Ablation of the Leptin Receptor in the Hypothalamic Arcuate Nucleus Abrogates Leptin-Induced Sympathetic Activation

Short Communication

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Rationale: **The hypothalamic arcuate nucleus (ARC) is considered a major site for leptin signaling that regulates several physiological processes.**

Objective: **To test the hypothesis that leptin receptor in the ARC is required to mediate leptin-induced sympathetic activation.**

Methods and Results: **First, we used the ROSA Cre-reporter mice to establish the feasibility of driving Cre expression in the ARC in a controlled manner with bilateral microinjection of adenovirus-expressing Cre-recombinase (Ad-Cre). Ad-Cre microinjection into the ARC of $ObR^{flox/flox}$ mice robustly reduced ObR expression and leptin-induced Stat3 activation in the ARC but not in the adjacent nuclei, confirming the efficacy and selectivity of the ARC deletion of ObR. Critically, deletion of ObR in the ARC attenuated brown adipose tissue and renal sympathetic nerve responses to leptin. We also examined whether ObR in the ARC is required for the preserved leptin-induced increase in renal sympathetic activity in dietary obesity. We found that deletion of ARC ObR abrogated leptin-induced increases in renal sympathetic discharge and resolved arterial pressure elevation in diet-induced obese $ObR^{flox/flox}$ mice.**

Conclusions: **These data demonstrate a critical role for ObR in the ARC in mediating the sympathetic nerve responses to leptin and in the adverse sympathoexcitatory effects of leptin in obesity. (*Circ Res*. 2011;108:808-812.)**

Key Words: leptin ■ arcuate nucleus ■ sympathetic tone ■ obesity ■ hypertension

Leptin is considered a critical signal that feeds back to inform the brain about the status of peripheral energy reserves.[1] Consistent with this, leptin increases sympathetic nerve activity (SNA) to thermogenic brown adipose tissue (BAT). In addition, leptin action in the brain increases SNA subserving cardiovascular organs such as the kidneys leading to blood pressure elevation.[2] In common obesity, there is partial resistance to the actions of leptin on food intake and body weight, but the ability of leptin to activate renal SNA and increase arterial pressure remains intact.[2,3]

The arcuate nucleus (ARC) of the hypothalamus is considered a major site for the regulation of physiological processes by leptin. The ARC contains the highest concentration of the long signaling form of the leptin receptor (ObRb) and is the most responsive brain nucleus to leptin in terms of activation of intracellular signaling pathways associated with ObRb (eg, Stat3 [signal transducer and activator of transcription-3 protein]).[4] Furthermore, lesioning the ARC abolishes the feeding response to leptin,[4] whereas restoration of ObRb expression in the ARC of leptin receptor–deficient Koletsky rats[5] or in mice that have a leptin receptor–null allele[6] decreases food intake and body weight. However, recent evidence demonstrating ObRb expression and leptin actions in several other brain regions has led to the concept of a distributed brain network of leptin action.[7,8]

Here, we tested the importance of leptin receptors in the hypothalamic ARC in mediating leptin-induced increases in regional sympathetic outflow.

Methods

An expanded Methods section is available in the Online Data Supplement at http://circres.ahajournals.org.

Adenovirus expressing an enhanced green fluorescence protein (Ad-GFP), used as control, or both Ad-Cre and Ad-GFP (referred to as Ad-Cre) were microinjected bilaterally into the ARC of $ObR^{flox/flox}$

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mice. Leptin activation of Stat3 was analyzed by immunohistochemistry. Recording of SNA was obtained in anesthetized mice. Obesity was induced by high-fat (45% kcal) feeding for 10 weeks.

Non-standard Abbreviations and Acronyms	
Ad	adenovirus
ARC	arcuate nucleus
BAT	brown adipose tissue
Cre	Cre-recombinase
DIO	diet-induced obese
GFP	green fluorescence protein
ICV	intracerebroventricular
ObR	leptin receptor
Stat3	signal transducer and activator of transcription-3 protein
SNA	sympathetic nerve activity

Results

Visualization of Cre-Mediated Recombination in the ARC

To assess the feasibility of driving Cre expression in a spatiotemporal controlled manner with an adenoviral vector, we used the ROSA Cre reporter mice. Brain sections of ROSA mice that received Ad-Cre in the ARC revealed β-galactosidase (β-gal) staining as early as 4 days after injection (Online Figure I). Peak β-gal expression levels were detected at 8 days, with no further increase at 15 or 21 days. Importantly, β-gal staining was restricted to the ARC because it was not found in the adjacent nuclei. These results demonstrate the feasibility of deleting a loxP flanked gene specifically in the ARC by bilateral microinjection of Ad-Cre into this nucleus.

Cre-Mediated Deletion of ARC ObR in ObR$^{flox/flox}$ Mice

Using quantitative RT-PCR, we found that within the mediobasal hypothalamus, the ARC contains the highest levels of ObR (Online Figure II), which is consistent with the known expression profile of this receptor.[4–7] Moreover, quantitative RT-PCR analysis confirmed the robust reduction (≈71%) in ObR expression in the ARC of ObR$^{flox/flox}$ mice after Ad-Cre microinjection into this nucleus (Figure 1A). In contrast, there was no difference in the expression levels of ObR in the adjacent ventromedial or lateral hypothalamic nuclei between ObR$^{flox/flox}$ mice that received Ad-Cre versus Ad-GFP (Figure 1A).

As expected, in ObR$^{flox/flox}$ mice that did not receive adenovirus microinjection into the ARC (referred to as naïve), leptin administration caused a robust increase in phospho-Stat3 immunostaining in the mediobasal hypothalamus including the ARC (Online Figure III). Microinjection of Ad-Cre into the ARC of ObR$^{flox/flox}$ mice substantially reduced leptin-induced activation of Stat3 in this nucleus, but not in the adjacent nuclei (Figure 1B and 1C). Of note, GFP expression was restricted to the ARC (Figure 1B). Microinjection of Ad-GFP alone was associated with normal leptin-induced activation of ARC Stat3, indicating that viral microinjections do not alter ObRb signaling. Additionally, we found no difference in the effect of leptin treatment (60 μg, twice daily for 3 days) on food intake and body weight between littermate C57BL/6J mice (no floxed ObR allele) that had undergone bilateral ARC-specific microinjection of Ad-Cre versus mice without microinjections (Online Figure IV). These data strongly support the conclusion that viral injection into the ARC does not, by itself, affect ObRb signaling.

Loss of ARC ObR Abrogates the Thermogenic Sympathetic Effects of Leptin

In naïve ObR$^{flox/flox}$ mice, intravenous administration of leptin (120 μg) resulted in a significant ($P<0.001$ versus vehicle) increase in BAT SNA (268±45%, Figure 2B). Similarly, leptin administration caused a robust increase in BAT SNA (275±77%) in ObR$^{flox/flox}$ mice that received Ad-GFP microinjection into the ARC (Figure 2A and 2B). In striking contrast, Ad-Cre microinjection into the ARC of ObR$^{flox/flox}$ mice significantly ($P<0.001$) attenuated BAT SNA response to leptin (-26±15%). The BAT SNA response to leptin in ObR$^{flox/flox}$ mice that underwent Ad-Cre micro-

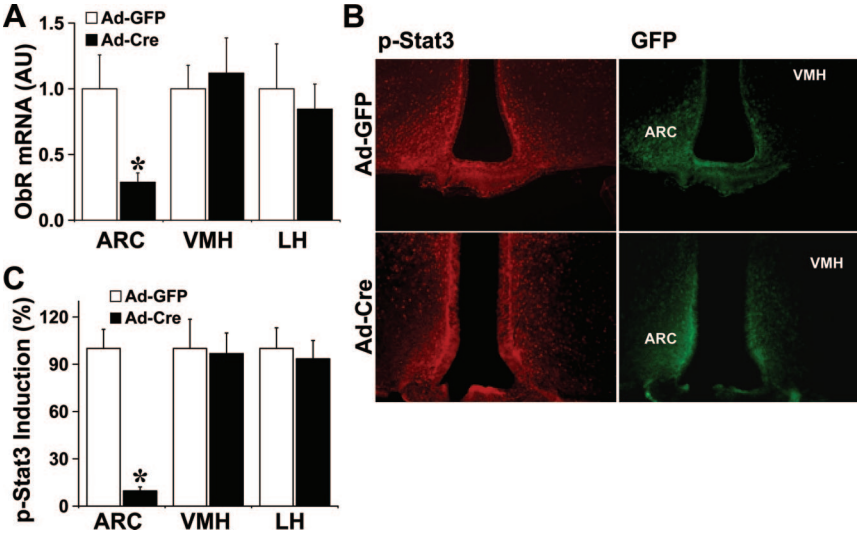

Figure 1. ARC-specific deletion of ObR in ObR$^{flox/flox}$ mice after Ad-Cre microinjection into this nucleus as determined by quantitative RT-PCR analysis of ObR expression (A) and leptin-induced increase in phospho-Stat3 in the ARC and adjacent ventromedial (VMH) and lateral hypothalamic (LH) nuclei (B and C). *$P<0.05$ vs Ad-GFP; n=4 to 5 per group.

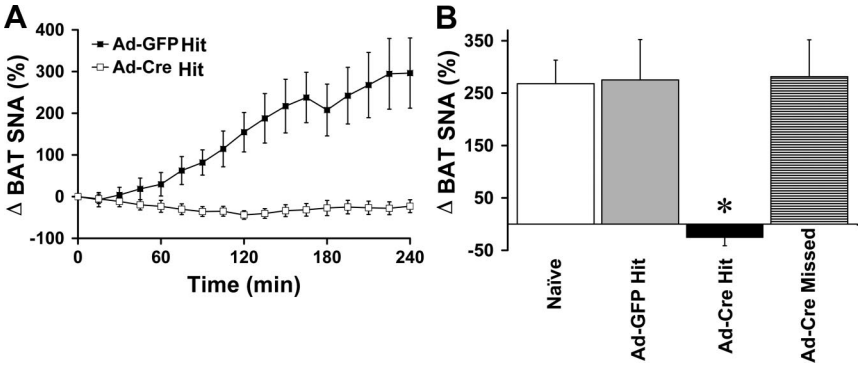

Figure 2. Deletion of ObR from the ARC abolishes leptin-induced BAT sympathetic activation. Time course **(A)** and average of last hour **(B)** of BAT SNA responses to intravenous leptin (120 μg) in ObR$^{flox/flox}$ mice after Ad-GFP vs Ad-Cre microinjections that "hit" or "missed" the ARC. *$P<0.001$ vs other groups; n=8 per group.

injection where the injection missed the ARC did not differ from leptin-induced increases in BAT SNA in naïve ObR$^{flox/flox}$ mice or after Ad-GFP microinjection (Figure 2B).

Next, we assessed the metabolic implications of impaired leptin-induced SNA to thermogenic BAT following deletion of ARC ObR. Leptin treatment induced substantial weight loss in ObR$^{flox/flox}$ mice that received Ad-GFP in the ARC (Online Figure V). This response to leptin was significantly ($P<0.01$) attenuated in the ObR$^{flox/flox}$ mice that received Ad-Cre in the ARC. There was a trend for an attenuated leptin-induced weight loss in the ObR$^{flox/flox}$ mice that received Ad-Cre in which we missed the ARC, but this was not statistically significant ($P=0.08$).

ARC ObR Is Necessary for Leptin-Induced Renal Sympathetic Activation

Intravenous administration of leptin (120 μg) caused a robust increase in renal SNA in naïve ObR$^{flox/flox}$ mice (287±47%; Figure 3B). Microinjection of Ad-GFP into the ARC of ObR$^{flox/flox}$ mice did not alter the renal SNA response to leptin, as indicated by the comparable increase in renal SNA (321±79%) following intravenous leptin (120 μg) (Figure 3A). In striking contrast, ObR$^{flox/flox}$ mice that have undergone Ad-Cre microinjection into the ARC had a significantly ($P<0.001$) blunted renal SNA response to leptin (15±20%). When the Ad-Cre microinjections missed the ARC, the renal SNA response (282±48%) to leptin was similar to the responses obtained in the naïve ObR$^{flox/flox}$ mice or after Ad-GFP microinjection (Figure 3B).

In line with our previous data in anesthetized mice,[3] leptin (as compared with vehicle) caused no significant change in arterial pressure or heart rate in naïve ObR$^{flox/flox}$ mice or after microinjection of Ad-GFP or Ad-Cre (data not shown).

ARC ObR Mediates Renal Sympathetic Activation to Leptin in Obesity

To examine the involvement of the leptin receptor in the ARC in the preserved renal SNA response to leptin in obesity, we studied the effect of deleting the ARC ObR on leptin-induced renal sympathetic activation in diet-induced obese (DIO) ObR$^{flox/flox}$ mice. High-fat feeding for 10 weeks caused obesity in ObR$^{flox/flox}$ mice, as indicated by the significantly ($P<0.05$) increased body weight and fat mass (Online Figure VI).

Consistent with our previous finding,[3] ICV injection of leptin caused comparable ($P=0.49$) increase in renal SNA in lean (108±30%) and DIO (96±28%) ObR$^{flox/flox}$ mice that underwent Ad-GFP microinjection into the ARC (Figure 4A and 4B). In contrast, Ad-Cre microinjection into the ARC of the DIO ObR$^{flox/flox}$ mice abolished the renal SNA response to ICV leptin (−16±11%; Figure 4A and 4B). As above, the renal SNA response to leptin was preserved in the DIO ObR$^{flox/flox}$ mice where the Ad-Cre microinjection missed the ARC (Figure 4B).

Analysis of hemodynamic parameters, measured under anesthesia during the SNA studies, revealed that relative to lean controls, DIO ObR$^{flox/flox}$ mice had significantly ($P=0.02$) elevated arterial pressure (Figure 4C). Of note, using radiotelemetry, we have previously shown similar arterial pressure elevation in DIO C57Bl/6J mice.[3] Interestingly, Ad-Cre microinjection into the ARC of obese ObR$^{flox/flox}$ mice eliminated the higher arterial pressure when the ARC was "hit," but not when this nucleus was "missed" (Figure

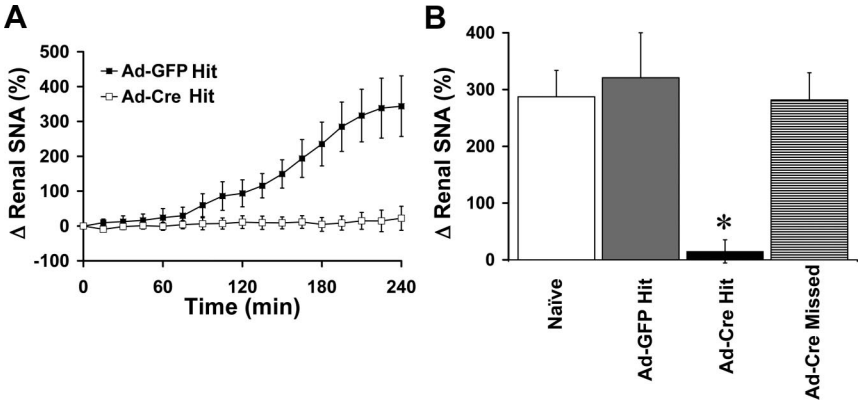

Figure 3. Deletion of ARC ObR abrogates leptin-induced renal sympathetic activation. Time course **(A)** and average of last hour **(B)** of renal SNA responses to intravenous leptin (120 μg) in ObR$^{flox/flox}$ mice after Ad-GFP vs Ad-Cre microinjections that "hit" or "missed" the ARC. *$P<0.001$ vs other groups; n=8 per group.

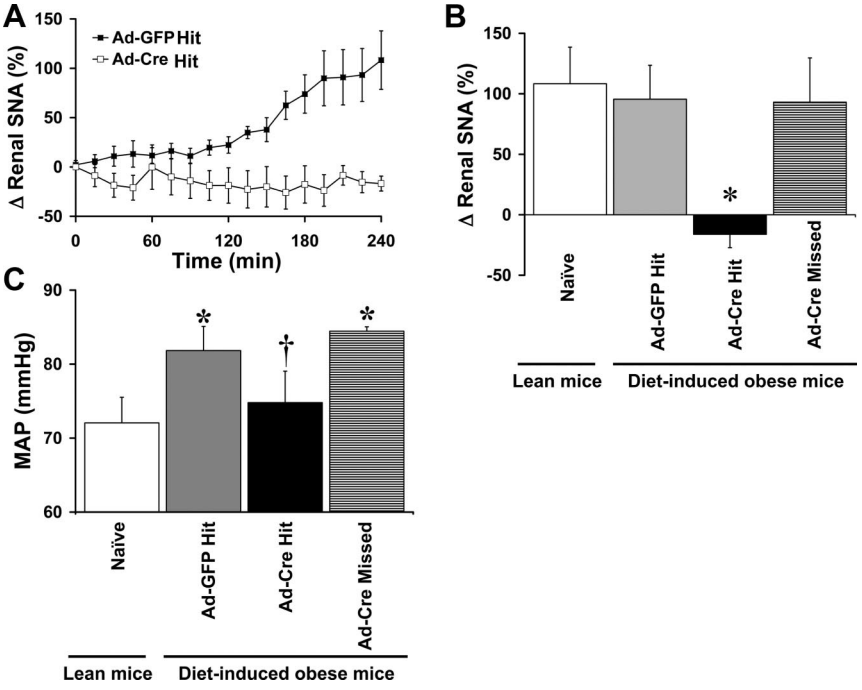

Figure 4. ARC ObR mediates the preserved leptin-induced renal sympathetic activation and arterial pressure elevation in obesity. A, Time course of renal SNA response to ICV leptin (2 μg) in DIO ObR$^{flox/flox}$ mice after Ad-GFP vs Ad-Cre microinjections that hit the ARC. **B and C,** Average of last hour of renal SNA responses to leptin **(B)** and baseline mean arterial pressure (MAP) **(C)** in naïve lean and DIO ObR$^{flox/flox}$ mice after Ad-GFP vs Ad-Cre microinjections that "hit" or "missed" the ARC. *$P<0.05$ vs naïve lean; †$P<0.05$ vs obese-Ad-GFP; n=5 per group.

4C). No significant differences in heart rate were detected among the various groups of ObR$^{flox/flox}$ mice (data not shown).

Discussion

The major finding from this study was the demonstration that leptin receptors in the hypothalamic ARC are necessary for leptin-induced increases in BAT and renal sympathetic discharge. Deleting leptin signaling in the ARC is sufficient to abolish leptin-induced sympathetic activation to both BAT and kidney. In addition, we show that leptin receptors in the ARC are critical for the preserved action of leptin on renal SNA and elevated arterial pressure in obesity. These data demonstrate that the ARC is an important site for the sympathetic effects of leptin in physiological and pathological states.

Our finding that deletion of the ARC leptin receptors abrogates leptin-induced sympathetic activation to BAT demonstrates the significance of leptin signaling in the ARC for the control of thermogenesis. This finding extends previous studies demonstrating that electrolytic lesioning of the ARC blunted the BAT sympathetic activation induced by systemic administration of leptin[2] and that direct injection of leptin into the ARC increased BAT SNA.[9]

Abolition of the renal SNA response to leptin following ARC-specific deletion of the leptin receptor is also consistent with our previous finding that microinjection of leptin into the ARC increased renal SNA and arterial pressure.[9] Despite the resistance to the anorectic and weight-reducing effects of leptin, DIO mice have intact renal sympathetic activation to leptin, which translates into a preserved leptin-induced increase in arterial pressure.[3] We found that ARC leptin receptors are critical in mediating the preserved leptin-induced renal sympathetic activation and for maintaining elevated blood pressure in dietary obesity. These findings represent major progress in our understanding of obesity-associated leptin resistance that occurs in the ARC and should facilitate the search for the mechanisms that account for selectivity in leptin resistance. In contrast to the prevailing view, our data indicate that in obesity, leptin receptor–containing neurons in the ARC are not uniformly resistant to leptin. The ARC neurons that mediate the renal SNA and cardiovascular effects leptin appear to escape leptin resistance. Additional studies will be required to reveal the identity of these neurons.

In conclusion, our data demonstrate the importance of ARC leptin receptors in mediating the SNA responses to leptin and in the adverse sympathoexcitatory effects of leptin in obesity.

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Disclosures

None.

References

1. Zhang Y, Proenca R, Maffei M, Barone M, Leopold L, Friedman JM. Positional cloning of the mouse obese gene and its human homologue. *Nature*. 1994;372:425–432.
2. Rahmouni K, Haynes WG. Leptin and the cardiovascular system. *Recent Prog Horm Res*. 2004;59:225–244.
3. Rahmouni K, Morgan DA, Morgan GM, Mark AL, Haynes WG. Role of selective leptin resistance in diet-induced obesity hypertension. *Diabetes*. 2005;54:2012–2018.

4. Schwartz MW, Woods SC, Porte D Jr, Seeley RJ, Baskin DG. Central nervous system control of food intake. *Nature*. 2000;404:661–671.
5. Morton GJ, Niswender KD, Rhodes CJ, Myers MG Jr, Blevins JE, Baskin DG, Schwartz MW. Arcuate nucleus-specific leptin receptor gene therapy attenuates the obesity phenotype of Koletsky (fa(k)/fa(k)) rats. *Endocrinology*. 2003;144:2016–2024.
6. Coppari R, Ichinose M, Lee CE, Pullen AE, Kenny CD, McGovern RA, Tang V, Liu SM, Ludwig T, Chua SC Jr, Lowell BB, Elmquist JK. The hypothalamic arcuate nucleus: a key site for mediating leptin's effects on glucose homeostasis and locomotor activity. *Cell Metab*. 2005;1:63–72.
7. Grill HJ. Distributed neural control of energy balance: contributions from hindbrain and hypothalamus. *Obesity*. 2006;14:216–221.
8. Myers MG, Munzberg H, Leinninger GM, Leshan RL. The geometry of leptin action in the brain: more complicated than a simple ARC. *Cell Metab*. 2009;9:117–123.
9. Rahmouni K, Morgan DA. Hypothalamic arcuate nucleus mediates the sympathetic and arterial pressure responses to leptin. *Hypertension*. 2007;49:647–652.

Novelty and Significance

What Is Known?

- Leptin is an adipocyte-derived hormone that is crucial for the regulation of body weight.
- Activation of the sympathetic nervous system by leptin increases blood pressure.
- Obesity is associated with selective leptin resistance (eg, intact cardiovascular effects of leptin despite loss of its metabolic actions).
- Leptin receptors are located in different regions of the brain regions; highest levels are found in the hypothalamic arcuate nucleus.

What New Information Does This Article Contribute?

- Arcuate nucleus–specific disruption of leptin receptors abolishes the sympathetic effects of leptin and lowers elevated blood pressure.
- The arcuate nucleus mediates sympathetic and cardiovascular effects of leptin in obesity.

Identification of the neuroanatomical sites of leptin action on sympathetic traffic will enhance our understanding of the secondary cardiovascular complications of obesity. This study indicates that leptin receptors in the arcuate nucleus are essential for the sympathetic activation evoked by leptin and for mediating the preserved leptin-induced renal sympathetic activation and maintenance of elevated blood pressure in diet-induced obesity. Preserved sensitivity of cardiovascular effects of leptin in obesity, which is mediated by the arcuate nucleus, suggests that leptin resistance in this nucleus is not uniform. The finding narrows down the search for molecular mechanisms of selective leptin resistance associated with obesity.