Neuroscience 170 (2010) 580–588

REVERSIBLE SUPPRESSION OF FOOD REWARD BEHAVIOR BY CHRONIC MU-OPIOID RECEPTOR ANTAGONISM IN THE NUCLEUS ACCUMBENS

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Abstract—Overindulgence in easily available energy-dense palatable foods is thought to be an important factor in the current obesity epidemic but the underlying neural mechanisms are not well understood. Here we demonstrate that mu-opioid receptor signaling in the nucleus accumbens may be important. Protracted suppression of endogenous mu-opioid receptor signaling focused on the nucleus accumbens shell for several days by means of microinjected β-funaltrexamine (BFNA) diminished both "liking" of sucrose, as indicated by fewer positive hedonic orofacial responses, and the incentive reinforcement value ("wanting") of a food reward, as indicated by lower completion speed and increased time being distracted in the incentive runway. BFNA-treatment also decreased responding to sucrose and corn oil in the brief access lick paradigm, a test measuring a combination of mainly taste-guided "liking" and low-effort "wanting", as well as 4 h intake of sucrose solution. These effects were not due to nonspecific permanent neuronal changes, as they were fully reversible. We conclude that endogenous mu-opioid signaling in the nucleus accumbens is necessary for the full display of palatable food-induced hyperphagia through mechanisms including hedonic, motivational, and reinforcement processes. Development of obesity could be the result of predisposing innate differences in these mechanisms or overstimulation of these mechanisms by external factors.

Key words: reward, ventral striatum, taste reactivity, hedonic value, working for food, obesity.

Parallels have recently been drawn between food and drug addiction, and attention directed to the vulnerability of a common underlying neural circuitry to habitual overindulgence that may result in the development of overweight and obesity (Kelley and Berridge, 2002; Volkow and Wise, 2005; Volkow et al., 2008). The psychological construct of reward is complex and efforts have been made to parse it into distinguishable components with specific neural pathways and mechanisms (Berridge, 1996; Schultz et al., 1997; Wise, 2005; Ikemoto and Panksepp, 1999; Carelli, 2002; Kelley and Berridge, 2002; Salamone et al., 2009). One view distinguishes "liking" (hedonic value or pleasure) from "wanting" (goal-directed action), and learning about rewards (Berridge, 1996; Berridge and Robinson, 2003). The major task of the "liking" system is to evaluate sensory input regarding its ability to evoke immediate pleasure or more extended well-being, while the major task of the "wanting" system is selection of behavioral action that optimally serves current needs. The neural system encoding "liking" is distributed across the neuraxis, including pathways of taste perception in the brainstem and pons, the nucleus accumbens, ventral pallidum, and prefrontal cortex (Grill and Norgren, 1978b; Pecina and Berridge, 2000; Kringelbach, 2004; Berridge and Kringelbach, 2008). The mesolimbic dopamine system with projections from the ventral tegmental area to the nucleus accumbens, frontal cortex, amygdala, and hippocampus has been implicated in both reinforcement and motivational ("wanting") processes of reward-seeking behavior (Mogenson et al., 1980; McFarland and Ettenberg, 1998; Cardinal et al., 2002; Everitt and Robbins, 2005; Wise, 2005; Berridge, 2007).

Opioid signaling, particularly through the mu-receptor, has long been known to be involved in the expression of reward behaviors (Morley et al., 1983; Cooper et al., 1985; Mucha and Iversen, 1986; Glass et al., 1999). The pioneering work of the group around the late Anne Kelley has demonstrated the powerful effects of mu-opioid stimulation of the nucleus accumbens on intake of palatable foods such as sucrose and high-fat diet in rats (Zhang et al., 1998; Will et al., 2003). Injection of the selective mu-opioid receptor agonist [D-Ala2, N-MePhe4, Gly-ol]-enkephalin (DAMGO) not only elicited voracious ingestion of high-fat diet and sucrose solution (Zhang and Kelley, 1997; Will et al., 2003, 2006), but also made rats work harder for a given food reward as assessed by the break point for progressive ratio lever pressing, often used as a measure of "wanting" (Zhang et al., 2003). Furthermore, a small hedonic hot spot within the accumbens shell was identified where DAMGO amplified positive orofacial hedonic reactions ("liking") to the taste of sucrose (Pecina and Berridge, 2005). Thus, the nucleus accumbens, particularly its shell, is one area where encoding for both "liking" and "wanting" appears to be represented.

While these mainly agonist-based studies have provided important insights into the neural organization of food reward, they are not ideally suited to elucidate the role

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Abbreviation: BFNA, β-funaltrexamine.

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doi:10.1016/j.neuroscience.2010.07.017

of endogenous opioid-signaling because they do not take into account ongoing signaling in the basal state, and blocking strategies seem more promising. The irreversible mu-opioid receptor antagonist β-funaltrexamine (BFNA) has proven to be particularly useful as it suppresses endogenous mu-opioid receptor signaling for several days (Takemori et al., 1981). Injection of BFNA into the cerebral ventricles leads to sustained suppression of food intake and body weight in rats (Cole et al., 1995, 1997) and injection into the nucleus accumbens reduces sucrose preference in rabbits (Ward and Simansky, 2006). Furthermore, we have previously shown that prolonged suppression with repeated injections of BFNA into the nucleus accumbens significantly reduced palatable food intake and slowed development of diet-induced obesity (Lenard et al., 2010).

Together, these studies suggest that mu-opioid signaling in the nucleus accumbens, particularly its shell, is necessary for the expression of reward-driven overconsumption of palatable foods, contributing to the development of dietary obesity, but it remains unclear which behavioral components of food reward behavior are involved. The aim of the present study was to examine specific components of food reward behavior both during and after mu-opioid receptor blockade and to test the hypothesis that chronic blockade of nucleus accumbens mu-opioid receptor signaling reduces palatable food intake by decreasing both hedonic evaluation ("liking") and motivation or incentive salience ("wanting").

EXPERIMENTAL PROCEDURES

Animals

Male Sprague–Dawley rats weighing ~250 g were purchased from Harlan Industries (Indianapolis, IN, USA) and housed individually in wire-mesh cages at a constant temperature of 21–23 °C with a 12-h light/dark cycle (light on at 6:00, off at 18:00). Animals were provided with regular chow *ad libitum* throughout the study unless specified otherwise. All protocols were approved by the Institutional Animal Care and Use Committee at the Pennington Biomedical Research Center in accordance with guidelines established by the National Institutes of Health.

Brain cannulation, accumbens injections, and verification of injection sites

Rats were pretreated with atropine (1 mg/kg i.p.) and anesthetized with ketamine-acepromazine-xylazine cocktail (80/1.6/5.4 mg/kg s.c.). Cannulas (24 gauge) were aimed bilaterally at the nucleus accumbens shell (AP+1.5, ML±1.0, DV −5.3) and secured to the skull using dental cement and three stainless steel screws. Rats were allowed to recover from surgery for 10 days, during which they were maintained on chow, but familiarized to the taste of sucrose and corn oil.

For intra-accumbens injections of mu-opioid receptor antagonist or vehicle, rats were briefly anesthetized with isoflurane and a volume of 0.5 μl was slowly injected over 2 min through an injector cannula protruding the guide cannula by 2 mm. The injector was left in place for another 2 min to prevent backflow and was then replaced by an obturator.

At the end of experiments, blue dye (0.5 μl of 1% Chicago Blue, Sigma, St. Louis, MO, USA) was injected through each cannula before euthanasia and perfusion. Brains were extracted and 30 μm-thick frontal sections were examined under the microscope. Injection sites were mapped on the nearest plates from the Paxinos and Watson rat stereotaxic atlas (Paxinos and Watson, 1986).

Behavioral testing

The taste reactivity test of Grill and Norgren (Grill and Norgren, 1978a) was used to quantify "liking" (Berridge, 2000). A 200 μl volume of sucrose solution was placed on the transparent floor of a cylindrical test cage, and the rat's orofacial expressions were videotaped from below. The number of characteristic tongue protrusions was assessed by inspection of slow motion videos (Berridge, 2000), averaged over three consecutive bouts of ingestion and for three ascending concentrations of sucrose (0.01, 0.1, and 1.0 M) administered on separate days in random order. Positive hedonic orofacial responses included midline tongue protrusions, lateral tongue protrusions, and paw licking. Only a few rats, and only during first exposure, demonstrated one or two aversive responses, such as headshakes, gapes, and forelimb flails.

The incentive runway was used to measure "wanting" (Pecina et al., 2003). The runway consisted of start and goal boxes connected with a 158 cm long running alley and a video camera mounted above. Rats were habituated to the runway during two daily 10 min sessions. During two additional sessions, overnight food deprived rats were enticed to eat a small food reward (Fruit Loop cereals, ~2 g, Kellogg's, Battle Creek, MI, USA) in the goal box. Runway behavior was then assessed in the non food-deprived state in daily sessions of two consecutive trials over a period of 20 days. After placing the rats in the start box, the door was opened and the time to reach the goal box and start consuming the reward (completion time) was measured and transformed into completion speed, so as to reflect the level of performance ("wanting"). In addition, the latency to leave the start box, the times spent walking/running forward, standing still (pauses), and moving backwards (reversals), as well as the net running speed were assessed by replaying the video recordings in slow motion. Video analysis was initially conducted by two independent observers and after establishing good agreement was continued by a single observer, blind to the stimulus condition.

The brief-access lick test (Davis MS-160, DiLog Instruments, Tallahassee, FL, USA) was used to measure taste-guided reward behavior (Spector et al., 1996). Brief access (10 s) to the spout, allowing a limited number of licks for each concentration, minimizes modulation of reward behavior by postingestive learning. Rats were first adapted to the special cage and trained to lick from the spout filled with highly palatable chocolate Ensure under conditions of mild food and water deprivation. On test days, non-deprived animals were presented with increasing concentrations of either sucrose (0, 0.001–1.5 M) or corn oil emulsions (0, 0.06–32%, in 1% Emplex emulsifier and distilled water). Each concentration was available for 10 s with 5 s intervals in two consecutive ascending series and the number of licks/10 s was averaged for each concentration.

Experimental protocol

After recovery from cannula implantation, rats were matched for body weight and assigned to either bilateral BFNA (10 nmol/0.5 ul saline/DMSO; Tocris Cookson, Ellisville, MO, USA) or vehicle injection as described above (Fig. 1). The dose of BFNA was chosen based on the effectiveness to block DAMGO-induced food intake (Ragnauth et al., 2000) and suppress development of high-fat diet-induced obesity (Lenard et al., 2010). Six days before the first injection, rats began habituation and pre-training in the incentive runway and daily sessions continued after the first injection. Two days after injection, when incentive runway performance was already suppressed by BFNA, taste reactivity tests and brief access lick tests were administered daily for 3 days. A second injection of BFNA or