Behavioural Brain Research 197 (2009) 225–229

Contents lists available at ScienceDirect

Behavioural Brain Research

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

Short communication

Muscarinic receptor antagonism causes a functional alteration in nucleus accumbens μ-opiate-mediated feeding behavior

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ARTICLE INFO

Article history:
Received 28 April 2008
Received in revised form 25 July 2008
Accepted 4 August 2008
Available online 12 August 2008

Keywords:
Accumbens
Muscarinic
Opioid
Feeding
Scopolamine
Eating
Acetylcholine

ABSTRACT

Intra-nucleus accumbens (Acb) infusion of cholinergic muscarinic antagonist, scopolamine (10 μg/0.5 μl), markedly reduced fat intake elicited by intra-Acb treatment of the μ-opioid receptor agonist, DAMGO, with 30 min and 4 h pretreatment intervals. Intra-Acb scopolamine infusions also reduced food intake in food-deprived rats, but not water intake in water-deprived rats. Hence, Acb muscarinic manipulations exhibit some specificity for feeding, perhaps via interactions with the striatal opioid system.

Obesity is currently a major health problem in the world today. While a great deal of research has focused on mechanisms controlling food intake and appetite, there has been little success in developing effective pharmacological treatments. Considerable progress has been made in better understanding metabolically driven feeding, particularly the communication between the periphery (i.e. leptin and ghrelin signals) and the brain (i.e. hypothalamus). However, homeostatic controls are not the only factors controlling ingestive behavior. Consideration of food cravings and the consumption of high calorie foods driven by palatability, events controlled by motivational and hedonic processes, must be taken into account.

The ventral striatum (nucleus accumbens, Acb) has received much attention as a component of higher order control of food motivation [11]. This forebrain region receives afferent projections from affect-related 'limbic' structures while sending efferent projections to the extrapyramidal motor control regions. These anatomical arrangements contributed to the hypothesis that the Acb plays a pivotal role in the translating of motivational signals into behavioral output [14]. The Acb also contains multiple neurotransmitter systems that have been shown to play critical, yet dissociable, roles in the control of appetitively-motivated behaviors [11,12]. For example, stimulation of Acb-localized μ-opioid receptors induces voracious eating of highly palatable foods in satiated animals and augments operant responding for food reward [1,15,20,26,29]. In contrast, the blockade of glutamate input or enhancement of GABA within the Acb shell, a subregion of the ventral striatum, increases food consumption [23] without a concomitant increase in food-reinforced instrumental responding [28]. Also, augmentation of Acb dopamine release increases operant responding for food and food-associated stimuli, but does not strongly affect intake [2–5,21]. Thus, striatal connections and discrete neurotransmitter systems both play important roles in the forebrain control of feeding and food-seeking behavior.

To expand our understanding of intra-striatal neurochemical interactions in the control of feeding, our laboratory has begun examining the role of the striatal acetylcholine (ACh) system. The cholinergic interneurons in the striatum comprise only about 1–2% of the neurons in the striatum, but their large dendritic and axonal

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[3] Unfortunately, Dr. Kelley passed away last August. While this paper was written after her death, the bulk of the work was carried out while she was still alive, under her supervision.

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doi:10.1016/j.bbr.2008.08.002

processes allow for possible influence of large striatal areas [30]. The ACh terminals synapse onto Acb neurons containing the opioid peptide enkephalin (ENK), and μ-opioid receptor mRNA has been found in ACh interneurons [8,18,19]. Thus, Ach and ENK neurons are positioned to undergo reciprocal interactions.

Recent data from our laboratory and others has suggested that striatal ACh mediates consumption of food, including opioid-mediated fat intake. It has been shown that intra-striatal infusion of scopolamine, a general muscarinic antagonist, can decrease the amount of sucrose an animal will consume and reduce the break point in a progressive ratio schedule while increasing locomotor activity immediately following drug treatment [16]. It has also been demonstrated that chow consumption is decreased and that preproenkephalin (PENK) mRNA is reduced for a 24 h period, suggesting a relatively long lasting effect of scopolamine treatment [17]. Recently we have shown that co-infusion of the μ-opioid specific agonist D-Ala2, NMe-Phe4, Glyol5-enkephalin (DAMGO) and scopolamine within the Acb decreased DAMGO stimulated fat-intake over a 1-h test session [27]. In the present study, we tested the hypothesis that scopolamine may exert a relatively long lasting effect on μ-opioid-mediated feeding behavior. To analyze the duration of the effect of scopolamine on opioid-induced feeding, we designed a time course study in which animals received intra-Acb infusions of scopolamine followed by intra-Acb DAMGO at varying post-scopolamine intervals. Additionally, we aimed to determine whether the effect of intra-Acb scopolamine is specific to food intake, or affects other types of ingestive behaviors, such as drinking.

Forty-two male Sprague–Dawley rats (Harlan, Madison, WI, USA) were housed in pairs in clear plastic cages. Rats received food and water *ad libitum*, unless otherwise specified by the experimental design, and were maintained on a 12-h light/dark cycle (lights on at 07:00 h). Procedures and animal care were performed according to NIH guidelines on the use of animals in research, following the approval of the University of Wisconsin-Madison Medical School Animal Care and Use Committee. All animals underwent standard aseptic surgery for implantation of 10-mm stainless steel guide cannulae (30 gauge) bilaterally above the Acb (1.3 mm anterior and ±1.7 mm lateral to bregma; 5.3 mm ventral to skull surface). A Ketamine–Xylazine mixture (100–10 mg/kg) was used for anesthesia. Guide cannulae were affixed to the skull with the use of screws and dental acrylic and stylets were placed to prevent cannulae from becoming occluded. Animals received an IM injection of buprenorphine (0.30 ml) for pain and recovered for at least 7 days prior to behavioral testing.

For all experiments, the competitive muscarinic acetylcholine receptor antagonist scopolamine methyl bromide (Sigma) (1 or 10 μg/0.5 μl/side), and the μ-opioid specific agonist D-Ala2, NMe-Phe4, Glyol5-enkephalin (DAMGO) (Bachem Biosciences Inc. King of Prussia, PA) (0.25 μg/0.5 μl/side), were each dissolved in sterile 0.9% saline. Drugs were administered bilaterally using a microdrive pump (Harvard Apparatus, South Natick, MA) connected via polyethylene tubing (PE-10) while animals were gently hand-held. Thirty-three gauge injectors were used, extending 2.5 mm beyond the end of the guide cannulae. The final injection site was 7.8 mm ventral from skull. Total volume infused was 0.5 μl. Injectors then were removed and stylets were replaced before placing the subjects in the test cages. Following behavioral testing, brains were removed and placed in 10% formalin–20% sucrose overnight. Frozen serial sections (60 μm) were collected through the entire extent of the injections site, mounted on gelatinized slides, stained with Cresyl violet and cover slipped. Cannulae placements were then assessed with light microscopy by an observer blind to the behavioral results of the animals. Photomicrographs of representative acceptable placements are shown in Fig. 1.

To assess the duration of scopolamine's effect on DAMGO-induced feeding, animals were placed in a room separate from the animal colony containing automated locomotion/feeding cages (Med Associates, St. Albans, VT). Food intake monitors (Med Associates) were mounted on the sides of the cage and were able to measure food weight with an accuracy of 0.1 g. Two arrays of infrared photobeams were mounted on the front and back of the

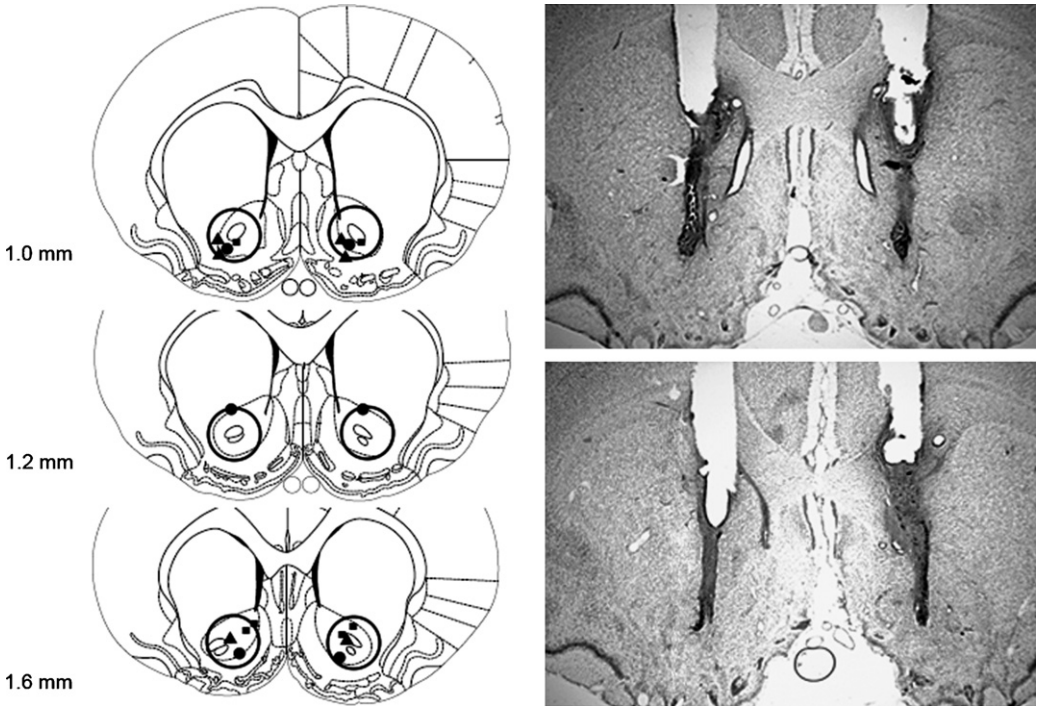

Fig. 1. Photomicrographs showing representative cannulae placements (right). Schematic diagrams displaying injection sites of three animals from each experiment (left) (▲ = Experiment 1 sites; ● = Experiment 2 sites; ■ = Experiment 3 sites). The stereotaxic coordinates shown are in mm anterior of bregma.