**Adiponectin potentiates the acute effects of leptin in arcuate Pomc neurons**

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Adiponectin potentiates the acute effects of leptin in arcuate Pomc neurons

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ABSTRACT

Objective: Adiponectin receptors (AdipoRs) are located on neurons of the hypothalamus involved in metabolic regulation — including arcuate proopiocortin (Pomc) and Neuropeptide Y/Agouti-related peptide (NPY/AgRP) neurons. AdipoRs play a critical role in regulating glucose and fatty acid metabolism by initiating several signaling cascades overlapping with Leptin receptors (LepRs), However, the mechanism by which adiponectin regulates cellular activity in the brain remains undefined.

Methods: In order to resolve this issue, we utilized neuron-specific transgenic mouse models to identify Pomc and NPY/AgRP neurons which express LepRs for patch-clamp electrophysiology experiments.

Results: We found that leptin and adiponectin synergistically activated melanocortin neurons in the arcuate nucleus. Conversely, NPY/AgRP neurons were inhibited in response to adiponectin. The adiponectin-induced depolarization of arcuate Pomc neurons occurred via activation of Phosphoinositide-3-kinase (PI3K) signaling, independent of AMP-activated protein kinase (AMPK) activity. Adiponectin also activated melanocortin neurons at various physiological glucose levels.

Conclusions: Our results demonstrate a requirement for PI3K signaling in the acute adiponectin-induced effects on the cellular activity of arcuate melanocortin neurons. Moreover, these data provide evidence for PI3K as a substrate for both leptin and adiponectin to regulate energy balance and glucose metabolism via melanocortin activity.

Keywords Melanocortin; Obesity; Diabetes; Energy balance; Patch-clamp; Electrophysiology

1. INTRODUCTION

Obesity is characterized by a chronic imbalance between energy intake and expenditure [1]. Multiple factors including hormonal regulation, fuel availability, and behavior contribute to energy utilization [2]. In the central nervous system, neuropeptide Y/agouti-related peptide (NPY/AgRP) and proopiocortin (Pomc) neurons receive and integrate information about energy availability via circulating adipokines/hormones (e.g. leptin and adiponectin) [3–5]. Leptin receptors (LepRs) in arcuate Pomc neurons are required and sufficient for the proper regulation of energy balance and glucose homeostasis (including systemic insulin sensitivity and hepatic glucose production) [6]. Similarly, adiponectin receptors (AdipoRs) play a critical role in regulating glucose and fatty acid metabolism in peripheral tissues [7–9]. AdipoRs are also located in the central nervous system including neurons of the hypothalamus involved in metabolic regulation such as arcuate Pomc and NPY/AgRP neurons [10,11]. However, the effect of adiponectin in the brain to regulate energy balance has been contentious, with studies showing differential effects on food intake [10,12,13] while increasing energy expenditure [14]. Importantly, adiponectin may be beneficial in the absence of leptin [8]. Moreover, adiponectin potentiated the effects of leptin on thermogenesis, body weight, fat, and V02 utilization, effects dependent upon melanocortin activity [13]. These data support an important regulation of metabolism...
by adiponectin which requires activity within the central nervous system to potentially melanocortin neurons. Together, these observations highlight a potential melanocortin-dependent compensatory/additive role for AdipoRs in the absence/presence of leptin. LepRs and AdipoRs initiate several overlapping signaling cascades including Janus kinase 2/Signal transducer and activator of transcription 3 (JAK2/STAT3), Insulin receptor substrate 1/2 (IRS1/2), Forkhead box protein O1 (FOXO1), AMP-activated protein kinase (AMPK), and phosphoinositol-3-kinase (PI3K) [15–18]. Of note, PI3K is required for the acute cellular effects of leptin in arcuate Pomc and NPY/AgRP neurons [5,19–22]. Additionally, PI3K in arcuate Pomc neurons is required for the acute effect of leptin to reduce food intake [20]. In contrast, much of the activity of adiponectin has focused on its cognate effects through AMPK resulting in little understanding of the signaling mechanisms including PI3K which may be required for the acute effects of adiponectin in the central nervous system.

In the current study, we tested the hypothesis that adiponectin and leptin may synergistically activate melanocortin neurons. To test this hypothesis we utilized transgenic and Cre-Lox technology to identify NPY/AgRP and Pomc neurons which express LepRs. We found that leptin and adiponectin modulate neuronal excitability of melanocortin neurons in an additive manner, an activity that requires PI3K signaling. Together, these data provide evidence for PI3K as a substrate for both leptin and adiponectin to regulate metabolism via melanocortin activity.

2. METHODS

2.1. Animals
All mice were housed under standard laboratory conditions (12 h on/off; lights on at 7:00 a.m.) and temperature-controlled environment with water and food available ad libitum. All experiments were performed in accordance with the guidelines established by the National Institute of Health Guide for the Care and Use of Laboratory Animals and approved by the University of Texas Institutional Animal Care and Use Committee. To identify POMC neurons with or without leptin receptors, we generated Pomc-hrGFP::LepR-cre::tdtomato (PLT) mice as previously described [23] anatomically restricted to the arcuate nucleus of the hypothalamus. NPY/AgRP neurons anatomically restricted to the arcuate nucleus of the hypothalamus were targeted from NPY-hrGFP mice [24].

2.2. Electrophysiology studies

2.2.1. Slice preparation and whole-cell recordings
Brain slices were prepared from young adult male mice (5–7 weeks old) as previously described [20,23,25]. Briefly, male mice were deeply anesthetized with i.p. injection of 7% chloral hydrate and transcardially perfused with a modified ice-cold artificial CSF (ACSF) (described below). The mice were then decapitated, and the entire brain was removed and immediately submerged in ice-cold, carbogen-saturated (95% O2 and 5% CO2) ACSF (126 mM NaCl, 2.5 mM CaCl2, 1.25 mM MgCl2, 1.25 mM NaH2PO4, 26 mM NaHCO3, and 5 mM glucose). For some experiments, slices were perfused with ACSF containing 0.5 mM or 3 mM glucose by replacing glucose with equiosmolar amounts of sucrose [26]. Coronal sections (250 µm) were cut with a Leica VT1000S Vibratome and then incubated in oxygenated ACSF at room temperature for at least 1 h before recording. The slices were bathed in oxygenated ACSF (32°C–34°C) at a flow rate of ~2 ml/min. All electrophysiology recordings were performed at room temperature.

The pipette solution for whole-cell recording was modified to include an intracellular dye (Alexa Fluor350 hydrazide dye) for whole-cell recording: 120 mM K-glucuronate, 10 mM KCl, 10 mM HEPES, 5 mM EGTA, 1 mM CaCl2, 1 mM MgCl2, and 2 mM MgATP, 0.03 mM Alexa Fluor 350 hydrazide dye (pH 7.3). Epifluorescence was briefly used to target fluorescent cells, at which time the light source was switched to infrared differential interference contrast imaging to obtain the whole-cell recording (Zeiss Axioskop FS2 Plus equipped with a fixed stage and a QuantEM:512SC electron-multiplying charge-coupled device camera). Electrophysiological signals were recorded using an Axopatch 700B amplifier (Molecular Devices), low-pass filtered at 2–5 kHz, and analyzed offline on a PC with pCLAMP programs (Molecular Devices). Membrane potential and firing rate were measured by whole-cell current clamp recordings from Pomc neurons in brain slices. Recording electrodes had resistances of 2.5–5 MΩ when filled with the K-glucuronate internal solution. Input resistance was assessed by measuring voltage deflection at the end of the response to a hyperpolarizing rectangular current pulse steps (500 ms of −10 to −50 pA). Leptin (100 nM; provided by A.F. Parlow, through the National Hormone and Peptide Program), adiponectin (10 nM; Phoenix pharmaceuticals), AdipoRon (0.1 µM–10 µM; Tocris) were added to the ACSF for specific experiments. Solutions containing drug were typically perfused for 5 min. A drug effect was required to be associated temporally with peptide application, and the response had to be stable within a few minutes. A neuron was considered depolarized or hyperpolarized if a change in membrane potential was at least 2 mV in amplitude. Neurons were voltage-clamped at −75 mV (for excitatory postsynaptic currents) and −15 mV (for inhibitory postsynaptic currents). Frequency and peak amplitude were measured by using the Mini Analysis program (Synaptosoft, Inc.)

2.3. Drugs
TTX, SKF96365, and 2-APB were obtained from Tocris; LY294002 (10 µM) was obtained from Calbiochem. All solutions were made according to manufacturer’s specifications. Stock solutions of SKF96365, 2-APB, were made by dissolution in DMSO (Sigma). The concentration of DMSO in the external solution was <0.1%. Stock solutions of leptin were made by dissolution in D-PBS (Gibco). Stock solutions of TTX were made by dissolution in de-ionized water.

2.4. Analysis and statistics
A value of twice the mean peak-to-peak noise level for a given recording in control solutions was used as the detection limit for minimal PSC amplitude (i.e. typically 5–10 pA). For spontaneous EPSCs and IPSCs (sEPSCs and sIPSCs), at least 2 min of activity was examined to identify effects on amplitude and frequency distributions. Effects on spontaneous PSC frequency before and during drug application were analyzed within a recording using the Kolmogorov–Smirnov (K–S) test (a nonparametric, distribution-free goodness-of-fit test for probability distributions). Typically, 100–300 events were compared for each condition. Pooled results from responding cells were analyzed using a paired t test; multiple groups of cells were compared using ANOVA. Proportions of responding cells from different groups were analyzed using a χ² test of independence. Effects on spontaneous PSC amplitude were analyzed using a paired two-tailed t test. Results are reported as the mean ± SEM unless indicated otherwise; where n represents the number of cells studied. Significance was set at p < 0.05 for all statistical measures.
Figure 1: Adiponectin depolarizes LepR-positive Pmc neurons via activation of a mixed cation conductance. (A–E) Brightfield illumination (A) of Pmc-hrGFP::LepR-cre::tdtomato neuron from PLT mice. (B) and (C) show the same neuron under FITC (hrGFP) and Alexa Fluor 594 (tdtomato) illumination. Complete dialysis of Alexa Fluor 350 from the intracellular pipette is shown in (D) and merged image of targeted Pmc neuron (E). Arrow indicates the targeted cell. Scale bar = 50 μm. (F) Current-clamp record demonstrates a Pmc-
3. RESULTS

3.1. Adiponectin depolarizes LepR-positive Pomc neurons via activation of a mixed cation conductance

In order to better identify adiponectin-induced effects on the membrane potential of Pomc neurons, whole-cell patch-clamp recordings were performed on Pomc neurons, which either express or do not express leptin receptors (LeptR) from Pomc-hrGFP::LepR-cre::tdTomato (PLT) mice [23,25]. Pomc neurons targeted for recording in the current study were anatomically restricted to the arcuate nucleus of the hypothalamus. Alexa Fluor350 hydrazide dye was added to the intracellular pipette solution for real-time confirmation that hrGFP-positive neurons were targeted for recording (Figure 1A—E). A majority of LepR-positive Pomc neurons were depolarized by local perfusion of adiponectin in a dose dependent manner (10 nM, 67%, ±5.9 ± 0.2 mV; resting membrane potential: −48.5 ± 2.4 mV; n = 12; Figure 1F,P and Supplemental Figure 1A,B). Application of adiponectin on LepR-positive Pomc neurons increased the action potential firing frequency (1.8 ± 0.5 Hz in normal ACSF; 5.1 ± 0.7 Hz in adiponectin; n = 7 Supplemental Figure 1B).

Adiponectin dose-dependently excites or inhibits neurons of the subfornical organ and the paraventricular hypothalamus [27–29]. However, the conductance involved has largely remained undefined. In order to better define the conductance, which contributes to the adiponectin-induced depolarization of LepR-expressing Pomc neurons, rectangular current steps (400 ms; ±50 pA) were applied to the membrane in order to obtain a current—voltage (I–V) plot. The depolarization was concomitant with a decrease in input resistance (27%, 1073 ± 54 MΩ for control, n = 6; 785 ± 95 MΩ for adiponectin, n = 6, Figure 1G). Subsequent linear regression analysis revealed the reversal potential of the adiponectin-induced depolarization to be −26.0 mV ± 5.1 mV (n = 6; Figure 1H), suggesting an activation of a mixed-cation conductance.

Importantly, pretreatment with TTX (2 μM) failed to abrogate adiponectin-induced depolarization of Pomc neurons (60%, ±5.8 ± 0.4 mV; resting membrane potential: −46.3 ± 1.2 mV; n = 5; Figure 1I,P), which supports a direct membrane depolarization independent of action-potential-mediated synaptic transmission. Analogous results were obtained using the synthetic small molecule agonist of the adiponectin receptors (AdipoRon). In particular, AdipoRon depolarized LepR-expressing Pomc neurons in a dose dependent manner (0.1–10 μM 5.6 ± 0.5 mV; resting membrane potential: −46.1 ± 2.2 mV; n = 4; Figure 1J,K,P, and Supplemental Figure 1B). Similar to the effects of adiponectin, the AdipoRon-induced depolarization was concomitant with an increase in the frequency of action potentials (5 μM: 2.0 ± 0.4 Hz in normal ACSF to 5.4 ± 0.9 Hz in AdipoRon, n = 4, Supplemental Figure 1B). Conversely, the majority (10 of 11) of LepR-negative Pomc neurons were unaffected by adiponectin (−0.4 ± 0.9 mV, 11 of 12 neurons, Figure 1L,M). The remaining 1 neuron was hyperpolarized (−6 mV). Together, these data suggest that the excitatory effects of adiponectin on Pomc neurons is largely selective to those which express leptin receptors (Figure 1M,P).

3.2. Adiponectin potentiates the leptin-induced excitation of Pomc neurons

Adiponectin rescued many of the metabolic deficits observed in ob/ob mice; an effect that required melanocortin signaling [8,13,30]. Moreover, adiponectin potentiates leptin’s effect to stimulate thermogenesis, fatty acid oxidation, and insulin sensitivity [13]. Thus, we hypothesized that leptin and adiponectin may have additive effects on the excitability of arcuate Pomc neurons. Consistent with previous reports, nearly 83% of LepR-positive Pomc neurons were depolarized and increased action potential firing in response to leptin superfusion (100 nM, −6.2 ± 0.3 mV; resting membrane potential: −50.0 ± 2.9 mV; n = 6; Figure 1N,P and Supplemental Figure 1B). Notably, combined administration with adiponectin (10 nM) and leptin (100 nM) resulted in a larger depolarization of LepR-positive Pomc neurons (55%; ±8.9 ± 0.4 mV; resting membrane potential: −48.0 ± 2.3 mV; n = 12; p < 0.05; Figure 1O,P, and Supplemental Figure 1B). Similarly, application of AdipoRon combined with leptin also resulted in a robust depolarization of LepR-positive Pomc neurons (75%; ±9.2 ± 1.0 mV; resting membrane potential: −47.4 ± 3.7 mV; n = 4; p < 0.05; Figure 1P and Supplemental Figure 1B). Combined administration of adiponectin or AdipoRon with leptin also resulted in the potentiation of the action potential frequency (for adiponectin + leptin: 2.0 ± 0.6 Hz in normal ACSF to 6.7 ± 1.0 Hz in adiponectin + leptin, n = 8; for AdipoRon + leptin: 1.7 ± 0.2 Hz in normal ACSF to 6.2 ± 1.2 Hz in AdipoRon + leptin, n = 3; Supplemental Figure 1B). These data suggest that adiponectin can enhance the leptin-induced excitatory actions on LepR-positive Pomc neurons (Figure 1P).

Glucose concentrations within the brain have reported between 0.1 and 5 mM [31–33]. Our data suggest that adiponectin excites Pomc neurons in 5 mM glucose. In order to examine the effect of adiponectin throughout this physiological range, extracellular glucose concentrations were lowered to 3 mM or 0.5 mM. Interestingly, adiponectin and leptin-induced depolarizations were preserved in both 3 mM and 0.5 mM extracellular glucose concentrations. For 3 mM glucose, adiponectin depolarized 57% of neurons recorded (+5.8 ± 0.4 mV; from resting membrane potential of −52.4 ± 1.7 mV, n = 7; Supplemental Figure 2). Similarly leptin depolarized 57% of neurons recorded (+5.5 ± 0.5 mV; from resting membrane potential of −51.4 ± 2.5 mV, n = 7; p < 0.05; Supplemental Figure 2). Moreover, combined administration with adiponectin (10 nM) and leptin (100 nM) led to an enhanced depolarization of LepR-positive Pomc neurons (67% of neurons targeted; +8.8 ± 0.3 mV; from resting membrane potential of −53.6 ± 4.8 mV; n = 6; p < 0.05; Supplemental Figure 2). Similar results were obtained at 0.5 mM extracellular glucose with adiponectin depolarizing 83% of LepR-positive Pomc neurons (+6.7 ± 1.0 mV; from a resting membrane.
adiponectin-stimulated depolarization of POMC neurons. (D) Pretreatment with the non-selective AMPK inhibitor, compound C, does not abrogate the adiponectin-induced depolarization of POMC neurons (2-APB: 100%; 0.5 μM). Pretreatment with the PI3K antagonist (LY294002, 50 μM) failed to alter the adiponectin-induced depolarization of POMC neurons (58%; 6.0 ± 0.4 mV; resting membrane potential: −47.1 ± 2.7 mV; n = 12; Figure 2B,E). These data suggest that adiponectin activates Pomc neurons that express leptin receptors independent of AMPK activity. Interestingly, pretreatment with the PI3K antagonist (LY294002, 10 μM) abrogated the adiponectin-induced depolarization of LepR-positive Pomc neurons (100%; 0.5 ± 0.4 mV; resting membrane potential: −47.8 ± 3.3 mV; n = 9; Figure 2C,E). Leptin depolarized Pomc neurons via a PI3K-dependent activation of TrpC channels [20,37]. Given the analogous effects of adiponectin observed in the current study, we hypothesized that adiponectin might also depolarize Pomc neurons via TrpC channels. Pretreatment with the non-selective TrpC channel inhibitors, 2-APB and SKF 96365, prevented the adiponectin-induced depolarization of LepR-positive Pomc neurons (2-APB: 100%; +1.5 ± 0.5 mV; resting membrane potential: −46.1 ± 3.3 mV; n = 17; SKF: +1.7 ± 0.7 mV; resting membrane potential: −48.1 ± 4.3 mV; n = 5; Figure 2D,E). Together, these data highlight a common mechanism in the metabolism of Pomc neurons where adiponectin stimulates depolarization via a PI3K-dependent activation of TrpC channels.
which adiponectin and leptin activate arcuate Pomc neurons (Figure 2E).

3.4. Adiponectin effects synaptic input organization of hypothalamic Pomc neurons

Pomc neurons receive and integrate afferent inputs from a variety of nuclei within and outside of the hypothalamus. In order to better describe the effects of adiponectin on the synaptic organization of arcuate Pomc neurons in the absence of voltage fluctuations, neurons were voltage-clamped at −75 mV (for excitatory postsynaptic currents) and −15 mV (for inhibitory postsynaptic currents). With a similar time course to the adiponectin-induced membrane depolarization, adiponectin (10 nM) decreased the frequency of spontaneous IPSCs in 10 of 14 LepR-positive Pomc neurons from 1.13 ± 0.36 Hz in normal ACSF to 0.4 ± 0.1 Hz in adiponectin (64.6% decrease from control; p < 0.05; paired t test; n = 10; Figure 3A−C). The amplitude of spontaneous IPSCs was decreased from 26.798 ± 3.91 pA in normal ACSF to 16.765 ± 1.99 pA in adiponectin (61.6% decrease from control; p < 0.05; paired t test; n = 10; Figure 3C). Oppositely, adiponectin (10 nM) resulted in a slight but not statistically significant decrease in spontaneous EPSCs (Figure 3D−F). The amplitude

![Figure 3](image-url)
of spontaneous EPSCs was also unaffected by adiponectin administration (15.39 ± 0.98 pA in normal ACSF; 14.92 ± 0.98 pA in adiponectin n = 11; Figure 3F).

3.5. Adiponectin inhibits the excitability of hypothalamic NPY/AgRP neurons

NPY/AgRP neurons, which commonly co-express the inhibitory neurotransmitter GABA, are immediately adjacent to arcuate Pomc neurons and are a known source for inhibitory synaptic input to arcuate Pomc neurons. Given that adiponectin selectively suppressed inhibitory synaptic activity to arcuate Pomc neurons, we examined whether adiponectin may suppress the excitability of NPY/AgRP neurons. NPY/AgRP neurons anatomically restricted to the arcuate nucleus of the hypothalamus were targeted from NPY-hrGFP mice [24]. Real-time visualization under fluorescence microscopy and Alexa Fluor350 hydrazide dye were used to dialyze neurons recorded (Figure 4A–D).

Consistent with the decreased inhibitory synaptic activity to Pomc neurons, adiponectin perfusion hyperpolarized a subpopulation of NPY neurons (33.3%, 8 of 24 NPY neurons, −8.9 ± 1.3 mV; resting membrane potential: −44.3 ± 1.4 mV; n = 24; Figure 4E,H). Additionally, current–voltage relationships demonstrated a reversal potential of −80.3 mV ± 5.7 mV (n = 5; Figure 4F).

Subsequent application of tolbutamide (200 μM) following adiponectin administration completely reversed the hyperpolarization of LepR-positive NPY neurons induced by adiponectin (n = 5). Furthermore, pretreatment of the slice with TTX (2 μM) failed to diminish the adiponectin-induced hyperpolarization of NPY/AgRP neurons (42.8%, −7.4 ± 0.4 mV resting membrane potential: −43.9 mV ± 2.3 mV; n = 7; Figure 4G,H), which indicates a direct membrane hyperpolarization independent of action-potential-mediated synaptic transmission. Overall, these data indicate that adiponectin hyperpolarizes NPY neurons via activation of an ATP-sensitive potassium conductance (KATP) independent of action-potential-mediated synaptic transmission (Figure 4H).

4. DISCUSSION

The predominant effect of adiponectin in Pomc-expressing neurons of the arcuate nucleus was excitatory, acting to depolarize neurons, decrease inhibitory synaptic inputs, and increase their responsiveness. Leptin potentiated these effects on Pomc cellular activity such that the leptin and adiponectin combined resulted in an enhanced
A novel observation in the current study is that adiponectin directly regulates the cellular activity of arcuate Pomc neurons that do not express leptin receptors. Previous reports have suggested that adiponectin failed to alter the expression of various hypothalamic neuropeptides including Pomc and NPY/AgRP [13]. However, altered neuropeptide expression is a poor correlate of cellular activity. Similarly, adiponectin failed to induce expression of the immediate early gene c-fos in the arcuate nucleus suggesting that adiponectin failed to activate these neurons [13]. Although, c-fos has been associated with increased cellular excitability, this is at best an indirect measure of activity. In the current study, the adiponectin-induced activation of Pomc neurons occurred almost exclusively in leptin receptor expressing neurons. This is in agreement with the ability of adiponectin to potentiate the effects of leptin on thermogenesis, insulin sensitivity, body weight, fat, and V02 utilization [13]. Moreover, adiponectin and leptin together enhanced UCP-1 mRNA expression in BAT [13]. Notably, melanocortin activity is an important regulator of the aforementioned alterations in energy balance and glucose homeostasis [38]. Accordingly, agouti (Ay/a) mice failed to respond to adiponectin or leptin, indicating the melanocortin pathway may be a common target [13]. We also found that adiponectin directly inhibited NPY/AgRP neurons further providing an indirect disinhibition (activation) of arcuate Pomc neurons. Although adiponectin suppressed NPY/AgRP neuronal activity, Pomc neurons that do not express leptin receptors failed to depolarize in response to adiponectin. This may suggest that the decreased GABA-ergic activity from NPY/AgRP neurons alone was not sufficient to modify the activity of these neurons. Alternatively, adiponectin may decrease inhibition of NPY/AgRP neurons to specific subsets of arcuate Pomc neurons as yet undefined. The current data may suggest that this subset would include Pomc neurons that express leptin receptors as Pomc neurons that do not express leptin receptors failed to respond to adiponectin. Although an intriguing hypothesis, this requires future investigation. Together, these data detail a cellular mechanism which supports an additive effect of leptin and adiponectin in melanocortin-expressing neurons.

To date, research on adiponectin receptors has focused primarily on the requirement of AMP-activated protein kinase (AMPK) signaling in peripheral tissues to regulate most aspects of metabolism [39]. Additional mechanisms widely studied include the effects of adiponectin and its receptors on sphingolipid metabolism, specifically with respect to lowering cellular ceramide levels [40]. However, there is a lesser-appreciated role for adiponectin’s pleiotropic effects to activate pathways, which may either converge on or diverge from AMPK, including PI3K [41]. The adiponectin-induced activity of PI3K might also be interwined with AMPK as inhibition of PI3K can abolish adiponectin-stimulated AMPK activation [42,43]. Unfortunately, little is known about the role of PI3K signaling in response to adiponectin in the central nervous system. However, the PI3K pathway in arcuate Pomc neurons has been demonstrated to be an important regulator of both thermogenesis and hepatic glucose production [20,44]. In particular, Pomc specific deficiency for the PI3K catalytic subunit p110alpha led to a hypometabolic phenotype contributing to increased weight gain and adiposity [20,44]. Alternatively, increased PI3K activity in Pomc neurons improved insulin sensitivity, whereas decreased PI3K signaling resulted in impaired glucose regulation [20,44]. Notably, these observations of PI3K signaling in Pomc neurons phenocopy the acute effect of adiponectin in the hypothalamus [13]. Accordingly, we found that the adiponectin-induced activation of arcuate Pomc neurons required PI3K signaling, independent of AMPK. Although currently unclear, these data support, at least in part, a cellular/molecular mechanism for adiponectin to alter energy balance and glucose metabolism via PI3K activity in melanocortin neurons.

Another salient finding is the temporal relationship of the electrophysiological responses observed. Similar to leptin, adiponectin activated arcuate Pomc neurons within minutes of application. These data suggest that adiponectin may parallel leptin activity in Pomc neurons; rapidly altering energy and glucose homeostasis via Pomc neuronal activity. In particular, previous work suggested that reduced PI3K activity in Pomc neurons alone was sufficient to block acute effect of leptin to activate Pomc neurons and reduce food intake up to 24 h after administration [20]. This supports a potential role of PI3K-dependent changes in cellular activity alone in Pomc neurons contributes to energy balance. Moreover, reports have suggested that chemogenic and/or optogenetic stimulation of arcuate Pomc neurons reduces food intake within a range of a few hours to days after continual stimulation [45—47]. Given that arcuate Pomc cells have varying projection

**Figure 5:** Schematic representation of the effects of adiponectin on hypothalamic Pomc and NPY/AgRP neurons. Adiponectin binds AdipoRs on Pomc neurons to depolarize Pomc neurons via PI3K mediated activation of TrpC channels independent of AMPK activity. Adiponectin also inhibits NPY/AgRP neurons via a PI3K-dependent activation of KATP channels. This results in decreased inhibitory postsynaptic currents to neighboring Pomc neurons. Together, this proposed model may provide a molecular/cellular mechanism by which adiponectin decreases food intake and increases energy expenditure via integrating its effects on hypothalamic Pomc and NPY/AgRP neurons.
patterns, which may separately influence feeding or autonomic behaviors [48–52], this highlights a need to understand which subpopulation of Pomc neurons are activated in order to better delineate the temporal regulation of metabolism. Together, while we cannot exclude other cellular processes; an adiponectin-induced activation of arcuate Pomc neurons may be sufficient to drive beneficial effects on energy balance and glucose homeostasis. It should be noted that while adiponectin receptors are widely distributed in the CNS. A rise in CSF levels was detected after iv administration of adiponectin [13]. Peripheral administration of adiponectin stimulates cell signaling pathways in the hypothalamus of mice to improve metabolism [10,13]. Moreover, we have previously demonstrated the presence of the lower molecular weight complexes of adiponectin in the CSF at approximately 0.1% of serum concentration [53], which is within the concentration range of adiponectin used in the current study. However, others have failed to identify adiponectin BBB transport mechanisms and suggest that the brain endothelium may play an important role in relaying peripheral adiponectin signaling [54,55]. In the current study, we have not delineated whether peripheral or central administration of adiponectin is sufficient to initiate these responses. Rather, these data demonstrate that activation of adiponectin receptors (be it physiologically or pharmacologically) may positively regulate melanocortin activity.

5. CONCLUSIONS

In summary, leptin and adiponectin synergistically activate arcuate Pomc neurons via a PI3K-dependent activation of TrpC channels, independent of AMPK activity. These data may provide evidence for PI3K as a substrate for both leptin and adiponectin to regulate energy balance and glucose metabolism via melanocortin activity.

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CONFLICT OF INTEREST

None declared.

APPENDIX A. SUPPLEMENTARY DATA

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.molmet.2016.08.007.

REFERENCES


