sucrose solution consumed compared to the total amount of solution consumed from both bottles. Bottles containing the sucrose solution were randomly placed on the left or right side of the compartment.

For Kir2.1 transgenic animal behavior, we have used two cohorts; one for sucrose preference test and tail-suspension test, and the other for elevated plus maze. At day 1, mice were