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AdipoR1 and 2 are expressed on warm sensitive neurons of the hypothalamic preoptic area and contribute to central hyperthermic effects of adiponectin

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Abstract

Adiponectin can act in the brain to increase energy expenditure and reduce body weight by mechanisms not entirely understood. We found that adiponectin type 1 and type 2 receptors (AdipoR1 and AdipoR2) are expressed in warm sensitive neurons of the hypothalamic preoptic area (POA) which play a critical role in the regulation of core body temperature (CBT) and energy balance. Thus, we tested the ability of adiponectin to influence CBT in wild-type mice and in mice deficient for AdipoR1 or AdipoR2. Local injection of adiponectin into the POA induced prolonged elevation of core body temperature and decreased respiratory exchange ratio (RER) indicating that increased energy expenditure is associated with increased oxidation of fat over carbohydrates. In AdipoR1 deficient mice, the ability of adiponectin to raise CBT was significantly blunted and its ability to decrease RER was completely lost. In AdipoR2 deficient mice, adiponectin had only diminished hyperthermic effects but reduced RER similarly to wild type mice. These results indicate that adiponectin can contribute to energy homeostasis by regulating CBT by direct actions on AdipoR1 and R2 in the POA.

Keywords

Adiponectin; Adiponectin receptor; Temperature; Homeostasis; Preoptic area; Warm sensitive neurons

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1. INTRODUCTIONS

Adiponectin is an important regulator of energy homeostasis proposed to be involved in metabolic and vascular diseases (Dridi and Taouis, 2009; Kadowaki and Yamauchi, 2005; Kadowaki et al., 2006; Kadowaki et al., 2007). Produced and secreted by adipose tissue, adiponectin regulates glucose and fatty acid metabolism in tissues such as muscle and liver (Berg et al., 2002), as well as insulin-sensitizing effects (Berg et al., 2001; Fruebis et al., 2001; Yamauchi et al., 2001). In rodents, peripheral administration of adiponectin enhanced both fatty acid oxidation and glucose uptake in muscle, and reduced hepatic glucose production (Berg et al., 2001; Fruebis et al., 2001; Qi et al., 2004; Shklyaev et al., 2003; Tomas et al., 2002; Yamauchi et al., 2001). Conversely, adiponectin deficiency leads to glucose intolerance, insulin resistance, dyslipidemia and increased susceptibility to vascular injury and atherosclerosis (Kubota et al., 2002; Maeda et al., 2002; Nawrocki et al., 2006).

The two structurally similar adiponectin receptors (AdipoR1 and AdipoR2) were found to be differentially expressed in different brain regions including the hypothalamus (Coope et al., 2008; Fry et al., 2006; Guillod-Maximin et al., 2009; Hoyda and Ferguson, 2010; Kos et al., 2007; Kubota et al., 2007; Psilopanagioti et al., 2009; Thundyil et al., 2011; Yamauchi et al., 2003). Bjursell generated and characterized mice null for AdipoR1 and R2 and concluded that while both receptors are involved in the regulation of energy metabolism, they mediate opposite effects (Bjursell et al., 2007).

Intracerebroventricular (icv) administration studies recapitulate the peripheral effects of adiponectin and suggested that this adipokine may have a central role in modulating energy homeostasis by influencing temperature and/or nutrient homeostasis (Qi et al., 2004). Qi and colleagues demonstrated that icv injection of adiponectin elevated energy expenditure by increasing the expression of the uncoupling protein 1 (UCP1) in brown adipose tissue (BAT), elevating colonic CBT and reducing body weight (Qi et al., 2004). A different group found that icv injection reduced food intake in fasted animals by 40% suggesting adiponectin may reduce energy intake acting as pro-anorexigenic. These effects were mediated by AdipoR1 and involved the classical insulin and leptin signaling pathways (Coope et al., 2008). Conversely, Kubota and colleagues reported that icv administration of the hexameric form of adiponectin increased food intake during re-feeding and that intravenous injection of full length adiponectin lowered oxygen consumption and BAT UCP1 expression, reducing energy expenditure without affecting body weight (Kubota et al., 2007). Finally, in experiments on agouti yellow mice, icv injection of adiponectin did not alter food intake or body weight (Masaki et al., 2003). The reason for these differences may, at least partially, be the type of adiponectin utilized and the fact the icv route of administration may target adiponectin receptors differently in distinct hypothalamic nuclei. Therefore mechanisms of central adiponectin action remain to be determined.

With the ultimate goal to measure the effects of adiponectin on energy homeostasis we tested the central effects of adiponectin by injection in the preoptic area (POA), a region containing warm (and cold) sensitive neurons critical in the regulation of temperature homeostasis and heat dissipation (Boulant, 2000; Nakayama et al., 1963). Core body temperature, respiratory exchange ratio, motor activity and energy expenditure were measured and compared in mice null for adiponectin receptor 1 or 2 and in their wild type littermates.

2. RESULTS

Warm sensitive neurons express adiponectin receptors type 1 and type 2

To investigate the possibility that adiponectin may be centrally active in modulating CBT, the presence of adiponectin receptors was investigated by RT-PCR in the hypothalamic POA as well as in POA warm sensitive neurons (WSN), which were identified by electrophysiological recording (Tabarean et al., 2005). Analysis on tissue dissected from naïve C57Bl/6 mice demonstrated that AdipoR1 as well as AdipoR2 receptors were expressed in the POA (Fig 1A). Single cell PCR and sequencing also showed that 1 and 2 out of 8 WSN contained mRNA for AdipoR1 and AdipoR2, respectively (Fig 1A). The expression of AdipoR1 was also demonstrated on POA neurons identified by retrograde transport from the raphe pallidus (RPa) (Fig 1B). Taken together these data suggested the possibility that AdipoR1 and R2 may influence CBT by modulating the activity of WSN.

Out of 10 temperature insensitive neurons tested none expressed Adipo R1 and 2 expressed AdipoR2 (Supplemental figure S1).

Hyperthermic action of adiponectin injection into the POA

Injection of adiponectin (12.5 ng in 0.5 μ l) into the POA of wild-type C57Bl/6 mice was followed by elevation of CBT while no increase was observed in animals receiving the same volume of artificial cerebrospinal fluid (ACSF) utilized as vehicle (Fig 2A, D). The dose used in the experiments was chosen as the lowest dose giving maximal effects in a dose curve response assessing the effects of injecting 1.25, 6.25, 12.5, 25 or 50 ng of adiponectin (Supplemental figure S2). Injections were performed at 4 hrs into the light part of the day when animals are normally not active and were associated with a typical stress induced CBT elevation that was similar in animals treated with vehicle or adiponectin. However, as CBT returned to basal level in animals receiving vehicle, it remained on average $\sim 1.2^{\circ}\text{C}$ higher in adiponectin treated mice for about 6 hrs as the average temperature for this period was $36.11 \pm 0.11^{\circ}\text{C}$ and $37.31 \pm 0.19^{\circ}\text{C}$ for vehicle and adiponectin, respectively (Fig 2A) ($n=6$; $p<0.01$). Difference in CBT disappeared at onset of darkness when animals became active and vehicle treated mice increased their CBT, and remained similar for the rest of the recording that was carried out for additional 12 hrs until termination of dark cycle. This effect of adiponectin was specific for the POA, since injections above the anterior commissure, at approximately 1 mm from the POA, didn't increase CBT.

The hyperthermic effects of adiponectin were then studied in mice lacking the type 1 or type 2 adiponectin receptors (AdipoR1 or AdipoR2 null) (Fig 2B-D). Comparative analysis demonstrated that basal CBT profile over the 24 hrs recording did not differ substantially in wild type, AdipoR1 or AdipoR2 null mice; average temperature during the light part of the day was $36.11 \pm 0.11^{\circ}\text{C}$, $36.21 \pm 0.13^{\circ}\text{C}$ and $36.19 \pm 0.18^{\circ}\text{C}$, respectively (Fig 2D) ($n=6$; NS). Small significant differences following vehicle treatment were observed in both AdipoR1 and AdipoR2 null mice at onset of dark and into the dark cycle when CBT was lower than in wild type mice (Figure 2D).

During the same period when CBT was elevated in adiponectin treated wild type mice, local injection of adiponectin into the POA caused only an average of 0.37°C increase of CBT in AdipoR1 null mice compared with the 1.2°C seen in wild type mice. The average temperature after adiponectin injection was $36.58 \pm 0.13^{\circ}\text{C}$ while in vehicle treated AdipoR1 null mice was $36.21 \pm 0.13^{\circ}\text{C}$ ($p<0.05$) (Fig 2B). The same treatment induced a 0.6°C CBT elevation in AdipoR2 null animals. The average temperature after injection was $36.16 \pm 0.18^{\circ}\text{C}$ and $36.79 \pm 0.15^{\circ}\text{C}$ for vehicle and adiponectin treated AdipoR2 null mice, respectively (Fig 2C).

Motor activity during the light part of the day did not change significantly in any of the conditions and groups tested (Fig 3). Consistent to what was previously reported (Bjursell et al., 2007) some significant differences were observed during the dark part of the day. Injection of adiponectin reduced motor activity during some part of dark but calculation of the area under the curve showed this was on average significant only in wt and AdipoR1 null (area under the curve in wild type, AdipoR1 null and Adipo2 null mice was 114.37 ± 7.27 , 112.15 ± 17.13 , 114.68 ± 119.38 in the saline group and 78.05 ± 7.59 , 79.55 ± 16.45 , 116.42 in the adiponectin group, respectively) (Fig 3A).

Effects of Adiponectin on RER and Heat production

In wild type mice, measurement of respiratory exchange ratio (RER) demonstrated that POA injection of adiponectin was followed by lowering of RER compared with vehicle treated mice ($p < 0.05$), indicating a switch from glucose metabolism to increased fatty acid oxidation (Fig.4A). The lower RER upon POA adiponectin injection compared with vehicle treated mice was prolonged and still present during the dark part of day when differences in CBT were not significant. The degree of differences fluctuated and appeared to be minimal during the transition phase from light to darkness with the largest difference during the dark phase to a maximum of 0.93 ± 0.017 vs 0.86 ± 0.012 in vehicle and adiponectin treated mice, respectively ($p < 0.05$). No significant differences were observed in the RER at any time during the 24 hrs recording when comparing AdipoR1 mice treated with vehicle or adiponectin. On the other hand, differences similar to those observed in wild type mice were recorded when comparing AdipoR2 mice. RER was 0.964 ± 0.022 and 0.876 ± 0.019 in AdipoR2 null mice treated with vehicle or adiponectin, respectively (Fig. 4C).

We also calculated the effects of adiponectin on heat production expressed as Kcal/hr/gr thus normalizing them per body weight. Consistently with the effects on RER adiponectin increased heat in wt mice. These effects were absent in AdipoR1 deficient mice and were reduced in mice lacking AdipoR2. Contrary to what was observed for RER and similarly to what was seen for CBT, these effects disappeared within 24 hrs of injection (Supplemental figure S4).

Feeding and body weight

Animals used for experiments were aged matched. Their body weight was similar in all but AdipoR1 deficient mice that were 10% heavier (Supplemental figure 5). As mentioned above, some groups reported that icv injection of adiponectin affected feeding (Coope et al., 2008; Kubota et al., 2007). In our experiments we found that a small, but not significant, increase of food intake (Supplemental figure S5). This may be due to the fact that the POA lies relatively far from the ventricles, also indicating that the primary action of adiponectin in the POA is hyperthermic.

3. DISCUSSION

We report that expression of AdipoR1 and AdipoR2 was detected in warm sensitive neurons (WSN) of the POA of the anterior hypothalamus, a region primarily responsible for the control of CBT. In addition, we found that local injection of adiponectin into the POA induced a robust hyperthermia that was associated with reduction of RER indicative of an increased oxidation of triglycerides over carbohydrates. These actions were mediated primarily by AdipoR1 and, to a minor degree, by AdipoR2.

Although adiponectin was not found to cross an intact blood brain barrier (Neumeier et al., 2007; Pan et al., 2006), a possible central action of adiponectin is suggested by the finding that it can be found in the cerebrospinal fluid (CSF) in both humans and rodents (Kos et al.,

2007; Kubota et al., 2007; Kusminski et al., 2007; Neumeier et al., 2007; Qi et al., 2004), and that both AdipoR1 and AdipoR2 are expressed in several brain regions including hypothalamic areas known to regulating nutrient homeostasis such as the arcuate, the paraventricular nucleus and the lateral hypothalamus (Coope et al., 2008; Fry et al., 2006; Guillod-Maximin et al., 2009; Hoyda and Ferguson, 2010; Kos et al., 2007; Kubota et al., 2007; Psilopanagioti et al., 2009; Thundyil et al., 2011; Yamauchi et al., 2003). Assessment of the effect of icv injection confirmed that adiponectin contributes to energy homeostasis and also suggested the possibility that it may do so by influencing food intake as well as CBT (Coope et al., 2008; Fry et al., 2006; Guillod-Maximin et al., 2009; Hoyda et al., 2007; Kos et al., 2007; Kubota et al., 2007; Psilopanagioti et al., 2009). Control of CBT is a tightly regulated physiological process and an important component of energy homeostasis: over several months, sustained 0.5°C reduction of CBT resulted in 15% change of energy expenditure leading to a 10% increase of body weight in mice (Conti et al., 2006). The finding here that injection of adiponectin into the POA induced a 1.2°C increase of CBT associated to changes in RER is indicative of the significance of this action for energy homeostasis.

Preoptic area WSN project distally to the dorsal raphe, either directly or via the dorsomedial hypothalamus, and can activate shivering as well as non shivering thermogenesis via brown adipose tissue activation and vasoconstriction (for comprehensive review see (Morrison and Nakamura, 2011)). Although the present study did not directly determine which thermoregulatory mechanisms were at play, shivering was not observed and the decrease in RER indicated increased oxidation of fatty acids suggesting activation of brown adipose tissue (BAT). This was similar to what was previously demonstrated by us on the central hyperthermic action of insulin (Sanchez-Alavez et al., 2010). Interestingly, RER remained lower even after no differences in CBT were observed between animals injected with vehicle or adiponectin. It should be noted that during the dark part of the day the lack of difference in CBT may be due to reduced motor activity observed in animals receiving adiponectin. However, RER was lower also during the following light period when no differences in MA or CBT were recorded. The reason for these prolonged effects of adiponectin on RER remain to be determined. Among the possibilities is that fat utilization remained elevated to replenish utilized BAT.

AdipoR1 and AdipoR2 were both found in a percentage of POA warm sensitive neurons (WSN) while only AdipoR2, but not AdipoR1, was expressed by a fraction of temperature insensitive neurons. None of the neurons tested showed co-expression of AdipoR1 and AdipoR2. The components of the neuronal circuitry proposed to regulate temperature homeostasis in the POA include both warm sensitive and temperature insensitive neurons. In this model, WSN excite heat loss effector neurons and inhibit heat production while temperature insensitive neurons act in the opposite manner (Boulant, 2000; Hammel, 1965). Our retrograde transport tracing demonstrated that AdipoR1 is expressed in neurons projecting to the dorsal raphe. Since in the POA, AdipoR1 was found only in WSN, we hypothesize that these cells are indeed temperature sensitive. However, this could not be conclusively demonstrated since to date there exists no specific marker for WSN. Similarly, although AdipoR2 was found in a fraction of temperature insensitive neurons, we could not prove that these were cells actually component of the temperature homeostasis circuitry. Despite these limitations, our data collectively indicate that adiponectin may affect CBT by direct modulation of the activity POA neurons.

In the hypothalamus, adiponectin was reported to have hyperpolarizing or depolarizing effects depending on the neuronal type or on the type of currents they can regulate (Hoyda et al., 2007; Hoyda and Ferguson, 2010). We previously showed that in the POA insulin exerted hyperthermic effects by inducing hyperpolarization and reducing firing rates of

WSN (Sanchez-Alavez et al.). It is thus possible that adiponectin may have similar effects on WSN inhibiting their activity thus triggering thermogenesis and limiting heat loss. With the data collected it is more difficult to predict the effects adiponectin may have on the activity of POA temperature insensitive neurons expressing AdipoR2 and how these, together with WSN, may influence thermogenesis or heat dissipation. The future determination of these actions and interactions may be of particular interest also because AdipoR1 and AdipoR2 were reported that mediate opposite effects on energy metabolism (Bjursell et al., 2007). Here, however, we found only that the contribution of AdipoR1 and AdipoR2 to CBT regulation differed only in intensity but not in direction. In fact, AdipoR1 appeared to play a prominent role in mediating hyperthermic effects as, in its absence, these were reduced by 70% and the effects on RER were blunted. In response to adiponectin, AdipoR2 null mice still showed increase of CBT equivalent to 50% that of wild type mice and had the same reduction of RER. This suggested that, overall in the POA, Adipo R1 and R2 might both mediate the hyperthermic effects of adiponectin.

The data presented here further demonstrates that hypothalamic WSN represents a heterogeneous population of neurons that may express different sets of receptors. Thus, in addition to their ability to respond to changes in local temperature, distinct subsets of temperature sensitive neurons may be specialized to respond to biochemical signals that can influence CBT. This indicates that these cells can provide a functional link between temperature and nutrient homeostasis, the two primary components of energy homeostasis, and that adiponectin may be one of the signals contributing to this regulation.

4. METHODS AND MATERIALS

Animals and Reagents

All procedures were approved by the Institutional Animal Care and Use Committee of the Scripps Research Institute. Animals were maintained on regular chow (Harlan Teklad LM-485 Diet 7012 (65% carbohydrate [kcal], 13% fat, metabolizable energy 3.41 kcal/g). Access to food and water was ad libitum and the light:dark cycle was 12:12 hrs with lights on at 6 a.m. AdipoR1 and AdipoR2 null mice were obtained from Deltagen (San Carlos, CA) (MGI:3604517). All animals were on C57B6 background. Male 3-4 month old mice were used in all experiments and wild-type littermates for each group were used ; n= 6 animals per groups.

Recombinant Mouse globular adiponectin (gAd, Catalog Number: 1119AC) was purchased from R&D systems (Minneapolis, MN, USA) and was resuspended in aCSF to give a solution of pH 7.2 – 7.3.

Slice preparation and patch clamp recording

The brain was quickly removed from C57BL/6 mice at 22–35 days old and submerged in ice-cold oxygenated (95% O₂/5% CO₂) normal aCSF; composition was (in mmol/l) NaCl 126, KCl 3.5, CaCl₂ 2, MgSO₄ 1, NaH₂PO₄ 1.25, NaHCO₃ 26, and glucose 10 (pH 7.4). Coronal slices (350 µm thick) containing the POA were cut by a microslicer (Vibratome). Standard tight-seal recordings were performed in current clamp mode (I-fast) with an Axopatch 200B amplifier to record spontaneous action potentials. The external recording solution was aCSF. In some recordings 9 mmol/l glucose was replaced by 9 mmol/l mannitol. The pipette solution used was (in mmol/l) 130 K-gluconate, 10 KCl, 10 HEPES, 2 MgCl₂, 0.5 EGTA, 2 ATP, and 1 GTP (pH 7.4). The electrode resistance after backfilling was 2–4 MΩ. The temperature of the external solution was controlled with an HCC-100A heating/cooling bath temperature controller (Dagan Corporation). Recordings were digitized using a Digidata 1320A interface and analyzed using Pclamp9 (Axon Instruments, Union

City, CA) software package. After establishing whole-cell configuration the spontaneous activity of the neuron was recorded for 2–4 min to determine its control behavior (at 36–37°C), after which it was tested for temperature sensitivity with a temperature cycle of at least 35–39°C. Warm sensitive or non sensitive neurons were identified following the same criteria utilized previously (Tabarean et al., 2005). The firing rate was determined for each 10-s interval and plotted against the temperature using Sigmaplot software. The thermal coefficient is defined as the slope of the linear regression of the firing rate plotted as a function of temperature. This plot was determined over a temperature range of $\geq 3^{\circ}\text{C}$ in which a neuron was most sensitive. A warm-sensitive neuron is defined as having a thermal coefficient $\geq 0.8 \text{ impulses} \cdot \text{s}^{-1} \cdot \text{C}^{-1}$. At the end of the recording the cytoplasm was harvested by applying negative pressure through the patch pipette under visual inspection.

Single cell PCR

Single warm sensitive neurons (WSN) were subject to linear RNA amplification according to the Eberwine method and as previously described (Eberwine, 2001; Eberwine and Crino, 2001; Sanchez-Alavez et al., 2010). cDNA was prepared by SuperScript III First-Strand Synthesis System (Invitrogen). The following primers were used: AdipoR1F-acg tcc agg ctt caa ata act t, AdipoR1R-gca gtg ata gca ggt ttc tt tt and AdipoR2F-cac aaa ctc tta ggg aac cga at, AdipoR2R-cac cga agg gaa gtt act gg designed to the 3' ends of the transcripts. The identity of the amplified targets was confirmed by sequencing.

Immunohistochemistry

Immunohistochemistry and retrograde staining were carried out on brain slices as previously described by us (Sanchez-Alavez et al., 2010). Briefly, Texas Red–conjugated Dextran beads (Invitrogen, Carlsbad, CA; 1 mg/ml) were injected into the raphe pallidus (RPa) (AP –6.12 mm, Lat 0.0 mm; V + 5.8 mm from the surface of the brain). One week after surgery animals were perfused via the ascending aorta with 10 ml of 0.9% NaCl followed by 50 ml ice-cold 4% (wt/vol) paraformaldehyde in 0.16 mol/l PBS. Brains were removed and postfixed for 2 hrs in the same fixative, then transferred to PBS containing 20% (wt/vol) sucrose and stored overnight at 4°C. Sections from the POA were cut on a cryostat (Leica) at 40 μm and transferred to individual wells containing PBS. Staining for Adiponectin receptor 1 was carried out with rabbit anti-AdipoR1 (1:200, Ab Phoenix #G-001-45) overnight, rinsed in PBS, and incubated with a Donkey anti-rabbit Alexa488 (1:200; Invitrogen) for 2 hrs at room temperature. Before mounting, sections were rinsed in PBS and incubated with 0.5 mol/l DAPI (Invitrogen) for 5 min. Confocal images were captured using a Zeiss laser confocal microscope using Zen 2009 Zeiss software suite (Carl Zeiss, Thornwood, NY). All serial optical images (0.3 μm interval step slices) were imported and spatially reassembled using Imaris (Bitplane, Saint Paul, MN) to generate a three-dimensional representation of the tissue and then maximum projected for two-dimensional images (gray scale panels) in Image Pro Plus (Media Cybernetics, Bethesda, MD).

Telemetry and POA injections

For the telemetry study, 3–6 month old male wild-type C57BL/6 mice, AdipoR1 null, AdipoR2 null and wild type littermates mice anesthetized with isoflurane (induction 3–5%, maintenance 1–1.5%) and surgically implanted with radio telemetry devices (TA-F20, Data Sciences, Inc., St Paul, MN) into the peritoneal cavity for core body temperature (CBT) and motor activity (MA) measurement as previously described (Sanchez-Alavez et al., 2010).

For brain injections, mice were first subject to stereotaxic placement of a guide cannula (26 Ga, 10 mm length) as previously described (Sanchez-Alavez et al., 2010). Coordinates for the POA were: anterior–posterior from bregma, +0.34 mm; lateral, at bregma; and ventral, 4.4 mm; for cannula placement above the anterior commissure: anterior–posterior from

bregma, +0.34 mm; lateral, at bregma; and ventral, 3.4 mm. Artificial cerebro-spinal fluid as vehicle, 12.5 ng mouse globular adiponectin (1119-Ac12, R&D, MN, USA) were injected directly to the target area through an implanted cannula using an injector (33 Ga, 10.5 mm length) connected to plastic tubing and a microsyringe in a volume of 0.5 μ l over a period of 5 min to allow diffusion of the drugs. The actual site of injection was determined by histology post mortem.

Mice were individually housed in a Plexiglas cage in a room maintained at $25 \pm 0.5^{\circ}\text{C}$ on a 12:12 hr light–dark cycle (lights on at 6:00 a.m.) with ad libitum access to food and water and allowed to recover from surgery for 2 weeks. The cages were positioned onto the receiver plates (RPC-1; Data Sciences) and radio signals from the implanted transmitter were continuously monitored and recorded. CBT and MA (number of horizontal movements) were continuously monitored with a fully automated data acquisition system (Dataquest A.R.T., Data Sciences, Inc., St Paul, MN) for at least 72 hrs for baseline levels of temperature, making sure that no ongoing febrile response confounds the results. 30 seconds before sacrifice mice were injected with dye 1% Pontamine Sky Blue to mark the site of injection.

Calculations were carried out with values collected 1 hr after injection in order to exclude the stress-associated increase of CBT that typically occur in all conditions around time of treatment. The levels of serum adiponectin were measured to ensure that central injection did not cause leakage and consequent increase of peripheral adiponectin: no changes were observed in any of the experiments (data not shown).

Indirect calorimetry

Indirect calorimetry was performed as we previously described (Sanchez-Alavez et al., 2010)(Sanchez-Alavez et al.) using the Comprehensive Lab Animal Monitoring System (CLAMS; Columbus Instruments, Columbus, OH) on acclimated (for 2-3 days), singly-housed mice using a computer controlled, open-circuit system (Oxymax System, Columbus Instruments, Columbus, OH). Respiratory exchange ratio (RER) was calculated as VCO_2/VO_2 . Data were recorded under ambient room temperature clamped at 25°C , beginning from the onset of the light cycle.

Heat production (kcal/hr/gr) was calculated using the formula = $\text{CV} * \text{VO}_2 * 1\text{L}/1000\text{ ml} * 1\text{kg}/1000\text{g}$ where CV represents the caloric value ($\text{CV} = 3.815 + 1.232 \times \text{RER}$) as described in (Gaidhu et al., 2011).

Statistics

Factorial analyses of variance (ANOVA) or Student's t-tests were used for comparisons involving >2 levels or exactly 2 levels, respectively. One-way ANOVA was used to determine differences in the mean between multiple groups. All results are expressed as mean $\pm$ SE.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

We found that the adiponectin receptors type 1 and type 2 receptors are expressed in preoptic hypothalamic neurons including warm sensitive neurons critical for the regulation of temperature homeostasis. When injected into the preoptic area of wild type mice adiponectin induced a robust and prolonged increase of core body temperature associated with an increased utilization of fatty acids over glucose. These effects were nearly absent in mice lacking the adiponectin type 1 receptor and greatly diminished in mice lacking the type 2 receptor. Our findings demonstrate that adiponectin can influence metabolism and energy homeostasis centrally by increasing energy dissipation via regulation of core body temperature via type 1 as well as type 2 receptors.

Fig 1. Expression of adiponectin type 1 and type 2 receptor in WSN and POA

(A) Ethidium bromide stained agarose gel of amplification products of PCR carried out on cDNA from the POA or from POA single WSNs subject to aRNA amplification. 1 out of 8 WSN expressed AdipoR1 transcript; 2 expressed AdipoR2 (B-G) Representative immunohistochemistry of adiponectin receptor 1 (green) (arrow) (B) in the POA after retrograde transport tracing with Texas red (red) (arrow) (C) and nuclear staining with DAPI (blue) (arrow) (D). Images were merged (E) and reconstructed in three dimension at same (arrow) (F) or at higher magnification (G).

Fig 2. Effects of adiponectin injection into the POA on CBT in wild type, AdipoR1 or AdipoR2 null mice

48 hrs profile of CBT recorded from wild type (A), AdipoR1 (B) null or AdipoR2 (C) null mice injected with adiponectin or aCSF used as vehicle (D) in the POA as indicated. Shaded area indicates dark part of the day. Injection was performed at time 4 hrs into the light part of the day (n= 6, p* < 0.05).

Fig 3. Effects of adiponectin injection into the POA on motor activity in wild type, AdipoR1 or AdipoR2 null mice

48 hrs profile of motor activity recorded from wild type (A), AdipoR1 (B) null or AdipoR2 (C) null mice injected with adiponectin or aCSF used as vehicle (D) in the POAs indicated. Shaded area indicates dark part of the day. Injection was performed at time 4 hrs into the light part of the day (n= 6, $p^* < 0.05$).

Fig 4. Effects of adiponectin injection into the POA on RER in wild type, AdipoR1 or AdipoR2 null mice

48 hrs profile of RER recorded from wild type (A), AdipoR1 (B) null or AdipoR2 (C) null mice injected with adiponectin or aCSF used as vehicle (D) in the POA as indicated. Shaded area indicates dark part of the day. Injection was performed at time 4 hrs into the light part of the day (n= 6, p* < 0.05).