Brief communication

MOLECULAR METABOLISM

Angiotensin type 1a receptors in the forebrain subfornical organ facilitate leptin-induced weight loss through brown adipose tissue thermogenesis

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ABSTRACT

Objective: Elevations in brain angiotensin-II cause increased energy expenditure and a lean phenotype. Interestingly, the metabolic effects of increased brain angiotensin-II mimic the actions of leptin, suggesting an interaction between the two systems. Here we demonstrate that angiotensin-type 1a receptors ($AT_{1a}R$) in the subfornical organ (SFO), a forebrain structure emerging as an integrative metabolic center, play a key role in the body weight-reducing effects of leptin *via* brown adipose tissue (BAT) thermogenesis.

Methods: Cre/LoxP technology coupled with targeted viral delivery to the SFO in a mouse line bearing a conditional allele of the *Agtr1a* gene was utilized to determine the interaction between leptin and SFO $AT_{1a}R$ in metabolic regulation.

Results: Selective deletion of $AT_{1a}R$ in the SFO attenuated leptin-induced weight loss independent of changes in food intake or locomotor activity. This was associated with diminished leptin-induced increases in core body temperature, blunted upregulation of BAT thermogenic markers, and abolishment of leptin-mediated sympathetic activation to BAT.

Conclusions: These data identify a novel interaction between angiotensin-II and leptin in the control of BAT thermogenesis and body weight, and highlight a previously unrecognized role for the forebrain SFO in metabolic regulation.

Keywords Leptin; Brown adipose tissue; Brain; Angiotensin; Sympathetic nervous system; Metabolic regulation

1. INTRODUCTION

The renin-angiotensin system (RAS), long known for its role in blood pressure and fluid balance regulation, is now emerging as a key regulator of metabolic control. Interestingly, while activation of the systemic RAS results in adipogenesis and body weight gain [1–3], recent evidence indicates that activation of the central nervous system (CNS) RAS has the opposite effect and promotes a lean phenotype. Brain infusion of angiotensin-II (Ang-II) in rats [4,5], or genetic overexpression of the RAS components in the CNS of mice [6], both result in an increase in thermogenic energy expenditure. While these findings highlight the importance of brain Ang-II in metabolic regulation, the underlying mechanisms and specific neural regions involved remain unclear.

Importantly, the energy expenditure effects of brain RAS activation are similar to the actions of the adipocyte-derived hormone leptin, suggesting that the metabolic influence of brain Ang-II may be due, in part, to an interaction with central leptin signaling. In line with this, a facilitatory leptin-RAS relationship has been demonstrated in the periphery [7,8]. Moreover, leptin and Ang-II type 1a receptors ($AT_{1a}R$) are co-expressed in a number of forebrain [9,10], hypothalamic [11,12] and brainstem [13–15] regions that are implicated in metabolism and energy expenditure [16]. Using AT_1R antagonists and global $AT_{1a}R$ knockout mice, Hilzendeger et al. [16] previously identified a brain interaction between leptin and RAS in the regulation of sympathetic nerve activity (SNA), but the brain region(s) or metabolic effects of this interaction remains unknown. Given that a number of diseases, including obesity and diabetes, are characterized by altered Ang-II and

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Abbreviations: Ang-II, angiotensin-II; $AT_{1a}R$, angiotensin type 1a receptor; BAT, brown adipose tissue; CNS, central nervous system; LepRb, leptin receptor; OVLT, organum vasculosum lamina terminalis; RAS, renin-angiotensin system; SFO, subfornical organ; SNA, sympathetic nerve activity

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leptin signaling in the CNS, investigation of brain sites that mediate a RAS-leptin metabolic interaction is critical.

Here we tested the hypothesis that Ang-II signaling through $AT_{1a}R$ in the brain is involved in the metabolic actions of leptin. We show that the subfornical organ (SFO), a tiny forebrain structure situated outside the blood–brain-barrier, dense with $AT_{1a}R$, and recently implicated as an integrative metabolic center [17–19], plays a previously unrecognized role in the control of body weight. Specifically, we demonstrate an interaction between SFO-$AT_{1a}R$ and CNS leptin in the regulation of brown adipose tissue (BAT) thermogenic metabolism and body weight. When $AT_{1a}R$ are selectively deleted from the SFO, sympathetically mediated BAT thermogenesis and decreases in body weight in response to leptin are significantly blunted, independent of changes in locomotor activity and food intake. In addition to revealing a previously unknown role for the forebrain SFO in metabolic regulation, these data identify a strong link between $AT_{1a}R$ in the SFO and CNS leptin in the control of energy expenditure and body weight.

2. METHODS

Detailed methods are available in the online supplement.

2.1. Animals

All animal protocols were approved by Animal Care and Use Committees at Cornell University and the University of Iowa. Studies were conducted in adult (8 weeks old) male $AT_{1a}R^{fl/fl}$ mice initially obtained from the colony of Dr. Thomas Coffman [20] and used to establish our own colony. Mice were fed standard chow and water *ad libitum* and were housed with a 12-h light/dark cycle.

2.2. Leptin administration

For experiments involving lateral ventricle (ICV) injection of leptin or vehicle (saline), mice were instrumented with an indwelling ICV cannula [21]. Murine leptin was injected ICV (2 μg daily) or i.p. (30 μg bi-daily) either over a 4-day period or acutely, as previously described [10,16,22].

2.3. Adenoviral targeting of Cre to the SFO and lateral ventricle cannulation

Targeting of the SFO with recombinant adenoviral vectors encoding AdCre (4×10^{10} plaque-forming units/ml) or titer-matched AdLacZ was performed as previously described in detail by our laboratory [21,23,24]. Viral targeting and ICV cannulation were performed in the same surgical setting.

2.4. Sympathetic nerve recording

Mice were instrumented for multifiber recordings of BAT-SNA as previously described [10,16,22] Briefly, following anesthesia, the nerves to BAT were identified, mounted on platinum–iridium recording electrodes and fixed with silicone gel. Following surgical procedures, the animals were allowed to stabilize prior to obtaining BAT measurements before and for up to 4 h following ICV leptin administration.

2.5. Quantitative real-time PCR

Micropunches of the SFO, organum vasculosum lamina terminalis (OVLT), arcuate nucleus, ventromedial hypothalamus, parventricular nucleus of the hypothalamus and somatosensory cortex were obtained using brain atlas coordinates [25] as described [10]. Tissue from two mice was pooled per biological sample. Total RNA was also individually isolated from BAT for thermogenic mRNA evaluation.

2.6. Data analysis

Data are expressed as mean ± SEM and were analyzed by a two-tailed unpaired t-test or two-way repeated measures ANOVA, with appropriate post-hoc comparisons when applicable. A value of $p < 0.05$ was considered statistically significant.

3. RESULTS

3.1. Cre-mediated ablation of $AT_{1a}R$ selectively in the forebrain SFO

Previous investigations that have examined a role for the brain RAS in metabolic regulation have used global knockout models, whole brain overexpression of RAS components or central infusion of Ang-II or its antagonists [4–6,16]. While beneficial, these approaches are limited as they do not allow for specific dissection of the neural regions involved [26]. Within the CNS, the actions of Ang-II are mediated primarily through $AT_{1a}R$, with very high expression of this receptor in the forebrain circumventricular SFO [15,17,18]. Interestingly, this CNS structure is emerging as important in metabolic regulation [9,19,27]. To investigate the metabolic role of $AT_{1a}R$ specifically in the SFO, we utilized Cre/LoxP technology coupled with brain site-selective viral delivery in a mouse line bearing a conditional allele of the *Agtr1a* gene ($AT_{1a}R^{fl/fl}$) [20]. Targeting of an adenoviral vector expressing Cre recombinase (AdCre) selectively to the SFO resulted in stable, robust, localized expression of Cre within this region (Figure 1A), consistent with our previous findings [10,23,24,28]. In line with this, quantitative real-time PCR of SFO micropunches demonstrated a ~95% reduction in $AT_{1a}R$ transcript levels in mice having undergone SFO-targeted transfer of AdCre compared to those that received a control vector (AdLacZ) (Figure 1B). Importantly, $AT_{1a}R$ levels remained unchanged in hypothalamic leptin-responsive regions, including the arcuate nucleus, ventromedial hypothalamus and paraventricular nucleus, as well as the circumventricular OVLT and somatosensory cerebral cortex (Figure 1B).

Since the long signaling form of the leptin receptor (LepRb) is also expressed within these neural regions [29–32], we verified that SFO-targeted AdCre did not alter *LepRb* mRNA in SFO, hypothalamic, OVLT or somatosensensory cortical regions following SFO-targeted AdCre ablation of $AT_{1a}R$ (Figure 1C). These findings are consistent with our previous reports [10,15,23] and demonstrate the effectiveness and selectivity of AdCre-mediated recombination of loxP-flanked *Agtr1a* in the SFO.

3.2. Ablation of $AT_{1a}R$ in the SFO attenuates leptin-induced weight loss independent of changes in food intake and locomotor activity

While the brain RAS has been implicated in the physiological regulation of energy metabolism and in an interaction with leptin, the brain region(s) and mechanisms of a brain RAS-leptin interaction have not been delineated. Given the abundance of $AT_{1a}R$ in the SFO and the emerging theory of the importance of this region in metabolic regulation [9,19,27], we examined the role of SFO-$AT_{1a}R$ on leptin-mediated control of body weight. $AT_{1a}R^{fl/fl}$ mice underwent SFO-targeted microinjections of AdCre or AdLacZ. Deletion of $AT_{1a}R$ in the SFO did not influence baseline body weight (AdCre vs. AdLacZ, 24.2 ± 0.4 vs. 24.6 ± 0.3 g, $n = 16–18$/group, $p > 0.05$). Next we administered leptin directly into the brain *via* an implanted ICV cannula [10,33], which allowed for investigation of brain leptin–$AT_{1a}R$ interactions without the confounding influence of leptin's peripheral metabolic actions. Daily ICV leptin administration caused a progressive and robust decrease in body weight over a 4-day period in mice with intact $AT_{1a}R$ in the SFO (AdLacZ, Figure 1D). By comparison, mice in

