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SCIENCE DIRECT
Peptides 26 (2005) 1712–1719
PEPTIDES
www.elsevier.com/locate/peptides

Melanocortin-4 receptor-null mice display normal affective licking responses to prototypical taste stimuli in a brief-access test

Shachar Eylam [a], Marcus Moore [b], Carrie Haskell-Luevano [b], Alan C. Spector [a,*]

[a] *Department of Psychology and the Center for Smell and Taste, University of Florida, P.O. Box 112250, Gainesville, FL 32611-2250, USA*
[b] *Department of Medicinal Chemistry, University of Florida, Gainesville, FL 32611, USA*

Received 5 August 2004; accepted 11 November 2004
Available online 1 July 2005

Abstract

We tested whether MC4R null mice display altered gustatory function relative to wild-type controls that may contribute to the characteristic hyperphagia and obesity associated with this gene deletion. Mice were tested for their licking responses to prototypical taste solutions (sucrose, NaCl, quinine, citric acid) in series of daily 30-min sessions in which a range of concentrations of each tastant was available in randomized blocks of 5-s trials. Notwithstanding some minor deviations, the concentration-response functions of the MC4R null and wild-type mice were basically the same for all of the prototypical compounds tested here. Thus, taste-based appetitive and avoidance behavior is expressed in the absence of the MC4 receptor, demonstrating that this critical component in the melanocortin system is not required for normal affective gustatory function to be maintained.

Keywords: Gustatory; Hedonics; Obesity; Hyperphagia; Neuropeptides; Sucrose; Quinine; NaCl; Citric acid; Knockout mice

1. Introduction

The use of several obese mouse models has led to the discovery of various genes (such as *ob* and *db*) that are associated with energy homeostasis and the control of food intake. The discovery of these genes has led to the identification of leptin, a hormone secreted by adipose tissue to convey information to the brain about body fat content [1,22,24,37,51]. Leptin receptors have been found in the brain (e.g. [5,13,33,40,47,51]). The identification of the *agouti* gene, the mutation of which results in obesity, brought about the identification of the melanocortin receptors and a neuronal pathway that appears to be associated with metabolism and food intake [18,27,29] which are part of the leptin system. Other downstream mediators of the leptin system include neurons expressing neuropeptides, such as pro-opiomelanocortin (POMC), which is the precursor to melanocortin receptor agonists, agouti-related protein (AGRP), a melanocortin antagonist, and neuropeptide Y (NPY), an orexigenic agent (e.g. [5,7,27,29,32]). POMC expression is stimulated by leptin, whereas AGRP and NPY expression is suppressed by leptin (e.g. [9]). Thus, the neurons expressing these various neuropeptides have opposing roles in the leptin system and project to a variety of melanocortin receptors in the brain (e.g. [8]).

Knock-out (KO) mice, lacking various melanocortin receptors and associated neuropeptides have been useful in distinguishing the contribution of these various components to the observed obesity. The phenotypes of these mice differ markedly depending on the protein that is absent, although they all exhibit obesity to some degree. Mice deficient in POMC exhibit morbid obesity, adrenal insufficiency, increased leptin production and hyperphagia but no hyperglycemia or hyperinsulinemia [50]. Mice deficient in melanocortin-3 receptors (MC3R) exhibit no hyperphagia but mild hyperinsulinemia and hyperleptinemia [3,6]. Mice deficient in melanocortin-4 receptors (MC4R) develop maturity-onset obesity associated with hyperphagia, hyperinsulinemia, hyperglycemia and display increased somatic

* Corresponding author. Tel.: +1 352 392 0601x288; fax: +1 352 392 7985.
E-mail address: spector@ufl.edu (A.C. Spector).

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doi:10.1016/j.peptides.2004.11.023

growth [3,6,27,48]. Surprisingly, these MC4R KO mice were found to respond normally to food deprivation [4]. Moreover, like wild-type mice, the MC4R KO mice respond to nutritional deficits by a decrease in body weight and return rapidly to their pre-deprivation weight after resumption of ad libitum feeding [4]. Unlike the wild-type mice, however, MC4R KO mice do not show a normal metabolic response to increased fat content of the diet, such as increased physical activity and thermogenesis [4].

Taste appears to play a role in the obesity displayed by mouse models deficient in any one of the various components of the leptin system affecting regulation of food intake and energy homeostasis. For example, NPY was demonstrated to potentiate intake of palatable solutions regardless of their caloric values [31,41,42]. Leptin itself has been previously suggested to target the tongue in addition to its many other peripheral organs and modulate taste receptor cells involved with signaling "sweet-tasting" stimuli [28,44]. Moreover, leptin receptors (Ob-R) were found in both posterior and anterior taste receptor cells of lean and leptin-deficient *ob*/*ob* mice [44], while mice deficient in the Ob-R (*db*/*db* mice) demonstrate larger chorda tympani nerve (CT) responses to sugars and non-sugar sweeteners but not other prototypical taste compounds compared with lean controls [35,36,38]. Behaviorally, the *db*/*db* mice showed greater preference for sugars compared with lean controls [35] while leptin injections suppressed licking responses to sugars and non-sugar sweeteners but not to other prototypical taste compounds in both lean controls and *ob*/*ob* mice, but not in *db*/*db* mice [44]. In addition, there is evidence that a variety of neuropeptides can be found in taste cells and that receptors for them exist in the taste buds as well. Neuropeptides not associated with the leptin system but affecting feeding (e.g. cholecystokinin [CCK], calcitonin gene-related peptide [CGRP] and substance P [SP]) have been demonstrated to modulate gustatory neural activity and their receptors have been found in taste cells (e.g. [25,30,45]).

More evidence supporting the interaction between taste and neuropeptides come from the neuropeptide modulation of taste-evoked responses in neurons of the in the nucleus of the solitary tract (NTS) [10,19], the first relay in the central gustatory pathway which receives gustatory and visceral input from the seventh, ninth and tenth cranial nerves (e.g. [23,43]). In addition, like the tongue, the NTS was demonstrated to express leptin receptors (e.g. [21,34]). Interestingly, the NTS also appears to be the site for a synergistic interaction between CCK and leptin in controlling food intake [14,26] while NPY in the NTS has an opposing modulatory effect [39].

At issue here is the role that taste plays in the control of food intake by the melanocortin receptors. Specifically, we investigated whether mice that have the *MC4r* gene deleted, and thus lack the receptor, would display altered gustatory function relative to their wild-type controls. To examine this, we used a brief-access taste test to behaviorally assess the affective responsiveness of these mice to four prototypical taste compounds thought to represent different taste qualities. This test involves the presentations of taste stimuli during very brief trials (i.e. 5 s) and the measurement of licking responses (e.g. [11,20]). Animals can initiate as many trials as possible during the session that is conducted in a specially designed gustometer. The fact that immediate responses during brief periods of stimulus access are measured, increases the confidence that the behavior is guided by the orosensory properties of the taste stimulus on any given trial.

2. Materials and methods

2.1. Subjects

MC4R KO mice were provided by Millennium Pharmaceutical Inc. [27] and were bred as heterozygotes. Mice were genotyped as previously described [27]. For these studies, 10 male MC4R null (−/−) and 10 male age-matched wild-type (WT) littermate were utilized. Upon arrival, the age of the WT mice was 90 (±3.18) days with mean body mass of 25.25 g (±0.82) and the age of the KO mice was 94 (±3.42) days with mean body mass of 32.58 g (±2.20). The mice were housed individually in polycarbonate shoebox cages in a colony room where the temperature, humidity and lighting (12 h light/12 h dark) were controlled automatically. All experimental manipulations took place during the lights-on phase of the lighting cycle. Subjects had free access to pellets of laboratory chow (LabDiet 5001, PMI Nutrition International Inc., Brentwood, MO) and purified water (Elix 10; Millipore, Billerica, MA). Three weeks after their arrival, the mice were put on a restricted water-access schedule where fluid was available only during the training and testing sessions on Monday–Friday (except on the sucrose testing week; see below). On weekends the mice received water and food ad libitum with the water bottles replaced on the home cage immediately following their respective session on Friday. The water bottles were removed on Sunday in a staggered manner starting at 8:30 a.m.; two water bottles were removed every half hour according to the testing order during the week, assuring an equal 24-h of water deprivation for all mice. While on the water-restriction schedule, mice that dropped below 85% of their body mass relative to that measured during ad libitum water access (hydrated body mass measured on Sunday before water bottle removal) received 1 ml supplemental water immediately following their respective daily testing session. All procedures were approved by the Institutional Animal Care and Use Committee at the University of Florida.

2.2. Taste stimuli

All solutions were prepared daily with purified water (Elix 10; Millipore, Billerica, MA) and reagent grade chemicals, and were presented at room temperature. Test stimuli consisted of five concentrations of sucrose (0.0625, 0.125,