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Angiotensin-converting enzyme inhibition reduces food intake and weight gain and improves glucose tolerance in melanocortin-4 receptor deficient female rats

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HIGHLIGHTS

- ACE inhibition reduced weight gain in both MC4R-deficient and wildtype rats.
- Food intake following ACE inhibition was only reduced in MC4R-deficient rats.
- MC4R-deficient rats were glucose intolerant; this was improved by ACE inhibition.

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ABSTRACT

Functional loss of melanocortin-4 receptor (MC4R) activity leads to hyperphagia and an obese, glucose intolerant phenotype. We have previously established that inhibition of angiotensin-converting enzyme (ACE) reduces food intake, body weight and glucose homeostasis in diet-induced obesity. The current study assessed the effect of ACE inhibitor treatment in MC4R-deficient female rats on body weight, adiposity and glucose tolerance. Rats homozygous (HOM) for a loss of function *Mc4r* mutation had an obese phenotype relative to their wildtype (WT) littermates. Inhibition of ACE for 8 weeks produced reductions in body weight gain in both HOM and WT rats; however, food intake was only reduced in HOM rats. Weight loss following ACE inhibitor treatment was specific to fat mass while lean mass was unaffected. HOM rats were severely glucose intolerant and insensitive to exogenous insulin injection, and treatment with an ACE inhibitor improved both glucose tolerance and insulin sensitivity in HOM rats although not fully to that of the level of WT rats. The current study indicates that HOM rats are sensitive to the anorectic effects of ACE inhibition, unlike their WT littermates. This resulted in a more rapid reduction in body weight gain and a more substantial loss of adipose mass in HOM animals, relative to WT animals, treated with an ACE inhibitor. Overall, these data demonstrate that MC4R signaling is not required for weight loss following treatment with an ACE inhibitor.

1. Introduction

The melanocortin-4 receptor (MC4R) is an important regulator of energy homeostasis [1–3] and functional loss of MC4R activity produces an obese phenotype in both animal models [4,5] and humans [6,7]. The obesity resulting from loss of MC4R function is associated with increased food intake, decreased energy expenditure, increased body weight and adipose tissue mass, and glucose intolerance/insulin resistance in both male and female animals [1,4–6,8]. MC4Rs are a part of the well-described peptide-signaling system that helps regulate energy balance. In particular, leptin and insulin act at their receptors in the arcuate nucleus of the hypothalamus (ARC) stimulating proopiomelanocortin (POMC) neurons that release α-melanocyte-stimulating hormone (αMSH), which in turn stimulates melanocortin receptors in several hypothalamic and other brain areas to reduce food intake and increase energy expenditure [9]. Leptin and insulin also act in the ARC to reduce activity of agouti-related peptide (AgRP)/neuropeptide Y (NPY) neurons [10]. Both AgRP and NPY are anabolic peptides. AgRP is an endogenous antagonist of MC4R and NPY is an agonist at Y1 and Y5 receptors, and both peptides cause increased food intake and body fat/weight [11].

The renin–angiotensin system (RAS) has recently been recognized as an important factor in the regulation of energy balance and glucose homeostasis [12–15]. Angiotensin-converting enzyme (ACE) inhibition [16–18], angiotensin receptor blockade [19–21], and genetic ablation of the RAS [22–24] each results in a phenotype of reduced body weight and improved glucose tolerance. Conversely, increased expression of components of the RAS can lead to increased adiposity and impaired glucose tolerance and insulin sensitivity [25–27]. The metabolic effects of the RAS appear to be independent of energy balance circuits relying on melanocortin or NPY/AgRP signaling [28]. The current study asked whether ACE inhibition reduces body weight in MC4R-deficient rats. Given that ACE inhibition acts independently of melanocortin and NPY/AgRP signaling, it was hypothesized that

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ACE inhibition would reduce body weight and adiposity and improve glucose tolerance in MC4R-deficient rats.

2. Methods

2.1. Animals

Female littermate offspring (n = 36) result from mating of rats heterozygous for a non-functional mutation in *Mc4r* (*Mc4r*^{K314X}) and that had been outcrossed for at least 7 generations [5]. Genotyping was performed by KBiosciences (Hoddesdon, UK) using the KASPar SNP Genotyping System, as we have previously described [29]. Wildtype (*Mc4r*$^{+/+}$ [WT]) and homozygous (*Mc4r*$^{-/-}$ [HOM]) animals were weaned at postnatal day 21 and group-housed (2–3 per cage) until 8 weeks of age, after which they were housed individually. The rats were maintained at the Metabolic Diseases Institute of the University of Cincinnati on a 12/12-h light/dark cycle at 25 ± 2 °C in an AAALAC-accredited facility. All rats had *ad libitum* access to water and a pelleted high-fat diet (HFD; D03082706, 4.54 kcal/g AFE, 15% calories protein, 46% calories carbohydrate, and 40% calories fat, Open Source Diets, New Brunswick, NJ) starting at 10 weeks of age. The rats had access to enrichment in their home-cages (red rat retreat; BioServe, MD, USA). The University of Cincinnati Institutional Animal Care and Use Committee approved all procedures for animal use.

2.2. Groups and treatment

At 10 weeks of age, half of the animals of each genotype were continued on normal water (control, CON: WT, n = 9; HOM, n = 9) and the other half were provided with drinking water containing the ACE inhibitor captopril (Sigma-Aldrich, St. Louis, MO) at a dose of 0.2 mg/mL (WT+, n = 9; HOM+, n = 9). The rats were maintained on this regimen for 8 weeks.

2.3. Food intake and body weight

Food intake and body weight of the rats were measured daily for the first 21 days of the experiment (only weekly data depicted). Subsequently, body weight and food intake were measured weekly for the remainder of the 8-week experiment.

2.4. Food intake following fasting

Following 5 weeks of control or ACE-inhibitor treatment, the animals were fasted for 24 h at the beginning of the dark phase. Food intake was assessed on a baseline day and after re-feeding at the 1-, 2-, 4- and 24-h time-points.

2.5. Body composition

Body composition (fat and lean masses) was assessed using nuclear magnetic resonance (NMR) technology (Echo NMR, Waco, TX) in conscious rats. This was performed prior to the commencement of ACE-inhibitor treatment and again at the completion of the experiment.

2.6. Intraperitoneal glucose- and insulin-tolerance tests

Glucose-tolerance was assessed after 6 weeks of treatment. Following a 14-h fast, the rats were given an intraperitoneal (i.p.) injection of 50% dextrose (1 g/kg). Blood glucose was assessed at baseline, 30, 60, 90 and 120 min (ACCU-CHEK; Roche Diagnostics, Indianapolis, IN). For the insulin tolerance test, performed a week later, fed-state animals were given an i.p. injection of human insulin (Humalin R, 0.5 U/kg). Blood glucose was assessed at baseline, 15, 30 and 60 min.

2.7. Statistical analyses

All data are displayed as mean ± S.E.M. Data were analyzed using Statistica 7 (StatSoft, Tulsa, OK, USA). Data were analyzed using two-way (genotype × drug) analysis of variance (ANOVA), or three-way with repeated-measures where appropriate. All ANOVAs were followed by Fisher's least significant differences (LSD) *post hoc* test if significant overall interactions were observed. The null hypothesis was rejected at the 0.05 level.

3. Results

3.1. Body weight

Deficiency of MC4R signaling produced an obese phenotype in female rats compared with their WT controls; body weight immediately prior to the commencement of the experiment was ~60% higher in HOM (334.2 ± 5.89) relative to WT (209.4 ± 6.6) rats (see Fig. 1A; $p<0.05$). ACE inhibition reduced body weight in HOM+ rats relative to HOM rats by week 2 of treatment ($p<0.05$), and this persisted throughout the experiment (see Fig. 1C). The parallel difference in body weight between WT+ and WT rats occurred later than in HOM rats, with a body weight reduction observed only after 8 weeks (see Fig. 1B; $p<0.05$). Over the course of the experiment body weight gain was significantly greater in HOM rats than in all other groups ($p<0.05$), HOM+ rats gained more weight than both WT groups ($p<0.05$), and WT rats gained more weight than WT+ animals (see Fig. 1D; $p<0.05$).

3.2. Food and water intake

Cumulative food intake was greater in HOM rats relative to WT rats irrespective of treatment with the ACE inhibitor ($p<0.05$). There was a significant reduction in food intake by HOM+ relative to HOM animals ($p<0.05$). Over the 8-week experimental period there were no differences observed in cumulative food intake between WT and WT+ groups (see Fig. 2A). Mean daily water intake was greater in ACE inhibitor-treated animals than CON animals regardless of genotype (see Fig. 2B; $p<0.05$).

3.3. Food intake following fasting

Following a 24-h fast, all the animals ate more than at baseline (see Fig. 2C and D; $p<0.05$). No significant differences in food intake were observed based on ACE inhibitor treatment or genotype after 2 or 4 h, but by 24 h after food return differences between genotypes were observed ($p<0.05$); ACE inhibition reduced 24-h intake in HOM but not WT animals ($p<0.05$).

3.4. Body composition

At the onset of the experiment there was significantly greater fat mass in HOM rats compared with WT rats (see Fig. 3A; $p<0.05$). Similarly, lean mass was elevated in HOM rats compared with that of the WT rats (see Fig. 3B; $p<0.05$). After 8 weeks of ACE inhibitor treatment fat mass was significantly reduced in both WT+ and HOM+ animals relative to the respective CON groups ($p<0.05$). ACE inhibitor treatment did not affect lean mass in either genotype.

3.5. Intraperitoneal glucose- and insulin-tolerance tests

There was no significant difference in fasting blood glucose among the groups. Glucose tolerance was impaired in HOM rats relative to WT rats, with elevated blood glucose at all time-points following glucose injection ($p<0.05$). ACE inhibition resulted in improved glucose