Brief communication

MOLECULAR METABOLISM

Sex difference in physical activity, energy expenditure and obesity driven by a subpopulation of hypothalamic POMC neurons

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ABSTRACT

Objective: Obesity is one of the primary healthcare challenges of the 21st century. Signals relaying information regarding energy needs are integrated within the brain to influence body weight. Central among these integration nodes are the brain pro-opiomelanocortin (POMC) peptides, perturbations of which disrupt energy balance and promote severe obesity. However, POMC neurons are neurochemically diverse and the crucial source of POMC peptides that regulate energy homeostasis and body weight remains to be fully clarified.

Methods: Given that a 5-hydroxytryptamine 2c receptor (5-HT_{2C}R) agonist is a current obesity medication and 5-HT_{2C}R agonist's effects on appetite are primarily mediated via POMC neurons, we hypothesized that a critical source of POMC regulating food intake and body weight is specifically synthesized in cells containing 5-HT_{2C}Rs. To exclusively manipulate *Pomc* synthesis only within 5-HT_{2C}R containing cells, we generated a novel *5-HT_{2C}R^{CRE}* mouse line and intercrossed it with Cre recombinase-dependent and hypothalamic specific reactivatable *Pomc^{NEO}* mice to restrict *Pomc* synthesis to the subset of hypothalamic cells containing 5-HT_{2C}Rs. This provided a means to clarify the specific contribution of a defined subgroup of POMC peptides in energy balance and body weight.

Results: Here we transform genetically programed obese and hyperinsulinemic male mice lacking hypothalamic *Pomc* with increased appetite, reduced physical activity and compromised brown adipose tissue (BAT) into lean, healthy mice via targeted restoration of *Pomc* function only within 5-HT_{2C}R expressing cells. Remarkably, the same metabolic transformation does not occur in females, who despite corrected feeding behavior and normalized insulin levels remain physically inactive, have lower energy expenditure, compromised BAT and develop obesity.

Conclusions: These data provide support for the functional heterogeneity of hypothalamic POMC neurons, revealing that *Pomc* expression within 5-HT_{2C}R expressing neurons is sufficient to regulate energy intake and insulin sensitivity in male and female mice. However, an unexpected sex difference in the function of this subset of POMC neurons was identified with regard to energy expenditure. We reveal that a large sex difference in physical activity, energy expenditure and the development of obesity is driven by this subpopulation, which constitutes approximately 40% of all POMC neurons in the hypothalamic arcuate nucleus. This may have broad implications for strategies utilized to combat obesity, which at present largely ignore the sex of the obese individual.

Keywords *Pro-opiomelanocortin (Pomc)*; 5-HT2c receptor; Obesity; Energy expenditure; Brown adipose tissue; Hyperinsulinemia; Sexual dimorphism; Hypothalamus

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1. INTRODUCTION

When energy intake exceeds energetic demands, energy is stored primarily as fat. Excess adipose accumulation is the hallmark feature of obesity. Though men and women are exposed to the same environmental conditions, the World Health Organization (WHO) reports higher rates of obesity in women worldwide, reaching twice the prevalence of men in some regions of the world [1]. Obesity has a significant and widespread impact on human health, placing it at the forefront of healthcare priorities and challenges of this century. Obesity medications are in general prescribed without attention to the sex of the obese individual, implying the absence of sex-based differences in the molecular regulation of energy balance.

Insights from genetic research have led to the discovery of key regulators of energy balance [2], such as the melanocortin peptides encoded by the pro-opiomelanocortin gene (*Pomc*) [3]. Humans and animals unable to synthesize melanocortins or the receptor through which they primarily signal to influence energy balance, the melanocortin4 receptor (*MC4R/Mc4r*), have dramatically increased food intake, reduced physical activity or energy expenditure and develop profound obesity [4,5]. *POMC/Pomc* function also has broader application to common obesity; in both high fat diet-induced obesity and middle-age associated obesity, POMC neuron activity within the arcuate nucleus of the hypothalamus (ARC) is diminished, which has been proposed to have a causal role in the increased acquisition of body weight and adiposity [6–8]. Treatment with a 5-hydroxytryptamine 2c receptor (5-HT_{2C}R) agonist, such as the new obesity medication lorcaserin (Arena Pharmaceuticals), restores diminished POMC neuron function and improves obesity [9–11]. Furthermore, inactivating 5-HT_{2C}Rs specifically on POMC neurons in mice, a genetic strategy employed to manipulate 5-HT_{2C}R expression, prevents the anorectic effect of 5-HT_{2C}R agonists [12], thereby revealing that 5-HT_{2C}R agonists modulate food intake via POMC neurons. Thus, POMC peptides are an important driver of body weight and POMC expressing neurons are amenable to pharmacological manipulation. Here, we sought to clarify the source of POMC peptides that critically mediate body weight using a newly developed genetic approach.

2. MATERIALS AND METHODS

2.1. Mice

5-$HT_{2C}R^{CRE}$ line. 5.6 kb of genomic DNA containing portions of the final exon and the 3′ UTR of the murine *Htr2c* gene was amplified by PCR from R1 ES cells [(129X1/SvJ × 129S1)F1 genetic background] and cloned into a plasmid for insertion of a FRT-NEO-FRT-IRES-CRE cassette between the STOP codon and the polyadenylation site, as previously described [17]. The targeting construct was linearized using NotI and electroporated into R1 mouse embryonic stem cells at the University of Michigan Transgenic Animal Model Core. Neomycin-resistant clones were analyzed by quantitative real-time PCR [18] for copy number of the native *Htr2c* allele and further confirmed by Southern blotting using an external probe. Correctly targeted ES cells were injected into C57BL/6J blastocysts to generate chimeras. Male chimeras were then bred to C57BL/6J females, and pups were genotyped to confirm insertion of IRES-Cre into the appropriate locus. These *5-$HT_{2C}R^{CRE}$* pups were then bred to a germline FlpO deleter strain (129S4/SvJae-Gt(ROSA)26Sortm2(FLP*)Sor/J; Jackson Laboratory) to remove the Neo cassette. Pups positive for FlpO and *5-$HT_{2C}R^{CRE}$* were genotyped for loss of the neo cassette in *5-$HT_{2C}R^{CRE}$* and further bred away from the FlpO allele.

5-$HT_{2C}R^{CRE}$ mice were then intercrossed with either ROSA26-stop-enhanced yellow fluorescent protein (YFP) (B6.129X1-*Gt(ROSA)26Sortm1(EYFP)Cos*/J; Jackson Laboratory) to create a reporter *5-$HT_{2C}R^{YFP}$* line or *$Pomc^{NEO}$* mice [13] to generate wild type, *5-$HT_{2C}R^{CRE}$*, ARC *Pomc* null (*$Pomc^{NEO}$*), and restored *Pomc* specifically in 5-HT_{2C}R expressing cells (*$Pomc^{5\text{-}HT2CR}$*) littermates.

All mice were group housed and maintained on a 12 h light/dark cycle with *ad libitum* access to water and standard laboratory chow diet. All experiments were in accordance with guidelines and approvals of the University of Michigan or the U.K. Animals (Scientific Procedures) Act 1986.

2.2. Immunohistochemistry (IHC)

Tissue was processed for endogenous POMC and for 5-$HT_{2C}R^{YFP}$ as previously described [9–11]. Briefly, under deep terminal anesthesia, mice were transcardially perfused with phosphate buffered saline (PBS) followed by 10% neutral buffered formalin (Sigma). Brains were extracted, post-fixed in 10% neutral buffered formalin at 4 °C, cryoprotected in 20% sucrose at 4 °C and then sectioned coronally on a freezing sliding microtome at 25 μm. Tissue was processed for POMC-immunoreactivity (IR) and 5-$HT_{2C}R^{YFP}$ (GFP-IR) as previously described [14,15] using rabbit anti-POMC primary antibody (1:1000; H-029-30, Phoenix Pharmaceuticals, Burlingame, CA, USA), chicken anti-GFP (1:500; ab13970, AbCam, Cambridge, UK) and Alexa Fluor secondary antibodies (1:500 A-11012, Life Technologies, Paisley, UK), respectively. Single and dual-labeled POMC-IR and GFP-IR cells were counted in the ARC [16]. Analysis was carried out on 7 levels of ARC (−1.46 to −2.18 from Bregma) for each mouse (n = 4/sex).

2.3. Quantitative PCR

Total RNA was purified from whole hypothalamus, brainstem and interscapular brown adipose tissue (BAT) using RNA STAT 60 (AMS Biotechnology, Abington, UK) according to the manufacturer's instructions and as previously described (9 months of age; n = 5–9/genotype/sex) [13]. cDNA was obtained by reverse transcription of 500 ng hypothalamic RNA, 1000 ng brainstem RNA and 500 ng BAT RNA. Real-time PCR analysis of cDNA was performed in duplicate on an ABI Prism 7900 sequence detection system using Taqman or Sybr assays for *Pomc* (ABI Taqman Gene expression assay Mm00435874_m1), *elongation of very long fatty acids-like 3* (*Elovl3*) and *peroxisome proliferator-activated receptor gamma coactivator-1alpha (Pgc-1a)*. Data for levels of target gene mRNAs are expressed in arbitrary units corrected to the geometric average of four housekeeping genes: *18s*, *36β4*, *βactin* and *glyceraldehyde-3-phosphate dehydrogenase (Gapdh)*. Sequences of primers and probes used are listed in Supplementary Table 1.

2.4. Metabolic profile

Body weight was measured from weaning up to 1 year of age (n = 7–17/genotype/sex). Home cage 24-h food intake was measured up to 6 months of age (n = 5–9/genotype/sex). At 9 months of age, a more detailed energy balance profile was performed, including light and dark cycle food intake, locomotor activity and energy expenditure assessment using indirect calorimetry in a Metabolic-Trace (Meta-Trace) system (Ideas Studio, UK; n = 5–9/genotype/sex). Body composition was also analyzed at 7–9 months of age using dual-energy X-ray absorptiometry (DEXA) Lunar PIXImus2 mouse densitometer (General Electric Medical Systems, Fitchburg, WI, USA; n = 5–11/genotype/sex).

Gonadal white adipose tissue (WAT) and interscapular BAT was dissected, fixed in 10% neutral buffered formalin, embedded in

