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Research report

Consequences of constitutive deletion of melanin-concentrating hormone-1 receptors for feeding and foraging behaviors of mice

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HIGHLIGHTS

- MCH_1 −/− mice exhibited increased motivation to obtain food compared to WT mice.
- Increased food-seeking in MCH1−/− mice was also observed when behavior was punished.
- Feeding in MCH_1 −/− mice contrasts in some important dimensions from data reported with MCH_1 receptor antagonism.

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ABSTRACT

In order to decipher the functional involvement of melanin-concentrating hormone 1 (MCH1) receptors in the control of feeding and foraging behaviors, mice with constitutive deletion of MCH1 receptors MCH1R −/− or knockout (KO) were studied and compared to age-matched littermate control mice (MCH1R +/+ or wildtype (WT)). Several challenges to food-motivated behaviors of food-restricted WT and KO mice were implemented. There were no differences between genotypes in the acquisition of a nose-poke response that produced food or in a discrimination between a response that produced food and one that did not. There were also no genotype differences in the rate of extinction of a food-motivated response. However, during the first day of extinction, foraging behaviors were increased significantly more in KO than in WT mice. Likewise, when the response requirement to obtain food was progressively increased, KO mice made significantly more food-directed responses than WT mice. Although adulteration of food with quinine did not suppress food-directed behavior in either genotype when the mice were food-restricted, manipulation of the degree of food-deprivation resulted in suppression of behavior of WT mice without suppressing the behavior of KO mice. Although response-produced foot shock suppressed food-maintained responding of both WT and KO mice, equipotent levels of shock (based upon psychophysical thresholds) suppressed behavior of WT mice without suppressing behavior of the KO mice. Finally, under a Vogel conflict procedure, KO mice had significantly higher levels of both punished and non-punished food maintained responding. Thus, the data from challenges with both appetitive and noxious stimulus challenges support the conclusion that mice with constitutive deletion of MCH1Rs have increased food seeking motivation that is coincident with their higher metabolism. The data also highlight important differences in the biological impact of MCH1 receptor KO and MCH1 receptor antagonism.

1. Introduction

Melanin-concentrating hormone (MCH), originally isolated from the salmon pituitary where it was found to modulate pigmentation [15], is a 19 amino acid cyclic peptide [41]. MCH is also expressed in mammals where it is localized with highest densities in the lateral hypothalamus and zona incerta suggesting an important role in the control of feeding [35,2]. In addition, extensive projections to a host of brain areas including hippocampus, amygdala, nucleus accumbens, and prefrontal cortex suggest a broad integrative role of MCH receptors in physiology and behavior [35,2]. Two subtypes of MCH receptors have been identified. MCH1 receptors (MCH1Rs) are widely expressed in numerous regions of the brain including prefrontal cortex, hippocampus, thalamus,

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midbrain, pons, olfactory bulb and hypothalamus [9]. MCH2R distribution overlaps that of MCH1R except that it is not expressed in the pituitary [10] or in rodents [30,40].

MCH has been implicated in a variety of physiological functions including regulation of the hypothalamic-pituitary-adrenal axis [13], reproduction [8,25], as well learning and memory [22,23]. One of the best documented actions of MCH is in energy balance regulation. MCH has been proposed to influence reward mechanisms based upon anatomical, electrophysiological, and behavioral data [5,12,7,33]. The hypothalamic MCH mRNA level is up-regulated in leptin deficient (ob/ob) mice and further increased by fasting [28]. Centrally-administered MCH stimulates food intake [28,29]. Overexpression of MCH in mice leads to obesity and insulin resistance [19] while MCH1 receptor deficient mice are hypermetabolic with decreased body weight and increased leanness [34,6]. Accordingly, MCH1R −/− mice are lean and resistant to diet-induced obesity. MCH1R deficient mice also exhibit higher metabolic rate that is associated with increased locomotor activities [4,20,36]. These data have further supported a role for MCH in the regulation of energy balance.

Although MCH1R null mice display hyperphagia relative to their body weight, the effect of MCH1R in controlling food-seeking behaviors under different conditions of metabolic and environmental pressures is largely unknown leaving unanswered the question of if and how increased metabolic demand is transcribed into behavioral sequences of food-seeking behaviors. The present study was designed to test the hypothesis that mice without MCH1 receptors would have increased motivational drive toward food-seeking compared to mice with intact MCH1 receptors. This was accomplished through multiple experiments in which various pressures were brought to bear on food-seeking using both appetitive demands and noxious stimulation. Specifically, we employed various operant procedures to determine whether MCH1R −/− and age-matched littermate control MCH1R+/+ mice differed in food motivated behaviors. In all cases, food-restricted mice were initially trained to nose poke using food as a reinforcer. The mice were subsequently challenged with either food withdrawal (extinction), response reinstatement, increasing numbers of nose-pokes required for food delivery (progressive ratio), food satiation, or the introduction of noxious stimuli (quinine-adulterated food or brief electrical stimulation). It was hypothesized that by studying a range of challenges, motivational differences in food-directed behaviors might be revealed.

2. Materials and methods

2.1. Mice

MCH1R −/− mice and their age-matched, littermate MCH1R +/+ controls were generated as previously described [4]. Mice were ∼10 weeks old at the start of the studies. Animals were individually-housed in a temperature- and humidity-controlled room maintained on a 12:12 light:dark cycle (lights on 0600 to 1800). All had free access to water and received standard mouse chow (Purina 5008 chow, Ralston-Purina, St. Louis, MO, USA) as described below. Prior to any manipulations of mild food restriction, mice were allowed to stabilize at free-feeding body weights. MCH1R −/− mice had free-feeding body weights of 24.8 + 0.4 g, whereas MCH1R +/+ mice had asymptotic levels of 30.1 + 0.5 g ($p > 0.05$).

All animal use in this study was conducted in compliance with approved institutional animal care and use protocols according to NIH guidelines (NIH Publication No. 86-23, 1985) as approved by internal animal care and use committees.

2.2. Behavioral methods

Several challenges to food-motivated behaviors of MCH1R −/− and MCH1R +/+ mice were implemented as described below.

2.2.1. Apparatus

Experiments were conducted in mouse operant conditioning chambers (MED Associates, Inc., St. Albans, VT). These chambers were equipped with two holes (13 mm diameter), 1 cm above a grid floor and spaced 9.7 cm apart. The holes could be illuminated with white light. A water bottle was located on the opposite wall. Nose pokes within the hole (>6 mm) were counted as responses and produced the audible click of a relay. Food pellets (20 mg, BioServe, Frenchtown, NJ) could be delivered to a food trough (centered between the nose-poke holes) coincident with an audible tone for 100 msec. The chambers were located within sound-attenuating cubicles supplied with ventilation and white noise to mask extraneous sounds. Scrambled electric shock could be delivered to the grid floor of the chamber by a constant current AC source (MED Associates). Experimental events and data were collected with MED-PC software (MED Associates).

In all experiments reported here, the left nose-hole was illuminated during experimental sessions when access to food was available. Nose-pokes into the right, non-illuminated hole could produce food whereas nose-pokes into the left hole could not. During food presentation, the food hopper was illuminated, a tone was delivered, and the left nose hole light was turned off. Thus, this constellation of stimuli defined the food-associated stimulus.

2.3. Non-Punishment procedures

2.3.1. Response acquisition

Mice were trained to nose poke under a fixed ratio (FR) 1 schedule of food presentation in which each nose poke into the right hole produced a food pellet. Mice were maintained at 85% of free-feeding weight throughout the study except where noted; they were weighed daily and body weights were maintained with daily food adjustments as required for this purpose. Upon stabilization at 85%, animals were introduced to the 20 mg food pellets used in testing for three days prior to nose-poke training. Mice were food deprived for 18 h prior to the first day of training then placed in the operant chamber in which 5 food pellets had been placed in the food trough by the experimenter. During the experimental session (1 h), nose-pokes in the right nose hole produced food under an FR1schedule where each nose poke produced a food pellet. Experimental sessions terminated after either the delivery of 100 food pellets or 1 h, whichever came first. This procedure was repeated for 7 experimental sessions.

2.3.2. Response extinction

For the next 5 experimental sessions, responding was extinguished by removing the response-reinforcer contingency; that is nose-pokes no longer produced food or food-associated stimuli. Experimental sessions terminated after 60 min. This procedure was repeated for 7 experimental sessions.

2.3.3. Response reinstatement

On the day following the final day of response extinction, reinstatement of extinguished responding was assessed. Under the reinstatement condition, the first 3 responses of each 5 min period of the 60 min experimental session produced a food pellet along with food-associated stimuli. For the remainder of the 5 min period, the 4th and subsequent responses produced only food-associated stimuli. After two days of no experimental sessions, the mice in this group were then retrained under the FR1 procedure of phase one