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Impaired mTORC2 signaling in catecholaminergic neurons exaggerates high fat diet-induced hyperphagia

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Abstract

Objective: Food intake is highly regulated by central homeostatic and hedonic mechanisms in response to peripheral and environmental cues. Neutral energy balance stems from proper integration of homeostatic signals with those “sensing” the rewarding properties of food. Impairments in brain insulin signaling causes dysregulation of feeding behaviors and, as a consequence, hyperphagia. Here, we sought to determine how the mammalian target of rapamycin complex 2 (mTORC2), a complex involved in insulin signaling, influences high fat feeding.

Methods: Rictor is a subunit of mTORC2, and its genetic deletion impairs mTORC2 activity. We used Cre-LoxP technology to delete Rictor in tyrosine hydroxylase (TH) expressing neurons (TH Rictor KO). We assessed food intake, body weight, body composition and DA dependent behaviors.

Results: TH Rictor KO mice display a high-fat diet specific hyperphagia, yet, when on low-fat diet, their food intake is indistinguishable from controls. Consistently, TH Rictor KO become obese only while consuming high-fat diet. This is paralleled by reduced brain DA content, and disruption of DA dependent behaviors including increased novelty-induced hyperactivity and exaggerated response to the psycho stimulant amphetamine (AMPH).

Conclusions: Our data support a model in which mTORC2 signaling within catecholaminergic neurons constrains consumption of a high-fat diet, while disruption causes high-fat diet-specific exaggerated hyperphagia. In parallel, impaired mTORC2 signaling leads to aberrant striatal DA neurotransmission, which has been associated with obesity in human and animal models, as well as with escalating substance abuse. These data suggest that defects localized to the catecholaminergic pathways are capable of overriding homeostatic circuits, leading to obesity, metabolic impairment, and aberrant DA-dependent behaviors.

Keywords: Rictor, mTORC2, Obesity, Dopamine, Amphetamine, High fat diet

Abbreviations: mTORC2: mammalian Target Of Rapamycin Complex 2, LF: Low fat, HF: High fat, AMPH: Amphetamine, DA: Dopamine

1. Introduction

A range of factors contributes to obesity, including nutritional trends, availability of highly palatable foods, changes to the built environment, economic stresses, and others [1]. Feeding is a centrally controlled complex biological behavior fine-tuned by both homeostatic metabolic drive (hunger or satiety) and hedonic motivational drive (reward and salience). Therefore, identifying the neurobiological circuits in which deficits lead to the development of both positive energy balance (i.e. “homeostatic” or “metabolic” obesity) and/or exaggerated responses to palatable food (i.e. “hedonic” obesity) is essential for defining mechanisms involved in the central regulation of food intake.

Akt is a key insulin dependent kinase that influences both peripheral endocrine responses and higher brain functions such as learning and memory, reward, and salience [2] [3] [4] [5] [6] [7]. Akt is activated by phosphorylation of two key residues, Thr308 and Ser473. Mammalian target of rapamycin (mTOR) complex 2 (mTORC2) is a multiprotein complex responsible for phosphorylation of Akt at Ser473 (pAkt-473). Within hypothalamic neurons, mTORC2 activity is implicated in mechanisms that control homeostatic neuroendocrine responses [8]. In the catecholaminergic system, mTORC2 function regulates monoamine turnover, DA neurotransmission, as well as psychostimulant action [9] [10] [11]. These processes are implicated in reward and salience [12]. This concept is also supported by data demonstrating that impairment in mTORC2 signaling leads to escalating morphine self-administration [13].

The mTORC2 complex consists of Rictor, mSIN1, mLST8, and mTOR. Recent studies show that conditional deletion of *Rictor* in hypothalamic centers leads to hyperphagia, impaired peripheral glucose homeostasis, and obesity [8]. In order to determine whether Rictor function specifically within catecholaminergic neurons plays a role in feeding behaviors and metabolism, we crossed *Rictor-Flox* mice with mice expressing *Cre recombinase* under control of the tyrosine hydroxylase (TH) promoter (TH Rictor KO). We show that disruption of mTORC2 signaling within catecholaminergic circuits supports exaggerated hyperphagia in response to palatable high-fat diets.

2. Materials and methods

All procedures were performed according to Vanderbilt University Institutional Animal Care and Use Committee approved procedures.

2.1. Experimental animals: generation and care

Mice were engineered as previously described [9] [10] [14]. Briefly, C57Bl6 mice with *floxedRictor* alleles were crossed to *TH-Cre* transgenic animals to produce TH-cell specific *Rictor* knockout (TH Rictor KO) mice. Control mice (CTR) were littermates that lacked the floxed *Rictor* allele. To genotype the animals, DNA from tail clippings was analyzed by PCR with primers for the *floxed*, *Cre recombinase*, and recombined alleles as previously described [15]. Male mice were studied from 8 to 18 weeks of age. Mice were housed in a temperature (22 °C) and light (12 h light/dark cycle) controlled room with free access to standard laboratory rodent chow diet (#5001, Lab Diet; St. Louis, MO) and water except where indicated.

2.2. Food intake and body composition analysis

Mice (male, 8–12 weeks old) were housed individually; diets (Research Diets, Inc., NJ, USA: high fat (HF) product number D12492; low fat (LF) product number D12450) were randomly assigned. Body weight was determined once per week, on a standard balance. Body composition was measured by nuclear magnetic resonance (NMR) in a Bruker Body Composition Analyzer (Bruker Optics; Billerica, MA). Adiposity (% body fat) was calculated as ((fat mass/lean mass)×100). Caloric intake (kcal) was determined daily for the first seven days of diet administration and weekly thereafter. Feed efficiency is the ratio of weight gained (total body weight, fat and lean), divided by calories consumed (kcal) over the indicated period.

2.3. Tissue harvest. Monoamine and their metabolites tissue content

Mice (male, 8–12 weeks old) were sacrificed by rapid decapitation under volatile isoflurane anesthesia, brains were removed and chilled on ice. Dorsal

striatum was dissected out from two hemispheres to create comparable samples for both monoamine content and immunoblotting. After dissection, tissue was frozen on dry ice and stored in -80°C until use. Monoamine content was determined at the Vanderbilt University Neurochemistry Core *via* high performance liquid chromatography (HPLC) with amperometric detection as described previously [16].

2.4. Locomotor behavior

Male mice (8–12 weeks old), were housed in temperature and humidity controlled rooms and kept on a 12-h light/dark cycle. Food and water were available *ad libitum*. Experiments were conducted in accordance with the NIH guidelines for the care and use of animals and were approved by the Vanderbilt University Institutional Animal Care and Use Committee. Initial handling lasted 5 days, with daily i.p. saline injections. On day 6 mice were tested for open field locomotor activity. Four-hour long sessions were performed using automated experimental chambers (27.9×27.9 cm; MED-OFA-510; MED Associates, Georgia, VT) under constant illumination in a sound-attenuated room. During days 7–12 mice were habituated to the chambers and saline injections: four-hour sessions with two saline injections daily (at times -120 min and 0 min). On day 13, mice were injected with saline at time -120 min and allowed to explore the chambers for two hours to settle to comparable baseline. At time 0 min, drugs or vehicle (saline) were administered i.p. (AMPH 2 mg/kg) and locomotion recorded for the next two hours. Analysis of open field activity, as well as stereotypic counts was performed using Activity Monitor (MED Associates).

2.5. Statistical analysis

All data are expressed as the mean \pm SEM. Mean differences between groups were determined using Student's *t* test or one- or two-way ANOVAs followed by *post hoc* testing when the main effect or interaction was significant at $p < 0.05$. Statistical analyses were conducted using Graph Pad Prism. The number of animals and specific statistical analyses used in each experiment are indicated in the and/or text.

3. Results

3.1. Rictor deletion within catecholaminergic circuits results in increased lean mass

We first assessed body weight and composition, as well as feeding in animals consuming a low fat diet for 8 weeks (LF, 10% fat). TH Rictor KO mice weigh more than control (CTR) mice (Fig. 1A; CTR mice were littermates of TH

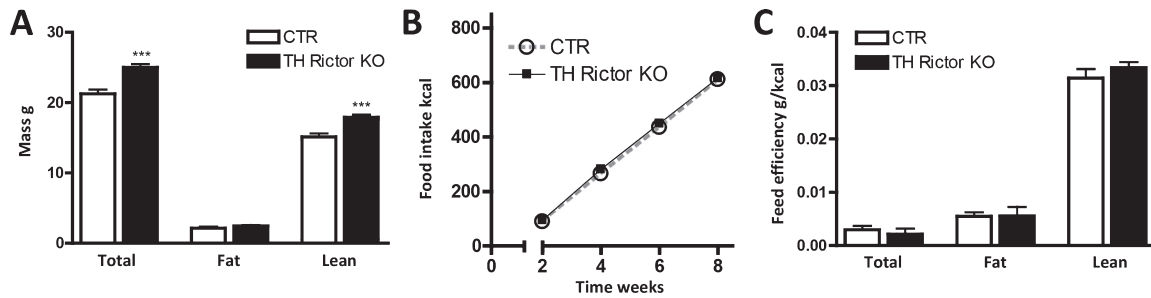


Fig. 1. Energy balance and body composition of TH Rictor KO mice on low-fat diet (LF). (A) Body composition of TH Rictor KO and CTR mice; $n = 8$ per genotype. (B) Cumulative food intake (LF diet) was measured over the course of eight weeks and was found to be indistinguishable from the LF food intake of CTR animals; $n = 6-7$ per genotype. (C) Feed efficiency over the corresponding eight week period was calculated as change in total body weight, fat, or lean mass in grams divided by total kcal consumed. Values represent mean \pm SEM; *** $p < 0.001$.

Rictor KO mice that lacked *Cre recombinase*). The increase in body weight is due to elevated lean mass as determined by NMR (Fig. 1A). DA neurons in the hypothalamus have been shown to regulate plasma growth hormone (GH) concentration [17]. The increase in lean mass, therefore, could stem from elevated GH levels. There were no differences in GH between genotypes (data not shown). Importantly, cumulative food intake was not different for TH Rictor KO mice relative to CTR animals (Fig. 1B) while on the LF diet. Feed efficiency, the change in total body weight, fat, or lean mass divided by the cumulative calories consumed over a period of time was not different (Fig. 1C).

These data strongly suggest that TH Rictor KO mice with disrupted mTORC2 signaling within catecholaminergic neurons have intact homeostatic energy regulation. However, since catecholamines are implicated in salience and reward, we next determined the role of mTORC2 signaling on the intake of a high fat (HF, 60% fat), palatable diet.

3.2. Conditional deletion of Rictorin catecholaminergic neurons results in escalating hyperphagia on high fat (HF) diet

The mesolimbic system is an essential component of the circuitry that evaluates saliency of natural rewards, including food [18] [19] [20] [21].

Catecholaminergic neurons are an essential component of this system. Given their role in food reward, we hypothesized that mTORC2 signaling in TH expressing neurons might play a pivotal role in regulating food consumption in animals exposed to palatable high fat diet (HF, 60% fat). Unlike on LF diet, TH Rictor KO mice consume significantly more calories than CTR animals on HF diet (Fig. 2A, inset). When excess HF diet intake is visualized cumulatively by

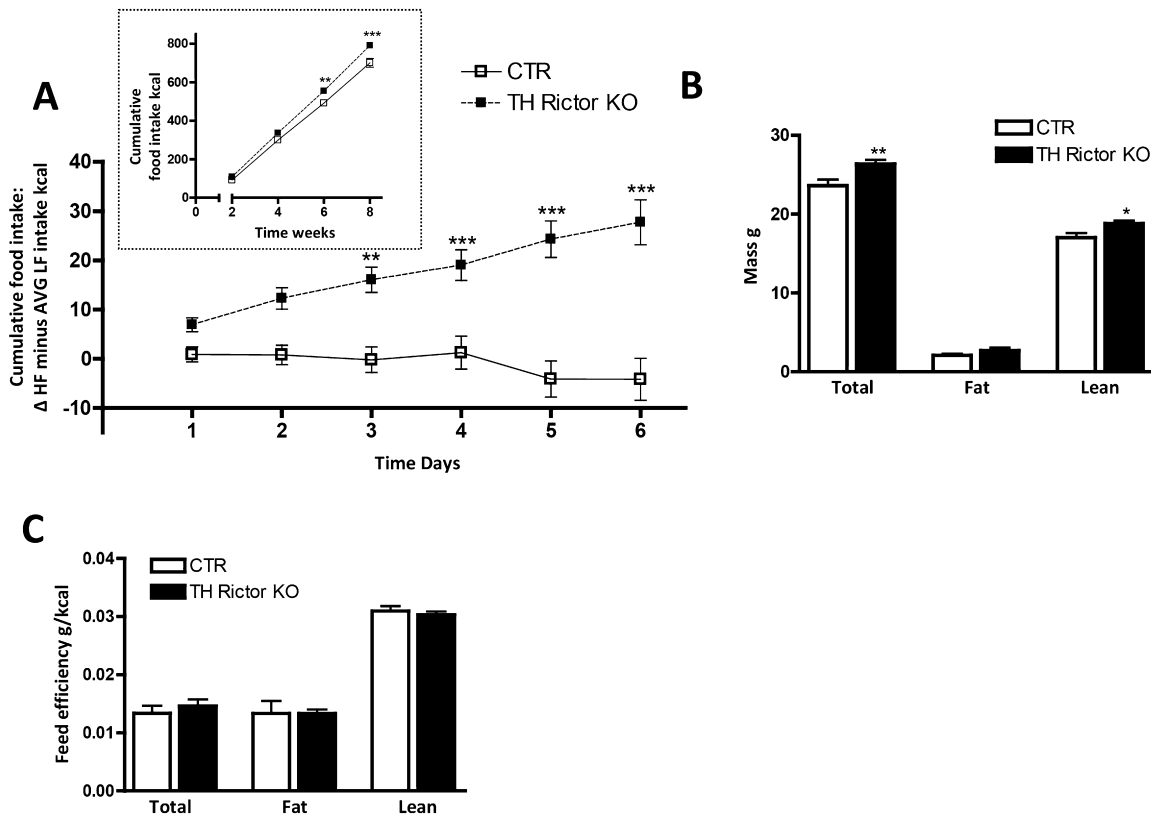


Fig. 2. Energy balance of TH Rictor KO mice on high-fat diet (HF). (A) Genotype-specific difference in cumulative consumption of HF food relative to LF food consumption (average low-fat food intake was subtracted from high-fat food intake in kcal); $n = 7-9$ per treatment per genotype. Inset: Cumulative food intake (high-fat diet) was measured over the course of eight weeks. In both graphs, high-fat food-induced escalating hyperphagia is shown for TH Rictor KO mice compared to CTR animals; $n = 6-7$ per genotype. (B) Body composition over the corresponding eight week period was measured and graphed to compare with CTR mice; $n = 8$ per genotype. (C) Feed efficiency over the corresponding eight week period was calculated as change in total body weight, fat, or lean mass in grams divided by total kcal consumed); $n = 4-5$ per genotype. Values represent mean \pm SEM; *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$.

subtracting average LF diet consumption from daily HF diet consumption, continuously escalating HF diet consumption is observed only in TH Rictor KO mice (Fig. 2A). This was calculated by subtracting the average caloric consumption on a low fat diet from the caloric consumption on the high fat diet of the correspondent genotype each day. Thus, CTR animals modulate their high-fat food intake, while TH Rictor animals continuously increase consumption of the high-fat diet over time (Fig. 2A). Concomitantly, TH Rictor KO mice show a rapid increase in body weight and lean mass after only six days on a high fat diet (Fig. 2B) without changes in feed efficiency (Fig. 2C).

3.3. Conditional deletion of Rictor in TH expressing neurons results in impaired DA neurotransmission and aberrant DA-dependent behaviors

The catecholaminergic system modulates motivated behaviors such as feeding, drinking, and locomotion as well as reward. TH Rictor KO mice exhibit normal food intake when exposed to a LF diet. However, they display exaggerated hyperphagia on HF diet. This hyperphagia could stem from disrupted DA neurotransmission in midbrain. To explore this possibility, we first analyzed DA tissue content in the ventral striatum. TH Rictor KO mice have reduced DA tissue content in the Nucleus Accumbens (NAc) relative to CTR animals (Fig. 3A), suggesting impaired mesolimbic DA tone in the conditional KO mice. Importantly, DA metabolites DOPAC, 3-MT, and HVA were not significantly changed in the TH Rictor KO mice (data not shown), suggesting altered DA homeostasis. This observation warrants further exploration.

Analysis of locomotor activity is an efficient method to evaluate the integrity of the DA neurotransmission in rodents. Novelty-induced hyperactivity is modulated by the mesolimbic DA pathway [22]. TH Rictor KO mice display exaggerated hyperlocomotion in a novel environment relative to CTR animals (Fig. 3B). Importantly, rodents that are prone to elevated novelty-induced hyperactivity, also exhibit heightened sensitivity to psycho stimulants, such as amphetamine (AMPH) [9] [10] [23]. Therefore, we challenged habituated (see Methods) TH Rictor KO mice with a single dose of AMPH. TH Rictor KO animals show increased AMPH-induced hyperactivity compared to their wild type counterparts (Fig. 3C).

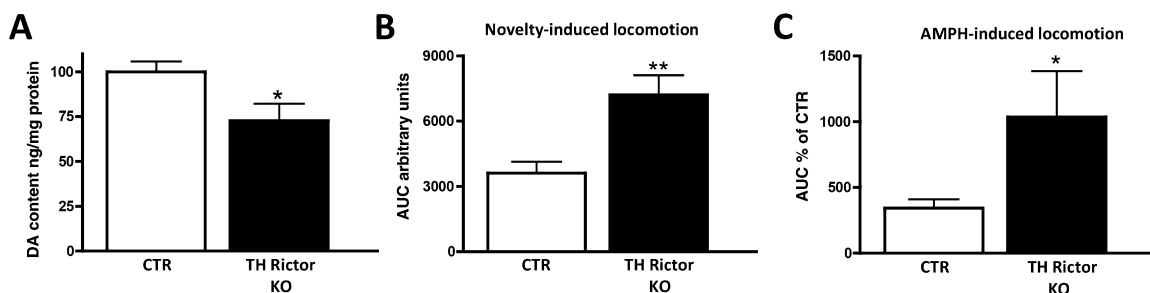


Fig. 3. Aberrant NAc DA tone and disrupted DA-dependent behaviors in TH Rictor KO mice. (A) DA tissue content as measured by HPLC in NAc homogenates; $n = 6-8$ per genotype. (B) Novelty-induced locomotion: horizontal movement measured in open field chambers in 5-min intervals; $n = 8$ per genotype. Data are represented as area under the curve (AUC) for the first 30 min in the chamber. (C) AMPH-induced locomotion; $n = 7-8$ per treatment per genotype. CTR and TH Rictor KO mice were habituated to saline injections and open field chambers for six days. On day seven AMPH (2 mg/kg) was administered i.p. and horizontal locomotor activity recorded in 5-min intervals. Data are represented as area under the curve (AUC) from time of injection to 30 min, expressed as percent of corresponding saline control. Values represent mean \pm SEM; ** $p < 0.01$, * $p < 0.05$.

These data support the notion that genetic deletion of *Rictor* in the TH expressing cells results in altered mesolimbic DA tone associated with exaggerated high fat hyperphagia and AMPH-induced hyperlocomotion.

4. Discussion

Regulation of energy balance is intricately regulated by the central nervous system. Food intake is controlled by both homeostatic and hedonic circuits, which rely not only on objective physiological cues supported by peripheral systems, but also on subjective experiences such as memory, motivation, and pleasure, all supported by environmental cues [12] [18] [21] [24] [25]. One of the key regulators of energy balance, the adiposity negative feedback hormone insulin, signals *via* mTORC2/Akt pathway in the brain, and modulates both homeostatic and hedonic neural circuits [8] [12] [26]. Proper integration of homeostatic signals with those “sensing” the saliency of food is necessary for appropriate energy balance regulation. Aberrant brain insulin signaling causes abnormal feeding behaviors, including hyperphagia [11] and has been shown to regulate catecholaminergic neurotransmission through Akt [10] [16].

Dopamine is essential in modulation of many vital behaviors including movement, cognition, motivation, and salience. Motivation to obtain natural rewards such as food is vital for the organismal survival, and was shown to depend on central DA neurotransmission. Indeed, motivation for seeking food as well as the reward and satiety we feel when we eat have been extensively studied in humans using imaging techniques [27] [28]. Mere consumption of food is a motivated behavior that is controlled by the DA signaling [29].

Our laboratory and others have previously shown that aberrant signaling through the Akt pathway which is stimulated by insulin and other neuropeptides, disrupts central DA neurotransmission [30] [31] [32] [33]. These data include *in vivo* studies demonstrating that aberrant peripheral insulin signaling caused by either high-fat diet or pharmacological interventions leads to altered central DA signaling [11] [34]. Therefore, in this study we sought to determine if disrupted mTORC2/rictor/Akt signaling specifically in TH expressing neurons, which include DA cells, leads to abnormal feeding and altered metabolism.

5. Conclusion

Here, we demonstrate that mTORC2 signaling in catecholaminergic neurons modulates brain DA homeostasis, and is implicated in DA-related behaviors such as novelty-induced hyperlocomotion, hypersensitivity to the psychostimulant AMPH, and, importantly, HF diet-specific hyperphagia. Importantly, studies in metabolic chambers did not reveal any differences in basal activity or locomotion between genotypes, suggesting that the increased

high-fat food intake was not caused by increased locomotor activity of the TH Rictor mice. Our data support a model in which disrupted mTORC2 signaling within catecholaminergic neurons creates aberrant striatal DA neurotransmission, and causes HF diet-specific exaggerated hyperphagia. These data suggest that mTORC2 signaling defects localized to the catecholaminergic pathways are capable of overriding homeostatic circuits, and drives aberrant DA-dependent behaviors.

Declarations

Author contribution statement

Olga Dadalko: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Aurelio Galli; Kevin Niswender: Conceived and designed the experiments; Wrote the paper.

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Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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