Original article

MOLECULAR METABOLISM

PGC-1α expression in murine AgRP neurons regulates food intake and energy balance

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ABSTRACT

Objective: Food intake and whole-body energy homeostasis are controlled by agouti-related protein (AgRP) and pro-opiomelanocortin (POMC) neurons located in the arcuate nucleus of the hypothalamus. Key energy sensors, such as the AMP-activated protein kinase (AMPK) or sirtuin 1 (SIRT1), are essential in AgRP and POMC cells to ensure proper energy balance. In peripheral tissues, the transcriptional coactivator PGC-1α closely associates with these sensors to regulate cellular metabolism. The role of PGC-1α in the ARC nucleus, however, remains unknown.
Methods: Using AgRP and POMC neurons specific knockout (KO) mouse models we studied the consequences of PGC-1α deletion on metabolic parameters during fed and fasted states and on ghrelin and leptin responses. We also took advantage of an immortalized AgRP cell line to assess the impact of PGC-1α modulation on fasting induced AgRP expression.
Results: PGC-1α is dispensable for POMC functions in both fed and fasted states. In stark contrast, mice carrying a specific deletion of PGC-1α in AgRP neurons display increased adiposity concomitant with significantly lower body temperature and RER values during nighttime. In addition, the absence of PGC-1α in AgRP neurons reduces food intake in the fed and fasted states and alters the response to leptin. Finally, both *in vivo* and in an immortalized AgRP cell line, PGC-1α modulates AgRP expression induction upon fasting.
Conclusions: Collectively, our results highlight a role for PGC-1α in the regulation of AgRP neuronal functions in the control of food intake and peripheral metabolism.

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Keywords PGC-1α; Agouti-related protein; Metabolism; Energy homeostasis; Pro-opiomelanocortin; Transcriptional regulation

1. INTRODUCTION

The arcuate nucleus of the hypothalamus receives and integrates different inputs from peripheral organs and subsequently controls food intake and energy expenditure according to the energy status of the body [1–4]. Two major cell populations in the ARC nucleus, namely the orexigenic agouti-related protein (AgRP) and an orexigenic pro-opiomelanocortin (POMC) neurons, secrete diverse neuropeptides including AgRP, neuropeptide Y (NPY), α-melanocyte stimulating hormone (α-MSH) and cocaine- and amphetamine-regulated transcript (CART), respectively. The activity of both AgRP and POMC neurons are regulated by hormonal inputs, such as ghrelin [5,6], leptin, and insulin [7], and nutrients such as glucose [8]. As a result, in the fasted state, AgRP neurons stimulate appetite and decrease energy expenditure while, in the fed state, POMC neuron activation leads to food satiety and enhanced energy production [8–10]. Interestingly, different energy sensors have been implicated in the cellular mechanisms in the ARC nucleus that ultimately regulate whole-body metabolism. For example, AMP-activated protein kinase (AMPK) is necessary for glucose sensing in both AgRP and POMC neurons and thereby for the control of energy balance [11,12]. Deletion of the NAD^+-dependent protein deacetylase sirtuin-1 (SIRT1) in AgRP neurons impairs the response to ghrelin and thus affects energy homeostasis [13]. Finally, the forkhead protein FoxO1 mediates leptin inhibition of AgRP expression and food intake [14].

The peroxisome proliferator-activated receptor γ coactivator 1α (PGC-1α) is a major coregulator of transcription factors involved in the control of cellular metabolism [15]. Intriguingly, key energy sensors that are part of the hypothalamic network controlling energy balance engage PGC-1α in peripheral tissues. For example, SIRT1 interacts with and deacetylates PGC-1α to induce the expression of gluconeogenic and mitochondrial genes in the liver [16]. Similarly, AMPK activation leads to transcriptional induction and activating phosphorylation events of the PGC-1α gene and protein, respectively [17]. In hepatocytes, Foxo1 engages PGC-1α in the context of insulin-regulated gluconeogenesis [18]. Importantly, global as well as brain specific deletion of PGC-1α protects mice from diet-induced obesity [19,20]. Furthermore, PGC-1α levels in the hypothalamus are increased in response to fasting [20], suggesting that PGC-1α may act as a metabolic integrator of different signaling pathways in the ARC

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Abbreviations: AgRP, Agouti-related protein; AMPK, AMP-activated protein kinase; FOXO1, Forkhead protein 1; PGC-1α, Peroxisome proliferator-activated receptor γ coactivator 1α; POMC, Pro-opiomelanocortin; SIRT1, NAD^+-dependent protein deacetylase sirtuin-1; TBP, TATA-binding protein

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nucleus for the regulation of whole body energy homeostasis [21]. However, due to the phenotypic complexity of global and brain-specific PGC-1α knockout animals, it is unclear whether this coactivator exerts a direct role in AgRP and POMC neurons.

The present study aimed to investigate the contribution of PGC-1α expression in the ARC nucleus to whole-body energy balance. We therefore generated mouse models with specific deletions of PGC-1α either in AgRP or in POMC neurons and studied their energy homeostasis and response to different metabolic challenges.

2. EXPERIMENTAL PROCEDURES

2.1. Animals

Male mice were kept under a 12 h/12 h light/dark cycle with lights on from 06:00 to 18:00 humidity-controlled rooms at 23 °C. All animals had free access to regular chow diet (Provimi Kliba 3432) or High Fat Diet (HFD) (Provimi Kliba 2127) and water. Animals with a specific PGC-1α knock-out in AgRP and POMC neurons (AgRP- and POMC-PGC1α KO) were generated by crossing PGC-1α$^{loxP/loxP}$ mice with transgenic AgRP$^{Cre/+}$ and POMC$^{Cre/+}$ mice, respectively. The PGC-1α$^{loxP/loxP}$ mice have been described previously [19]. AgRP$^{Cre/+}$ (Agrp$^{tm1(cre)Lowl}$, Jax #012899) and POMC$^{Cre/+}$ (STOCK tg(Pomc1-cre) 16Lowl/J, Jax #005965) were purchased from Jackson Laboratories. AgRP- and POMC-PGC1α KO or AgRP$^{Cre/+}$ and POMC$^{Cre/+}$ were crossed with Rosa26-EGFP reporter mice carrying an EGFP sequence in the Rosa26 locus to generate AgRP- or POMC-EGFP-Cre and AgRP- or POMC-EGFP-Cre-PGC1α KO mice expressing EGFP in AgRP or POMC neurons. Animals used in all experiments besides weight curves measurement were between 16 and 20 weeks old, except for ghrelin and leptin experiments, for which 8 weeks old mice were used. PGC-1α$^{loxP/loxP}$ littermates mice without AgRP$^{Cre/+}$ and POMC$^{Cre/+}$ sites were used as controls (ctr). The genotype of AgRP-, POMC-PGC1α KO and littermate control animals was assessed by PCR using specific primer pairs (listed in the DNA/RNA extraction and qPCR section) to detect the presence of AgRP$^{Cre/+}$, POMC$^{Cre/+}$ and loxP sites. Aberrant expression of the Cre transgene is sporadically detected in germ cells in both AgRP and POMC lines. Whole body PGC-1α knock-out animals were therefore identified by PCR and excluded from the experiments (approximately 50% of AgRP-PGC1α KO and 2% of POMC-PGC1α KO mice). All experiments were performed in accordance with the federal guidelines for animal experimentation and were approved by the Kantonales Veterinäramt of the Kanton Basel-Stadt.

2.2. Body weight curves and composition analysis

Body weight was measured the same day of the week in the morning from 4 to 22 weeks of age and subsequently every month until the age of 50 weeks. Body composition was evaluated with an EchoMRI-100 analyzer (EchoMRI Medical Systems). Fat and lean mass were normalized to body weight. A HFD experiment was started with 6 weeks old mice and body composition was evaluated after 8 weeks of HFD treatment.

2.3. COBAS blood analysis

Blood was harvested after the mice were killed. Blood samples were centrifuged for 10 min at 2000 g in tri-potassium-EDTA tubes and plasma was collected. Plasma glucose and triglycerides levels were measured with a COBAS c111 analyzer (Roche Diagnostics).

2.4. Glucose tolerance test

A bolus of 2 g (glucose)/kg (body weight) was injected intraperitoneally into mice fasted for 16 h. Blood glucose was measured in the tail vein 0, 15, 30, 45, 60, 90 and 120 min after glucose injection with a glucose meter (Accu-Chek, Roche Diagnostics). All mice were acclimatized to handling before the experiment.

2.5. Comprehensive laboratory animal monitoring system (CLAMS)

Whole body metabolism was assessed with an indirect calorimetric system (CLAMS, Columbus Instruments). Food intake, locomotor activity, VO_2 and VCO_2 were recorded in 15 min intervals. Data were analyzed after one day of acclimatization. The plotted values represent 3 days of measurements in fed, 24-h fasted and 24-h refed animals.

2.6. Voluntary wheel-running activity and body temperature measurements

Mice were given free access to running wheels. The number of wheel revolutions was recorded in 30 min intervals. Plotted values represent two weeks of measurements after two weeks of acclimatization. In separate experiments, Anipill capsules (Animal Monitoring) were implanted intraperitoneally under isoflurane anesthesia for body temperature measurements. After a recovery period of 2 weeks, body temperature was recorded in 15 min intervals.

2.7. Ghrelin and leptin sensitivity

Animals were acclimatized to handling before the experiment. Intraperitoneal injections were performed with 2 and 5 mg/kg body weight of rat *ghrelin* (Bachem H-4862) and rat *leptin* (R&D 498-OB-05M), respectively, in PBS vehicle. Vehicle control, ghrelin and leptin, respectively, were injected in subsequent experiments into the same animals. Food pellets were weighed and exchanged after injections. Ghrelin injections were done at 12:00. Food intake was measured 1, 2 and 3 h after injection. Two consecutive leptin injections were made at 17:30 and at 07:30 on the next day. Food intake and body weight were measured 16 and 24 h later.

2.8. Cell culture

The MHypoA-59 cell line (Bioconcept CLU468) was grown in monolayer cultures in regular DMEM (Sigma—Aldrich D 5796) supplemented with 5% fetal bovine serum (FBS) (HyClone Laboratories, Inc., Logan, UT), 4.5 mg/ml glucose and 1% penicillin/streptomycin. Cells were maintained at 37 °C with 5% CO_2. Cells were grown to 50% confluence before infection. PGC-1α knock-down was induced using adenoviral vectors expressing specific short hairpin RNA (shRNA) against PGC-1α or scrambled control shRNA. Both viruses expressed EGFP to allow infection efficiency monitoring. Two days after infection, regular growth medium was exchanged with fresh regular growth medium or with low glucose DMEM (1 mg/ml, Sigma—Aldrich D 6046) without FBS to induce cell starvation. After 4 h, the medium was exchanged with low glucose DMEM supplemented with 5% FBS to mimic refeeding. Cells were harvested 4 h after starvation and 1 h after refeeding. Cells exposed to normal growth medium were used as a fed state.

2.9. ARC nucleus punch isolation and imaging

Mice were killed by CO_2 inhalation. Mouse brains were harvested and directly frozen in 2-methylbutane (M32631). Brain tissue was embedded in optimal cutting temperature medium (OCT, Tissue-Tek 25608-930). For arcuate nucleus isolation, 100—200 μm sections containing the region of interest were cut with a cryostat (Leica). Sections were placed in RNA later solution (Qiagen 76104) and the hypothalamic region containing the ARC nucleus was isolated using a punch needle (Leica 39443001). For AgRP and POMC neuron imaging, 15 μm sections containing the arcuate nucleus of AgRP- or POMC-EGFP-Cre and AgRP- or POMC-EGFP-Cre-PGC1α KO mice

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