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Downregulation of GPR83 in the hypothalamic preoptic area reduces core body temperature and elevates circulating level of adiponectin

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Abstract

Objective—The G protein-coupled receptor 83 (GPR83) was recently demonstrated in warm sensitive neurons (WSN) of the hypothalamic preoptic area (POA) that participate in temperature homeostasis. Thus, we investigated whether GPR83 may have a role in regulating core body temperature (CBT) by reducing its expression in the POA. D issipation of energy in the form of heat is the primary mode of energy expenditure in mammals and can ultimately affect energy homeostasis. Thus, we also measured the level of important regulators of metabolism.

Matherials/Methods—Downregulation of GPR83 was obtained by lentiviral short-hairpin RNAs (shGPR83) vectors designed and selected for their ability to reduce GPR83 levels in vitro. Mice received POA injection of shGPR83 or non-silencing vectors and were monitored for CBT, motor activity, food intake body weight and circulating levels of IGF-1, insulin, leptin and adiponectin.

Results—Down-regulation of GPR83 in the POA resulted in a small (0.15°C) but significant reduction of CBT during the dark/active cycle of the day. Temperature reduction was followed by increased body weight gain independent of caloric intake. shGPR83 mice also had increased level of circulating adiponectin (31916 ± 952 pg/ml vs. 23474 ± 1507 pg/ml, $p<0.01$) while no change was observed for insulin, IGF-1 or leptin.

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Conclusions—GPR83 may participate in central thermoregulation and the central control of circulating adiponectin. Further work is required to determine how GPR83 can affect POA WSN and what are the long term metabolic consequences of it down-regulation.

Keywords

lentivirus; RNAi; shRNA; knockdown; warm sensitive neurons; preoptic area; G-protein coupled receptor 83; thermoregulation; body temperature; adiponectin; leptin; insulin; IGF-1

INTRODUCTION

The G protein-coupled receptor 83 (GPR83) (also known as GIR, GPR72 or JP05) is an orphan receptor originally isolated as the glucocorticoid-induced receptor (GIR) in thymoma [1,2] and confirmed to be abundantly expressed in regulatory T (Treg) cells and the central nervous system [2, 3]. Four different GPR83 isoforms have been described in mice and only the most abundant isoform-1 has been so far demonstrated in humans [2, 4]. Isoform-1 is a 423 amino acid long functional protein, while isoform-2, the second most predominant isoform, lacks 42 amino acids of the third transmembrane domain and is therefore thought to be non-functional. The other two isoforms contain inserts of 68 amino acids (isoform-3) and 20 amino acids (isoform-4) both in the second cytoplasmic loop. The amino acid insertion of isoform-4 contains the last 20 amino acids found in the isoform-3 insertion [2]. To date, no known function has been attributable to isoform-3 but isoform-4 was shown to be involved in expression of regulatory T cells [4]. In fact, although GPR83-deficient mice had no abnormalities in Treg development [5, 6], over-expression of isoform-4 was shown to play a role in the induction of $CD25^+Foxp3^+$ Treg and to be instrumental for their suppressive action *in vivo* [4, 7].

While the biological function of GPR83 was so far tested primarily in the immune system, isoform-1 is also expressed in different brain regions where its physiological role remains to be determined. Localization and distribution of brain GPR83 transcript in several regions including the cortex, the hypothalamus, the thalamus, the hippocampus and the amygdala, suggested it may have a role in the regulation of emotions, as well as of cognitive and neuroendocrine functions [8-12]. In addition, the finding that GPR83 transcript in the prefrontal cortex was elevated by amphetamine and remained high for several days following cessation of treatment, indicated a possible involvement in neuroadaptation and reward [12]. Finally, a role of central GPR83 in participating to the changes in glucocorticoids levels observed during stress or disease was also proposed [8]. Interestingly, the effects of glucocorticoids on GPR83 might be cell type specific as one study found that dexamethasone reduced rather than increased GPR83 transcript in several brain regions [8].

Molecular profiling of single neurons also demonstrated that GPR83 was expressed in warm sensitive neurons (WSN) of the preoptic area of the anterior hypothalamus (POA), which are important regulators of temperature and energy homeostasis [13]. These specialized neurons participate in central thermoregulation responding to local temperature increase, pyrogens, as well as nutrient signals and can regulate the amount of energy expenditure by influencing heat dissipation [14-17]. Thus, we hypothesized that GPR83 may participate in the regulation of temperature and energy homeostasis. Because no natural ligand, agonist or antagonist for GPR83 is yet available, we initiated testing this hypothesis by local knockdown with lentivirus-expressed short-hairpin RNAs (shRNAs) directed against all isoforms of GPR83. Towards investigating the effects that altered temperature, or energy expenditure, may have on metabolism we also measured the level of the four major metabolic hormones IGF-1, insulin, leptin and adiponectin.

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MATERIALS AND METHODS

In vitro testing of shRNA

DNA for mouse GPR83 was synthesized at DNA2.0 (Menlo Park, CA, USA) and subcloned into the expression vector, pcDNA5FRT/TO (Invitrogen Corp., Carlsbad, CA, USA). Three shRNA hairpins, shRNAs for mouse GPR83 (V2LMM_56683, V2LMM_54869, V2LMM_51223) were purchased from Open Biosystems (Huntsville, AL, USA). Transfection quality DNA preps were produced for all plasmids using Promega Wizard Midiprep Kit (Promega Corp., Madison, WI, USA). TLA-HEK293T cells (Open Biosystems, Huntsville, AL, USA) were co-transfected with the GPR83 expression vector and one or all of the shRNAmirs using Fugene HD (Roche, Indianapolis, IN, USA) following the manufacturer's instructions with the following modifications. Cells were plated at a density of 100,000/well in a 24 well tissue culture dish in 0.5 ml growth medium (DMEM) (Invitrogen #11995) supplemented with 10% heat-inactivated Fetal Bovine Serum (FBS), 1% penicillin-streptomycin (Invitrogen Corp, Carlsbad, CA, USA) and incubated overnight at 37°C/5% CO_2 the day prior to transfection. The day of the transfections, 50 ng of the GPR83 expression plasmid was mixed with 450 ng of total shRNAmir and then brought to 25 μl with serum-free medium and then 1.5 μl of FuGene HD was added. The DNA:FuGene was mixed and allowed to sit at room temperature for 15-30 minutes prior to adding drop wise to the cells. The incubation continued for another 48 hours at which time doxycycline (1 μg/ml final concentration) was added for the expression of GPR83. The following day, RNA was isolated using the Qiagen RNeasy kit with on-column DNase treatment (Qiagen, Valencia, CA, USA).

Lentivirus generation and expression

Lentivirus was produced according to the manufacturer's protocol (Open Biosystems, Huntsville, AL, USA). Briefly, TLA-HEK293T cells were plated overnight (4×10^6 cells/T75 flask) at 37 °C/5% CO_2. The following day, the medium was changed to UltraCulture serum-free medium (Invitrogen Corp., Carlsbad, CA, USA) and transfected with a pool of three shRNAmirs for GPR83 and the lentivirus packaging mix using FuGene HD. The transfection was allowed to proceed for 48 hours at which time greater than 90% of the cells stained positive for green fluorescent protein (GFP). The supernatants were transferred to a 50 ml conical tube and spun at 3000rpm/10°C/30 minutes to remove debris. The clarified supernatants were then passed through a Millex HV 0.45um PVDF filter then transferred to Amicon Ultra15 (100 kDa) concentration column and spun at 1500 rpm/15°C/75 minutes. The final volume was approximately 140 μl. The virus was frozen at −80°C in 10 μl aliquots.

The titer of the concentrated virus was determined by the following protocol. TLA-HEK23T cells were plated at a cell density of 50,000 cells/well in 24-well poly-d-lysine tissue culture plates and incubated overnight at 37°C/5% CO_2. Two wells from every plate were used to determine the number of cells/well the following day and this number was used to calculate the virus titer. The remaining wells were used for a serial dilution of virus. The medium was removed and replaced with UltraCulture medium containing 2μl virus and polybrene (8 μg/ml) in a total of 50μl. Once the virus was added to the cells, the plates were spun at 2000 rpm/room temp/1 hour followed by 3 hour incubation at 37°C/5% CO_2. The virus was then aspirated and replaced with complete medium and the incubation continued for 48 hours at which time the percentage of GFP positive cells was determined by FACS analysis. The virus titer was calculated using the following equation: [%GFP/100 x average cells/well x 4 x dilution factor x 500 = 293T transducing units/ml]. Critical to the success of this endeavor was the ability to generate a very high virus titer. Based on some previously published work [18-21], a titer of at least 10^9 virus particles/ml was necessary so that 5×10^5 least virus

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particles could be injected into the POA in a volume of 0.5 μl. For the GPR83 shRNA lentivirus, a titer of 3.4×10^{9}/ml was generated and for the non-silencing control shRNA lentivirus a titer of 1.4×10^{10}/ml was generated (data not shown).

Quantitative RT-PCR

RNA isolated from either the POA or transfected HEK293T cells was reverse transcribed using Superscript III reverse transcriptase (Invitrogen Corp, Carlsbad, CA, USA) and random hexamers (Applied Biosystems, Foster City, CA, USA) following manufacturer's protocol in a 20 μl reaction. After the reverse transcription, the cDNA was treated with RNase H (Invitrogen Corp, Carlsbad, CA, USA) for 20 minutes at 37°C. To assess in vitro knockdown in the transfected 293T cells, quantitative PCR was performed on 2 μl of reverse transcription reaction using the Lightcycler FastStart Kit for SYBR Green (Roche, Indianapolis, IN, USA) and the following conditions (95°C/10 min followed by 40 cycles of 95°C/10 sec; 60°C/20 sec; 72°C/30 sec/single acquisition followed by a melt cycle of 95°C/10 sec; 65°C/30 sec; then increase to 95°C at a rate of 0.1°C/sec with a continuous acquisition). The primers for GPR83 were 5′-ATG AAG GTT CCT CCT GTC CTG C-3′ and 5′-GGC ATG TGT CAT GTC AGT CGC TT-3′and the primers for GAPDH were 5′-CCA CAG TCC ATG CCA TCA C-3′ and 5′-TCC ACC ACC CTG TTG CTG TA-3′. Initial studies were done to make sure that cycling conditions caused a linear rate of amplification of a doubling/cycle. GPR83 expression was calculated relative to glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA. To assess in vivo knockdown in the POA, Taqman primer/probe (Invitrogen, Carlsbad, CA, USA) sets for either GPR83 or GAPDH were utilized following the manufacturer's protocol.

Animals

All procedures were approved by the Institutional Animal Care and Use Committee of the Scripps Research Institute and were carried out on 3- to 5-month-old male C57BL/6J mice. Animals were purchased from The Jackson Labs at 8 weeks of age and were maintained on Research Diets, Inc. D12492 (carbohydrate 20% kcal, fat 60%, protein 20%, metabolizable energy 5.2 kcal/g). Access to food and water was ad libitum, and the light:dark cycle was 12:12 h with lights on at 7:00 a.m. Body weights and food intake were measured weekly.

Telemetry

Male mice were anesthetized with isofluorane (induction 3-5%, maintenance 1-1.5%) and surgically implanted with radiotelemetry devices (TA-F20, Data Sciences, St. Paul, MN) into the peritoneal cavity for core body temperature (CBT) and locomotor activity (LA) evaluation. Following surgical implantation and appropriate wound closure, the animals were allowed to recover for 2 weeks and then submitted for freely moving telemetry recordings. Mice were individually housed in a Plexiglas cage in a room maintained at 25 ± 0.5°C. 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 LA were continuously monitored with a fully automated data acquisition system (Dataquest ART, Data Sciences, St. Paul, MN). Recordings were made for at least 5 days before treatment to ascertain that baseline levels of temperature were stable and that no ongoing febrile response confounds the results.

Lentiviral Injections

Injections of 500,000 lentivirus particles expressing shRNA were administered directly to the POA (anterior-posterior [AP] from bregma = −0.38 mm anterior to the bregma, lateral [Lat] =midline, ventral [V] = 4.2 mm, injector 26 GA) connected to plastic tubing and a

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microsyringe (10 μl) in a volume of 0.5 μl over a period of 5 min to prevent reflux and allow diffusion.

Hormone and adipokine measurements

Circulating levels of serum, insulin, leptin, and IGF-1, IL-6 and TNF were determined on serum using the multiplex system (Millipore, St. Charles, MO, USA) (Inter-assay: <12%; Intra-assay: <5%). Adiponectin was determined by ELISA (Millipore, St. Charles, MO, USA) (inter-assay: 1.4–10.8 %; intra-assay: 3.8–8.2%). Sensitivity for leptin and adiponectin was 16.71 pg/ml and 0.78 ng/ml, respectively.

Statistical analyses

Analysis was carried out comparing animals treated with non-silencing shRNA (n = 8) or silencing shRNA for GPR83 (n = 5). After removing time points where temperatures were not considered physiological (i.e., under 34°C and over 39°C), the average temperatures for each animal were calculated for each dark and light cycle (i.e., 7:00 p.m. to 6:55 a.m. and 7:00 a.m. to 6.55 p.m. respectively). Then, the average per day and group was assessed and reported for dark cycle and light cycle. To assess the significance of the difference in temperature between groups, an ANOVA model accounting for the repeated measure for each animal was built. In this model, terms as Period, Group and the interaction Group period were considered as fixed factors, whereas the animal factor was included as a random factor nested in the group factor. The significance level was settled at 0.05.

RESULTS

In vitro knockdown of GPR83

Three shRNAs directed against all isoforms of mouse GPR83 were tested alone and as a pool for knockdown of transiently expressed GPR83 in HEK293T cells. The locations of the shRNAs within GPR83 are shown in Fig. 1A. All three resulted in knockdown ranging from 43 ± 0.8 to 83 ± 3.1% with the pool of shRNAs causing the greatest amount of knockdown, 87 ± 5.3% when compared with non-silencing control shRNA (Fig. 1B). Based on these results, as well as the belief that attacking multiple sites within the gene could help increase the level of knockdown, the pool of shRNAs was chosen for lentivirus production and *in vivo* injection.

shRNA pool caused knockdown of GPR83 in the POA

Mice (n= 8 per group) were injected with 500,000 virus particles expressing either a non-silencing control hairpin or a pool of shRNAs directed at GPR83 (shGPR83). The injections were made into the POA. The injections were performed between 10:00 am and 11:30 am. The animals were then returned to their cages on a 12 h light:dark cycle with free access to food (high fat diet) and water. The animals were monitored for 3 weeks post injections at which time they were fasted overnight prior to isolating blood and sacrificed. Three weeks after the lentivirus injection, knockdown of GPR83 mRNA was detectable in five of the eight animals injected (Fig. 1C). The range of mRNA knockdown was 28.4 ± 3.4 to 52.4 ± 4.0% when compared to non-silencing control shRNA. Only these five animals were used in the assessment of GPR83 knockdown on the physiology of these animals. Lentiviral injection resulted in shRNA expression in a discrete region of size comparable but not larger than that of the preoptic area (Fig 1D)

Knockdown of GPR83 caused a decrease in dark cycle core body temperature

POA knockdown of GPR83 significantly reduced CBT during the dark cycle of day (average reduction was 0.15 ± 0.07°C from 37.36 ± 0.04°C in non-silencing to 37.21 ±

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0.05°C in GPR83 shRNA animals, $p<0.05$) (Fig. 2A). Reduction was also observed during the light:resting part of day although without statistical significance (average reduction was 0.09 ± 0.07°C from 36.51 ± 0.05°C in non-silencing vs. 36.42 ± 0.06°C in GPR83 shRNA animals $p=0.29$) (Fig. 2B). Compared to pre-treatment, CBT was on average 0.07°C higher treatment. Fever response to injection lasting approximately 8 hrs was aslo observed (not shown). No temperature differences were observed during the transition from dark to light and from light to dark. Motor activity profile was not different among groups across the entire day (data not shown).

Knockdown of GPR83 altered body weight gain and serum adiponectin level

GPR83 knockdown led to an increase in body weight gain, measured as percent weight gain from pretreatment weight, that was significant 3 weeks after lentiviral injection (13.76 ± 1.79% for the non-silencing shRNA vs. 19.97 ± 1.94% for the GPR83 shRNA, $p<0.05$) (Fig. 3A). Post mortem weighting of adipose tissue demonstrate that difference in body weight was due to the mass of inguinal and gonadal white adiposity. The increase in body weight gain was not associated with increased food intake (average daily intake during week 2 and 3 after treatment was 1.05 ± 0.03 kcal/$g^{0.75}$ and 1.10 ± 0.02 kcal/$g^{0.75}$, respectively for non-silencing shRNA and 1.05 ± 0.04 kcal/$g^{0.75}$ and 1.11 ± 0.03 kcal/$g^{0.75}$ for GPR83 shRNA, $p > 0.1$) (Fig. 3B).

There were no changes in fasted serum IGF-1 (529 ± 19 pg/ml for non-silencing shRNA vs. 556 ± 7 pg/ml for GPR83 shRNA, $p>0.1$), insulin (562 ± 46 pg/ml for non-silencing shRNA vs. 762 ± 77 pg/ml for GPR83 shRNA, $p>0.1$), or leptin (2817 ± 590 pg/ml for non-silencing shRNA vs. 2945 ± 507 pg/ml for GPR83 shRNA, $p>0.1$) (Fig. 4A) or glucose (135.0 ± 6.49 for non-silencing shRNA vs. 151.6 ± 4.59 mg/dl for GPR83 shRNA, $p>0.1$). Instead, fasted serum adiponectin was increased by 36% in GPR83 knockdown mice compared with the non-silencing control mice (31916 ± 952 pg/ml vs. 23474 ± 1507 pg/ml, $p<0.01$) (Fig. 4B). No differences in circulating IL-6 and TNF were found between groups (not shown).

DISCUSSION

GPR83 is expressed in several brain regions [11] and its transcript was recently identified in hypothalamic warm sensitive neurons suggesting it may have a role in central thermoregulation [13]. To initiate testing this hypothesis we suppressed GPR83 expression in the POA utilizing a shRNA lentiviral approach in mice. CBT and locomotor activity, an important contributor to thermogenesis, were measured in animals injected with silencing or non-silencing shRNAs vectors in POA. Successful long term down-regulation of the transcript was obtained with a single injection of virus giving the opportunity to carry out the proposed study and demonstrating that this approach can be utilized to investigate the role of GPR83 in other brain regions.

Despite incomplete knockdown of GPR83, and the relatively modest number of animals used in the study we observed a significant decrease in CBT of 0.15°C during the dark/ active part of the day. Importantly, there were no changes in either the CBT rhythmicity or locomotor activity suggesting that the effects on CBT were not due to a modification of the circadian CBT profile or to reduced physical activity. The possibility that these changes could be due to a reduced shivering or non-shivering thermogenesis was not addressed and the precise mechanisms mediating CBT reduction remain to be determined.

Lowering expression of GPR83 in the POA promoted increased body weight gain without affecting calorie intake. One possibility is that maintenance of a lower CBT required a diminished energy expenditure that, if not balanced by lowering caloric intake, may result in

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weight gain. While such hypothesis needs to be corroborated by accurate measurements of heat production and heat dissipation, this phenotype was similar to what was described for the transgenic “cool mice” that were designed to have reduced CBT by increasing local heat generation in the vicinity of the POA [22]. In fact, these mice showed reduction of CBT only during the dark/active part of the day and weight gain despite having the same calorie intake as their wt littermates. In addition, the modest (0.35°C) but prolonged reduction of CBT achieved in the “cool mice” increased longevity of up to 20%, indicating that reduced CBT alone could have beneficial effects in spite of the increased weight gain. Here we did not investigate the lifespan of GPR83 shRNA mice and thus cannot assume that a prolonged reduction of 0.15°C may be sufficient to increase longevity. In the future, when GPR83 antagonists are available, it will be interesting to test their effects on CBT, energy homeostasis and longevity.

Dissipation of energy in the form of heat is the primary mode of energy expenditure in mammals and, together with nutrient homeostasis, can influence metabolism. A substantial number of studies demonstrated that nutrient signals can act centrally, primarily in the hypothalamus, to regulate CBT and peripheral metabolism, reviewed in [13, 15, 16, 23]. Thus, we investigated whether shGPR83 mice had any difference in four major metabolic hormones also demonstrated or proposed to influence CBT: insulin, IGF-1, leptin and adiponectin [13, 15, 16, 23]. We found that the circulating levels of insulin, IGF-1 or leptin were similar in non-silencing and silencing groups. We cannot exclude that changes in the level of these molecules across the experimental groups may be observed at later time-points. In fact, in our experiments CBT reduction occurred after 5 days post injection and body weight increase was significant only after 14 days but measurements were not carried out after day 21 post-injection.

Instead, knock-down mice showed increased levels of circulating adiponectin already at week 3. Exposure to temperature reduction was previously shown to influence circulating levels of adiponectin in humans and rodents [24-28]. Although both increase and decrease in adiponectin was reported, it appears that these were induced by mild or large temperature differences, respectively. The source and the mechanisms responsible for the increase of adiponectin in shGPR83 mice remain to be determined. Among the possibilities are the elevation of fad pads and temperature-dependent modulation of sympathetic nerve activity and heat-shock proteins previously proposed to influence adiponectin level [24-28]. Circulating levels of adiponectin are normally inversely correlated with body mass index and adiposity [29, 30], as well as with metabolic and cardiovascular disorders [31, 32] suggesting that elevating adiponectin may be beneficial. These data indicate that antagonism of GPR83 might provide a target for elevating adiponectin in disease states where circulating levels of adiponectin are normally decreased.

“In summary, we present for the first time evidence that down-regulating the expression of GPR83 in the preoptic area reduced core body temperature in mice. These animals also gained body weight and had elevated circulating levels of adiponectin. Additional work is required to determine the role of GPR83 in regulating temperature homeostasis in POA warm sensitive neurons and to fully characterize the effects that CBT reduction can have on metabolism.”

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Abbreviations

GPR	G protein-coupled receptor
WSN	warm sensitive neurons
POA	preoptic area
CBT	core body temperature
shRNAs	short-hairpin RNAs
GIR	glucocorticoid-induced receptor
Treg	regulatory T cells

References

1. Harrigan MT, Baughman G, Campbell NF, et al. Isolation and characterization of glucocorticoid- and cyclic AMP-induced genes in T lymphocytes. Molecular and cellular biology. 1989; 9(8):3438–46. [PubMed: 2552295]
2. Harrigan MT, Campbell NF, Bourgeois S. Identification of a gene induced by glucocorticoids in murine T-cells: a potential G protein-coupled receptor. Molecular endocrinology. 1991; 5(9):1331–8. [PubMed: 1663214]
3. Sugimoto N, Oida T, Hirota K, et al. Foxp3-dependent and -independent molecules specific for CD25+CD4+ natural regulatory T cells revealed by DNA microarray analysis. International immunology. 2006; 18(8):1197–209. [PubMed: 16772372]
4. Hansen W, Westendorf AM, Toepfer T, et al. Inflammation in vivo is modulated by GPR83 isoform-4 but not GPR83 isoform-1 expression in regulatory T cells. Genes and immunity. 2010; 11(4):357–61. [PubMed: 20200545]
5. Lu LF, Gavin MA, Rasmussen JP, et al. G protein-coupled receptor 83 is dispensable for the development and function of regulatory T cells. Molecular and cellular biology. 2007; 27(23):8065–72. [PubMed: 17893329]
6. Toms C, Jessup H, Thompson C, et al. Gpr83 expression is not required for the maintenance of intestinal immune homeostasis and regulation of T-cell-dependent colitis. Immunology. 2008; 125(3):302–12. [PubMed: 18479351]
7. Hansen W, Loser K, Westendorf AM, et al. G protein-coupled receptor 83 overexpression in naive CD4+CD25− T cells leads to the induction of Foxp3+ regulatory T cells in vivo. Journal of immunology. 2006; 177(1):209–15.
8. Adams F, Grassie M, Shahid M, et al. Acute oral dexamethasone administration reduces levels of orphan GPCR glucocorticoid-induced receptor (GIR) mRNA in rodent brain: potential role in HPA-axis function. Brain research. Molecular brain research. 2003; 117(1):39–46. [PubMed: 14499479]
9. Brezillon S, Detheux M, Parmentier M, et al. Distribution of an orphan G-protein coupled receptor (JP05) mRNA in the human brain. Brain research. 2001; 921(1-2):21–30. [PubMed: 11720708]
10. Pesini P, Detheux M, Parmentier M, et al. Distribution of a glucocorticoid-induced orphan receptor (JP05) mRNA in the central nervous system of the mouse. Brain research. Molecular brain research. 1998; 57(2):281–300. [PubMed: 9675427]
11. Sah R, Pritchard LM, Richtand NM, et al. Expression of the glucocorticoid-induced receptor mRNA in rat brain. Neuroscience. 2005; 133(1):281–92. [PubMed: 15893650]
12. Wang D, Herman JP, Pritchard LM, et al. Cloning, expression, and regulation of a glucocorticoid-induced receptor in rat brain: effect of repetitive amphetamine. The Journal of neuroscience: the official journal of the Society for Neuroscience. 2001; 21(22):9027–35. [PubMed: 11698613]
13. Eberwine J, Bartfai T. Single cell transcriptomics of hypothalamic warm sensitive neurons that control core body temperature and fever response Signaling asymmetry and an extension of chemical neuroanatomy. Pharmacology & therapeutics. 2011; 129(3):241–59. [PubMed: 20970451]

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14. Griffin JD, Saper CB, Boulant JA. Synaptic and morphological characteristics of temperature-sensitive and -insensitive rat hypothalamic neurones. The Journal of physiology. 2001; 537(Pt 2): 521–35. [PubMed: 11731583]
15. Sanchez-Alavez M, Osborn O, Tabarean IV, et al. Insulin-like growth factor 1-mediated hyperthermia involves anterior hypothalamic insulin receptors. The Journal of biological chemistry. 2011; 286(17):14983–90. [PubMed: 21330367]
16. Sanchez-Alavez M, Tabarean IV, Osborn O, et al. Insulin causes hyperthermia by direct inhibition of warm-sensitive neurons. Diabetes. 2010; 59(1):43–50. [PubMed: 19846801]
17. Tabarean IV, Conti B, Behrens M, et al. Electrophysiological properties and thermosensitivity of mouse preoptic and anterior hypothalamic neurons in culture. Neuroscience. 2005; 135(2):433–49. [PubMed: 16112471]
18. Georgievska B, Kirik D, Bjorklund A. Aberrant sprouting and downregulation of tyrosine hydroxylase in lesioned nigrostriatal dopamine neurons induced by long-lasting overexpression of glial cell line derived neurotrophic factor in the striatum by lentiviral gene transfer. Experimental neurology. 2002; 177(2):461–74. [PubMed: 12429192]
19. Georgievska B, Kirik D, Bjorklund A. Overexpression of glial cell line-derived neurotrophic factor using a lentiviral vector induces time- and dose-dependent downregulation of tyrosine hydroxylase in the intact nigrostriatal dopamine system. The Journal of neuroscience: the official journal of the Society for Neuroscience. 2004; 24(29):6437–45. [PubMed: 15269253]
20. Grillo CA, Tamashiro KL, Piroli GG, et al. Lentivirus-mediated downregulation of hypothalamic insulin receptor expression. Physiology & behavior. 2007; 92(4):691–701. [PubMed: 17585961]
21. Jin J, Bao X, Wang H, et al. RNAi-induced down-regulation of Mecp2 expression in the rat brain. International journal of developmental neuroscience: the official journal of the International Society for Developmental Neuroscience. 2008; 26(5):457–65. [PubMed: 18396005]
22. Conti B, Sanchez-Alavez M, Winsky-Sommerer R, et al. Transgenic mice with a reduced core body temperature have an increased life span. Science. 2006; 314(5800):825–8. [PubMed: 17082459]
23. Schwartz MW, Porte D Jr. Diabetes, obesity, and the brain. Science. 2005; 307(5708):375–9. [PubMed: 15662002]
24. Bernabucci U, Basirico L, Morera P, et al. Heat shock modulates adipokines expression in 3T3-L1 adipocytes. J Mol Endocrinol. 2009; 42(2):139–47. [PubMed: 18996960]
25. Imai J, Katagiri H, Yamada T, et al. Cold exposure suppresses serum adiponectin levels through sympathetic nerve activation in mice. Obesity (Silver Spring). 2006; 14(7):1132–41. [PubMed: 16899794]
26. Imbeault P, Depault I, Haman F. Cold exposure increases adiponectin levels in men. Metabolism. 2009; 58(4):552–9. [PubMed: 19303978]
27. Iwen KA, Wenzel ET, Ott V, et al. Cold-induced alteration of adipokine profile in humans. Metabolism. 2011; 60(3):430–7. [PubMed: 20423746]
28. Puerta M, Abelenda M, Rocha M, et al. Effect of acute cold exposure on the expression of the adiponectin, resistin and leptin genes in rat white and brown adipose tissues. Horm Metab Res. 2002; 34(11-12):629–34. [PubMed: 12660872]
29. Arita Y, Kihara S, Ouchi N, et al. Paradoxical decrease of an adipose-specific protein, adiponectin, in obesity. Biochem Biophys Res Commun. 1999; 257(1):79–83. [PubMed: 10092513]
30. Hu E, Liang P, Spiegelman BM. AdipoQ is a novel adipose-specific gene dysregulated in obesity. J Biol Chem. 1996; 271(18):10697–703. [PubMed: 8631877]
31. Duncan BB, Schmidt MI, Pankow JS, et al. Adiponectin and the development of type 2 diabetes: the atherosclerosis risk in communities study. Diabetes. 2004; 53(9):2473–8. [PubMed: 15331562]
32. Gannage-Yared MH, Khalife S, Semaan M, et al. Serum adiponectin and leptin levels in relation to the metabolic syndrome, androgenic profile and somatotropic axis in healthy non-diabetic elderly men. Eur J Endocrinol. 2006; 155(1):167–76. [PubMed: 16793964]

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Figure 1.
(A) Sequence of mouse GPR83 isoform-1. The position and sequences corresponding to the three shRNA are shown. V2LMM_56683 corresponds to the third cytoplasmic loop, V2LMM_51223 corresponds to TM5 and V2LMM_54689 corresponds to TM7. (B) Histogram showing the tested ability of various hairpins to knockdown expression of mouse GPR83 mRNA transiently transfected into HEK293T cells. The level of GPR83 was normalized to GAPDH (***P<0.001 vs. non-silencing control). (C) Histogram showing GPR83 knockdown in the POA of mice injected with 500,000 lentivirus particles 3 weeks post injection. RNA was isolated during the light cycle and subjected to qRT-PCR. The level of GPR83 was normalized to GAPDH and compared to animals injected with the non-silencing control shRNA lentivirus (* p<0.05, **p<0.01 and ***p<0.001vs. non-silencing control). (D) Representative picture of GFP (Green) and DAPI staining (Blue) of the POA following injection.

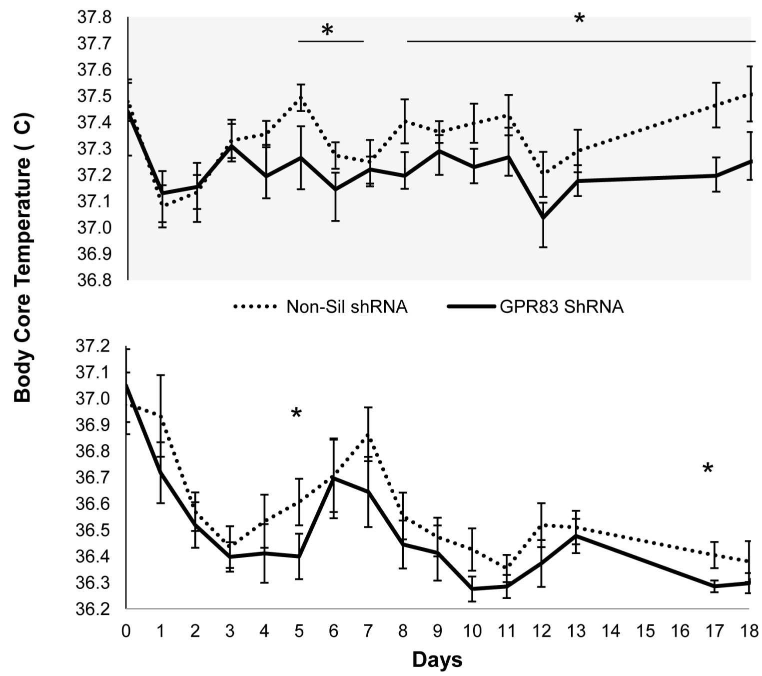

Figure 2.
Profile of average CBT profile during (A) dark and (B) light apart of the day GPR83 knockdown and control mice (* P<0.05).

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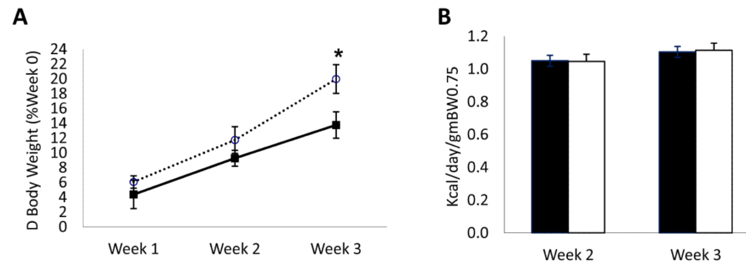

Figure 3.

(A) Percentage of body weight gain over time and (B) weekly food intake (not measured during the first week as the mice were recovering from the viral injections). Solid bars represent non-silencing control animals (n=8) and open bars represent GPR83 knockdown animals (n=5). Data are means ±SE (n=3). * P<0.05, **P<-0.01 vs. non-silencing shRNA.

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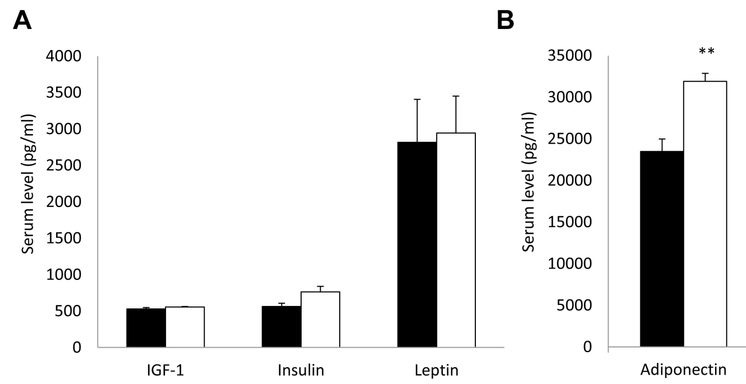

Figure 4.

Fasting serum levels of (A) IGF-1, insulin, and leptin and (B) adiponectin measured 21 days post lentivirus injection. Solid bars represent non-silencing control animals (n=8) and open bars represent GPR83 knockdown animals (n=5). Data are means ± SE (n=3). **P<0.01.