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AMPA RECEPTOR SUBUNIT GluR1 DOWNSTREAM OF D-1 DOPAMINE RECEPTOR STIMULATION IN NUCLEUS ACCUMBENS SHELL MEDIATES INCREASED DRUG REWARD MAGNITUDE IN FOOD-RESTRICTED RATS

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Abstract—Previous findings suggest that neuroadaptations downstream of D-1 dopamine (DA) receptor stimulation in nucleus accumbens (NAc) are involved in the enhancement of drug reward by chronic food restriction (FR). Given the high co-expression of D-1 and GluR1 AMPA receptors in NAc, and the regulation of GluR1 channel conductance and trafficking by D-1-linked intracellular signaling cascades, the present study examined effects of the D-1 agonist, SKF-82958, on NAc GluR1 phosphorylation, intracranial electrical self-stimulation reward (ICSS), and reversibility of reward effects by a polyamine GluR1 antagonist, 1-NA-spermine, in *ad libitum* fed (AL) and FR rats. Systemically administered SKF-82958, or brief ingestion of a 10% sucrose solution, increased NAc GluR1 phosphorylation on Ser845, but not Ser831, with a greater effect in FR than AL rats. Microinjection of SKF-82958 in NAc shell produced a reward-potentiating effect that was greater in FR than AL rats, and was reversed by co-injection of 1-NA-spermine. GluR1 abundance in whole cell and synaptosomal fractions of NAc did not differ between feeding groups, and microinjection of AMPA, while affecting ICSS, did not exert greater effects in FR than AL rats. These results suggest a role of NAc GluR1 in the reward-potentiating effect of D-1 DA receptor stimulation and its enhancement by FR. Moreover, GluR1 involvement appears to occur downstream of D-1 DA receptor stimulation rather than reflecting a basal increase in GluR1 expression or function. Based on evidence that phosphorylation of GluR1 on Ser845 primes synaptic strengthening, the present results may reflect a mechanism via which FR normally facilitates reward-related learning to re-align instrumental behavior with environmental contingencies under the pressure of negative energy balance.

Key words: SKF-82958, 1-NA-spermine, addiction, synaptic plasticity.

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Abbreviations: AL, *ad libitum* fed; CPu, caudate-putamen; DA, dopamine; FR, food-restriction; ICSS, intracranial electrical self-stimulation; NAc, nucleus accumbens; 1-NA-spermine, 1-naphthylacetyl spermine.

Regulation of drug rewarding effects by diet composition, energy balance, and body weight have become increasingly well substantiated, beginning with early demonstrations of enhancement by food restriction (Carroll et al., 1979; Carroll and Meisch, 1984) and more recent reports of inhibition by maintenance on high energy diets (Wellman et al., 2007; Davis et al., 2008). These effects are not surprising given the common involvement of ventral tegmental dopamine (DA) neurons and nucleus accumbens (NAc) microcircuitry in the incentive motivational and reward-related learning processes that underlie food- and drug-directed behavior (e.g., Kelley, 2004; Volkow and Wise, 2005; Hyman et al., 2006; Fields et al., 2007).

The enhancement of acute drug rewarding effects by chronic food restriction (FR) has been conceptualized as arising from neuroadaptations that otherwise facilitate foraging, procurement, and reward-related learning in the service of restoring energy balance and body weight (Carr, 2007). Indeed, a variety of changes in presynaptic DA dynamics (Pothos et al., 1995; Cadoni et al., 2003; Pan et al., 2006; Zhen et al., 2006) and postsynaptic intracellular signaling and transcriptional responses to DA receptor agonist administration (Carr et al., 2003; Haberny et al., 2004; Haberny and Carr, 2005a,b) are consistent with this hypothesis. One coherent set of reported changes consists of increased behavioral, intracellular signaling, and transcriptional responses to D-1 DA receptor stimulation in NAc.

Considering that the DA innervation of NAc is convergent with several major limbic forebrain glutamate inputs (Groenewegen et al., 1999; Kalivas et al., 2005), and the integration of DA- and glutamate-coded signals is involved in the regulation of medium spiny neuronal activity (e.g., Moyer et al., 2007; Surmeier et al., 2007), goal-directed behavior, reward-related learning, and addiction (Kelley, 2004; Malenka et al., 2004; Dalley et al., 2005; Hyman et al., 2006), the ability of D-1 receptor stimulation to regulate the phosphorylation state of specific NMDA and AMPA receptor subunits (Wang et al., 2006) is of general functional importance and may be of particular importance in understanding the mechanisms via which FR modulates behavioral responses to food and abused drugs.

Among the glutamate receptor types co-expressed with DA receptors in striatal neurons (Bernard et al., 1997; Glass et al., 2008), AMPA receptors mediate fast excitatory synaptic transmission (Hollmann and Heinemann, 1994; Barry and Ziff, 2002). AMPA receptors are tet-

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rameric and composed of combinations of four subunits, GluR1-4. GluR1 subunit-containing AMPA receptors undergo activity-dependent trafficking, and GluR1 homomers are Ca^{2+} permeable. Generally, insertion and removal of AMPA receptors from the neuronal membrane underlie changes in synaptic strength (Shi et al., 2001; Barry and Ziff, 2002; Derkach et al., 2007). Phosphorylation of GluR1 on Ser845 by D-1 receptor-regulated cAMP and NMDA receptor-regulated cGMP pathways enhances AMPA currents and facilitates rapid insertion into the postsynapse (Roche et al., 1996; Snyder et al., 2000; Banke et al., 2000; Man et al., 2007; Serulle et al., 2007). It is therefore of interest that *in vivo* administration of D-1 DA receptor agonists, cocaine, methamphetamine, and ingestion of sugar lead to a rapid increase in striatal GluR1 phosphorylation on Ser845 that is D-1 DA receptor-dependent (Rauggi et al., 2005; Snyder et al., 2000; Valjent et al., 2005).

AMPA currents are also increased by CaMKII- and PKC-mediated phosphorylation of GluR1 on Ser831 (Derkach et al., 1999), although D-1 agonists, psychostimulants, and sugar do not normally lead to phosphorylation of GluR1 on this serine residue. However, the increased phosphorylation of the NMDA receptor NR1 subunit in NAc of FR rats following D-1 DA receptor stimulation, and the consequent NMDA receptor-dependent activation of CaMKII (Haberny and Carr, 2005a), raise the possibility of GluR1 phosphorylation on Ser831, selectively in FR subjects.

The purpose of the present study was to determine whether *in vivo* administration of a D-1 DA receptor agonist leads to greater phosphorylation of GluR1 on Ser845 and/or Ser831 in NAc of FR relative to *ad libitum* fed (AL) subjects, and whether NAc microinjection of a polyamine GluR1 homomer antagonist attenuates the reward-potentiating effect of a D-1 DA receptor agonist and diminishes the difference otherwise observed between AL and FR subjects. Thus, Experiment 1 examines whether systemically administered SKF-82958, at a dose verified to produce a greater behavioral effect in FR relative to AL rats, increases phosphorylation of GluR1 on Ser845 and Ser831, with a difference between feeding groups. Experiment 2 examines the effect of sucrose ingestion on these measures. In Experiment 3, acute reward-potentiating effects of SKF-82958 microinjected into NAc are compared between AL and FR rats in an intracranial electrical self-stimulation (ICSS) paradigm, and the involvement of GluR1 is assessed by co-infusion of a polyamine GluR1 antagonist, 1-NA spermine. To ascertain the specificity of 1-NA spermine effects to D-1 agonist-induced behavioral responses, acute reward-potentiating effects of the D-2/3 agonist, quinpirole, with and without co-infused 1-NA spermine, are also determined. To assess whether an apparent D-1 DA receptor stimulation-dependent involvement of GluR1 in the enhanced reward-potentiating effect may instead be due to a D-1 receptor-independent increase in GluR1 function, Experiment 4 examines GluR1 abundance in the synaptosomal fraction of NAc tissue samples and the reward-potentiating effect of AMPA microinjection in NAc.

EXPERIMENTAL PROCEDURES

Subjects and surgical procedures

All subjects were male Sprague–Dawley rats (Taconic Farms, Germantown, NY, USA) weighing 350–400 g at the time of arrival in the central animal facility where they were housed in individual plastic cages, with free access to Purina rat chow (St. Louis, MO, USA) and water unless otherwise noted. The animal room was maintained on a 12-h light/dark cycle, with lights on at 07:00 h. Approximately half the subjects in each experiment were placed on a chronic food restriction regimen whereby daily food allotment was limited to 10 g of chow, delivered at 17:00 h, until a 20% decrease in body weight was attained. The remaining subjects continued to have *ad libitum* access to chow. In the biochemical experiments, in which no surgical preparation of subjects was required, food restriction was implemented 3–5 days after arrival of rats in the animal facility. In the behavioral experiments, in which rats were implanted with chronically indwelling stimulating electrodes and microinjection cannulas, food restriction was implemented 10–15 days following surgery. In all experiments, the target body weight of food-restricted rats, which took 15–20 days to achieve, was maintained by titrating the daily food allotment.

In preparation for the behavioral experiments, each rat was anesthetized with ketamine (100 mg/kg i.p.; Fort Dodge Animal Health, Fort Dodge, IA, USA) and xylazine (10 mg/kg i.p.; Lloyd Laboratories, Shenandoah, IA, USA) and stereotaxically implanted with a 0.25 mm diameter monopolar stimulating electrode (Plastics One, Roanoke, VA, USA) in the lateral hypothalamic medial forebrain bundle (skull flat coordinates, 3.0 mm posterior to bregma, 1.6 mm lateral to the sagittal suture, and 8.5 mm ventral to skull surface). An anterior ipsilateral stainless steel skull screw served as ground. Rats were also implanted with two chronically indwelling guide cannulas (26 ga) which were placed bilaterally 2.0 mm dorsal to injection sites in the NAc medial shell (1.6 mm anterior to bregma; 2.1 mm lateral to the sagittal suture, tips angled 8° toward the midline, 5.8 mm ventral to skull surface). The electrode, ground, cannulas, and three additional mounting screws were then permanently secured to the skull by flowing dental acrylic around them.

All experimental procedures were approved by the New York University School of Medicine Institutional Animal Care and Use Committee and were performed in accordance with the "Principles of Laboratory Animal Care" (NIH publication number 85-23). All efforts were made to minimize animal suffering, to reduce the number of animals used and to utilize alternatives to *in vivo* techniques.

Tissue preparation and western blotting

Whole cell homogenates. Brains were rapidly frozen in powdered dry ice, 500-μm sections were cut using an IEC Minotome cryostat, and NAc and CPu were micropunched, under an Olympus dissecting microscope, from a series of six consecutive frozen sections. The tissue was then sonicated in 2× Laemmli sample buffer (10 s, on ice), heated for 5 min at 95 °C, centrifuged (14,000 g, 5 min) and the supernatant stored at −80 °C until use.

Synaptosomal fraction. NAc was dissected from fresh brain on ice. NAc of two rats per treatment condition were pooled for fractionation. Protease inhibitor and PMSF were added to 0.32 M sucrose solution containing 1 mM NaHCO3, 1 mM $MgCl_2$ and 0.5 mM $CaCl_2$ (Solution A). Brain tissue was rinsed, homogenized and subsequently diluted to 10% wt/vol in Solution A. The homogenate was centrifuged at 1400 g for 10 min, after which intact cells and nuclei formed a pellet at the bottom of the tube. The supernatant was saved and the pellet resuspended in Solution A. The homogenate was again centrifuged at 1400 g for 10 min. The