0013-7227/07/$15.00/0
Printed in U.S.A.

Endocrinology 148(1):401–410

doi: 10.1210/en.2006-1019

Regulation of Pituitary Cell Function by Adiponectin

Francisca Rodriguez-Pacheco, Antonio J. Martinez-Fuentes, Sulay Tovar, Leonor Pinilla, Manuel Tena-Sempere, Carlos Dieguez, Justo P. Castaño, and María M. Malagon

Department of Cell Biology, Physiology and Immunology, University of Córdoba (F.R.-P., A.J.M.-F., L.P., M.T.-S., J.P.C., M.M.M.), 14014-Córdoba, Spain; and Department of Physiology, University of Santiago de Compostela (S.T., C.D.), S Francisco s/n, Santiago de Compostela (A Coruna) 15782, Spain

Adiponectin is a member of the family of adipose tissue-related hormones known as adipokines, which exerts antidiabetic, antiatherogenic, antiinflammatory, and antiangiogenic properties. Adiponectin actions are primarily mediated through binding to two receptors expressed in several tissues, AdipoR1 and AdipoR2. Likewise, adiponectin expression has been detected in adipocytes as well as in a variety of extra-adipose tissues, including the chicken pituitary. Interestingly, adiponectin secretion and adiponectin receptor expression in adipocytes have been shown to be regulated by pituitary hormones. These observations led us to investigate whether adiponectin, like the adipokine leptin, regulates pituitary hormone production. Specifically, we focused our analysis on somatotrophs and gonadotrophs because of the relationship between the control of energy metabolism, growth and reproduction. To this end, the effects of adiponectin on both GH and LH secretion as well as its interaction with major stimulatory regulators of somatotrophs (ghrelin and GHRH) and gonadotrophs (GnRH) and with their corresponding receptors (GHS-R, GHRH-R, and GnRH-R), were evaluated in rat pituitary cell cultures. Results show that adiponectin inhibits GH and LH release as well as both ghrelin-induced GH release and GnRH-stimulated LH secretion in short-term (4 h) treated cell cultures, wherein the adipokine also increases GHRH-R and GHS-R mRNA content while decreasing that of GnRH-R. Additionally, we demonstrate that the pituitary expresses both adiponectin and adiponectin receptors under the regulation of the adipokine. In sum, our data indicate that adiponectin, either locally produced or from other sources, may play a neuroendocrine role in the control of both somatotrophs and gonadotrophs. (*Endocrinology* 148: 401–410, 2007)

ADIPONECTIN IS A collagen-like 30-kDa protein that belongs to the family of adipose tissue-specific or enriched hormones termed adipokines, which play important roles in the regulation of food intake and energy homeostasis as well as in vascular homeostasis and immunity (reviewed in Refs. 1 and 2). Adiponectin, also known as acrp30 (3), apM-1 (4), GBP28 (5), or adipoQ (6), is the most abundantly secreted adipokine and, in fact, plasma adiponectin levels are considerably high (3–30 μg/ml in human, 3–6 μg/ml in rodents) (7). Accumulating experimental evidence indicates that this adipokine is involved in the regulation of multiple processes. Thus, adiponectin, whose circulating levels are reduced in obese and diabetic human and mice (6–8), is considered as an insulin-sensitizing factor because it reduces endogenous glucose production by increasing hepatic insulin sensitivity (9, 10), increases glucose uptake in adipocytes (11) and myocytes, and enhances fatty acid oxidation in muscle (12, 13). This adipokine has been also reported to modulate the endotelial inflammatory response and to exert a direct antiatherogenic effect (reviewed in Refs. 14 and 15). Finally, adiponectin has been also proposed to exert antiangiogenic and antitumoral actions (16, 17) as well as to regulate osteoblast proliferation and differentiation (18).

Recently, two adiponectin receptors, AdipoR1 and AdipoR2, were identified by expression cloning (19). Both receptors, which share 67% amino acid identity, appear to be integral membrane receptors containing seven transmembrane domains but, contrary to G protein-coupled receptors, their N terminus is intracellular and the C terminus is extracellular. Initial studies on the distribution of adiponectin receptors showed that AdipoR1 mRNA was most abundant in skeletal muscle, although it was also present in other tissues and organs such as heart, liver and brain, whereas AdipoR2 was predominantly expressed in liver (19). More recently, the expression of AdipoR1 and AdipoR2 has been also demonstrated in pancreatic β-cells (20), endothelial cells (21), bone-forming cells (22), hypothalamus (23), adipocytes (24), or placenta (25), which further supports the pleiotropic actions reported for this adipokine. In line with this notion, adiponectin mRNA has been also detected in multiple locations besides white and brown adipose tissue, including skeletal muscle (26, 27), osteoblastic cells (22), cardiomyocytes (28), and placenta (25) in murine and human. A recent report by Maddineni *et al*. (29) confirmed the widespread distribution of adiponectin in chicken tissues and showed that the adipokine is also abundantly expressed in the anterior pituitary. Interestingly, pituitary GH, which has important metabolic roles (30), has been shown to regulate adiponectin secretion and AdipoR expression in human and mouse adipocytes (24, 31). Based on these findings, in the present study we aimed at investigating whether adiponectin may play a reciprocal, endocrine role and regulate so-

First Published Online October 12, 2006

Abbreviations: dNTP, Deoxynucleotide triphosphate; FBS, fetal bovine serum; GHRH-R, GHRH receptor; GHS-R, the receptor for ghrelin/GH secretagogues; GnRH-R, GnRH receptor; HPRT, hypoxanthine-guanine phosphoribosyl-transferase; RT, reverse transcription.

***Endocrinology* is published monthly by The Endocrine Society (http://www.endo-society.org), the foremost professional society serving the endocrine community.**

matotroph cell function. We also investigated the response of pituitary gonadotrophs to this adipokine because of the known relationship between energy homeostasis and fertility and the demonstrated effects of other regulators of energy homeostasis such as the adipocyte-derived hormone leptin on reproduction (32). Specifically, the effect of adiponectin on both GH and LH release and its interaction with major stimulatory regulators of somatotrophs, ghrelin and GHRH (reviewed by Ref. 33), and gonadotrophs, GnRH (34), as well as with their corresponding receptors, was evaluated in rat pituitary cell cultures after either short- (4 h) or long-term (24 h) exposure to the adipokine. Further insight on adiponectin function at the pituitary was assessed by analyzing the expression of both adiponectin and adiponectin receptors in the cell cultures.

Materials and Methods

Reagents

Rat GH and LH were kindly supplied by Dr. A. F. Parlow (Pituitary Hormones and Antisera Center, Harbor-University of California-Los Angeles Medical Center, Torrance, CA). Fetal bovine serum (FBS) was obtained from Sera-Lab Ltd. (Crawley Down, UK). Tripure Reagent was purchased from Invitrogen (Paisley, Scotland, UK), PowerScript reverse transcriptase from BD Bioscience (Erembodegem, Belgium), QuiaQuick Gel Extraction Kit from QIAGEN GmBH (Hilden, Germany), and Eco-Taq polymerase from Ecogen (Barcelona, Spain). Human Pituitary Gland PolyA RNA was obtained from CLONTECH (Newark, NJ). Recombinant Mouse gAdiponectin/gACRP30 was purchased from R&D Systems Inc. (Minneapolis, MN), human ghrelin was purchased from Bachem Ltd. (Merseyside, UK), and GHRH (1–29) was obtained from UCB Bioproducts (Brain L'Alleud, Belgium). DMEM, collagenase type V, trypsin type I, soybean trypsin inhibitor I, deoxyribonuclease I, antibiotic-antimycotic solution, BSA, and all other reagents were purchased from Sigma Chemical Co. (London, UK), unless otherwise specified. Tissue culture products were obtained from Invitrogen (Grand Island, NY).

Animals

Male Sprague Dawley rats (200–250 g; Harlan Iberica, Barcelona, Spain) were housed in air-conditioned rooms (22–24 C) under a 12-h light, 12-h dark cycle and fed standard rat chow and water *ad libitum*.

Animals were killed by decapitation between 0900 h and 1300 h. Pituitary glands were immediately removed, the posterior lobes were discarded, and the anterior lobes were transferred to sterile cold (4 C) DMEM supplemented with 0.1% BSA and antibiotic-antimycotic solution. All the animal procedures were conducted according to the principles approved by the Cordoba University Ethical Committee for animal experimentation in accordance with the European Union normative for care and use of experimental animals.

Pituitary cell dispersion and culture

Isolated cells from rat anterior pituitary were obtained using a dispersion protocol previously described (35). Briefly, for each experiment, three to four anterior pituitaries were pooled, minced, and enzymatically dissociated by sequential incubation in DMEM supplemented with 0.3% trypsin (type I), 0.1% collagenase (type V), 0.1% soybean trypsin inhibitor I, 2 μg/ml deoxyribonuclease I, and Ca^{2+}/Mg^{2+}-free salt solution with EDTA (2 and 1 mM). Finally, the tissues were mechanically dispersed using a siliconized Pasteur pipette until a homogeneous cellular suspension was obtained. After each step, the cellular suspension was centrifuged at 60 $\times$ *g* for 5 min. Cellular viability, as estimated by the trypan blue test, was always above 90%.

Dispersed adenohypophyseal cells were plated at a density of 300,000 cells/200 μl DMEM onto 24-well culture plates and incubated at 37 C in a 5% CO_2 atmosphere in 1 ml culture medium supplemented with 10% FBS and 0.1% gentamycine sulfate. After 48 h of culture, medium was replaced by fresh DMEM-FBS. Cultures were maintained for 3 d before treatments. On the day of the experiment, medium was removed and cells were preincubated in 1 ml serum-free DMEM for 2 h to stabilize basal GH secretion. Medium was then replaced with fresh DMEM containing the test substances at the appropriate concentrations or the corresponding control vehicle, and incubated for either 4 h or 24 h at 37 C. Specifically, cultures were challenged with either adiponectin alone at doses ranging 10^{-7} to 10^{-9} M, or in combination with ghrelin, GHRH, or GnRH. For the combined treatments, a single dose of ghrelin, GHRH and GnRH (10^{-8} M) was chosen based on results obtained previously on rat pituitary cell cultures (36–38).

Medium samples were collected at the end of the experiments, centrifuged at 6000 $\times$ *g* for 5 min, and the supernatants were stored at -20

TABLE 1. Sequences of the primers employed for RT-PCR amplifications

Gene	Primer sequence (5′–3′)	GenBank accession no.
rGHS-R	F: GGACCAGAACCACAAGCAGA R: GGCTCGAAGGACTTGGAAAA	NM_032075
rGHRH-R	F: CACTGCCCCAGGAACTACAT R: TAGGAGATGTGGAGGCCAAC	NM_012850
rGnRH-R	F: CGATCTTCTCGCAATGTGTGACC R: GCACGGGTTTAGGAAAGCAAAG	NM 031038
rAdiponectin	F: CTCCACCCAAGGAAGCTTGT R: GGCTCGAAGGACTTGGAAAA	NM_144744
rAdipoR1	F: CTTCTACTGCTCCCCACAGC R: TCCCAGGAACACTCCTGCTC	NM 001037979
rAdipoR2	F: CCACACAACACAAGAATCCG R: CCCTTCTTCTTGGGAGAATGG	NM 207587
hAdiponectin	F: CCTAAGGGAGACACTGGTGA R: GTAAAGCGAATGGGCATGTT	NM_004797
hAdipoR1	F: TACCAGCCAGATGTCTTCCC R: AGTGTCAGTACCCGCACCTC	NM_015999
hAdipoR2	F: GGACCGAGCAAAAGACTCAG R: AAAGTGCATGACCGAAGAGC	NM_024551
rHPRT	F: CAGTCCCAGCTCGTGATTA R: AGCAAGTCTTTCAGTCCTGTC	NM_012583
h18S	F: CCCATTCGAACGTCTGCCCTATC R: TGCTGCCTTCCTTGGATGTGGTA	GI36162

F, Forward; R, Reverse; r, rat, h, human.

C until hormone determinations. Cells in the culture plates were processed for RNA extraction as indicated below.

Hormone measurement by RIA

LH and GH levels in culture media were measured in a volume of 25–50 μl using a double antibody method and RIA kits kindly supplied by the National Institutes of Health (Dr. A. F. Parlow). Rat GH-I-7 and LH-I-9 were labeled with ^{125}I using the chloramine-T method and Iodo-Gen precoated iodination tubes (Pierce, Rockford, IL), respectively. Hormone concentrations were expressed using the reference preparations LH-RP-3 and GH-RP-2 as standards. Intra- and interassay coefficients of variation were <6% and 9% for GH, and 8% and 10% for LH. The sensitivity of the assay was 5 pg/tube for GH and 20 pg/tube for LH.

Accuracy of hormone determinations was confirmed by assessment of rat serum samples of known hormone concentrations used as external controls.

RNA extraction and cDNA synthesis

Total RNA was isolated using Tripure Reagent from rat pituitary cultured cells and following manufacturer's instructions. Rat pituitary RNA and human pituitary PolyA RNA (2 μg) were reverse transcribed to cDNA by using PowerScript reverse transcriptase as recommended in the manufacturer's manual. Briefly, reverse transcription (RT) reactions were carried out in a 20 μl final volume by adding 2 μg total RNA, 1 μl of PowerScript, 10 μM deoxynucleotide triphosphate (dNTP) mixture and 250 ng of random hexamer primers. The reaction mixtures were incubated at 70 C for 10 min and at 42 C for 1 h. Finally, reactions were terminated by heating at 70 C for 15 min and cooling on ice.

PCR amplification

PCR analysis was applied to assess the expression of adiponectin and its two receptors, AdipoR1 and AdipoR2, in rat and human pituitary by using specific primers (Table 1). As internal control for RT, amplification of a 150-bp fragment of rat hypoxanthine-guanine phosphoribosobyl-transferase (HPRT) (for rat pituitary) or a 137-bp fragment of 18S ribosomal RNA (for human pituitary) was carried out in parallel in each sample. PCRs were performed in a 25-μl final volume using an iCycler IQ (Bio-Rad, Madrid, Spain) thermocycler by adding 1 μl of RT product (cDNA, 100 ng), 0.1 μM reverse and forward primers, 0.8 mM dNTPs, and 0.125 U EcoTaq DNA Polymerase. Temperature profiles for adiponectin, AdipoR1, AdipoR2, HPRT, and 18S were as follows: 95 C/30 sec, 60 C/30 sec, and 72 C/30 sec for 35 cycles. For all different primer pairs used, a negative control with identical amount of non-retrotranscribed total RNA was performed. PCR products were electrophoresed in a 2% agarose gel containing ethidium bromide and extracted using QuiaQuick Gel Extraction Kit. Identities of amplicons were confirmed by sequencing (Central Sequencing Service, University of Cordoba, Cordoba, Spain).

Real-time quantitative RT-PCR

To evaluate changes in gene expression in rat pituitary cell cultures exposed to adiponectin, real-time RT-PCR was performed using the iCycler iQ real-time PCR detection system (Bio-Rad Laboratories, Hercules, CA) according to the manufacturer's instructions. PCR primers for amplification of the receptor for GHRH (GHRH-R), the receptor for ghrelin/GH secretagogues (GHS-R), the receptor for GnRH (GnRH-R), adiponectin, AdipoR1, AdipoR2, and HPRT were based on the corresponding rat and human mRNA sequences published in GenBank and are shown in Table 1. The primers used amplify PCR products between 100 and 500 bp long.

The 25-μl amplification mixture contained 2 μl cDNA (corresponding to 50 ng cDNA), 12.5 μl of 2× iQ Supermix containing 50 mM KCl, 20 mM Tris-HCl, 0.2 mM dNTPs, 3 mM $MgCl_2$, 2.5 U iTaq DNA polymerase, and SYBR Green I as fluorescent dye. PCRs consisted of an initial activation and denaturing cycle at 95 C for 5 min, followed by 35 cycles at 95 C/30 sec, 60 C/30 sec and 72 C/30 sec. The amount of PCR products formed in each cycle was estimated on the basis of SYBR Green I fluorescence dye. No-template controls were included in all assays, yield-

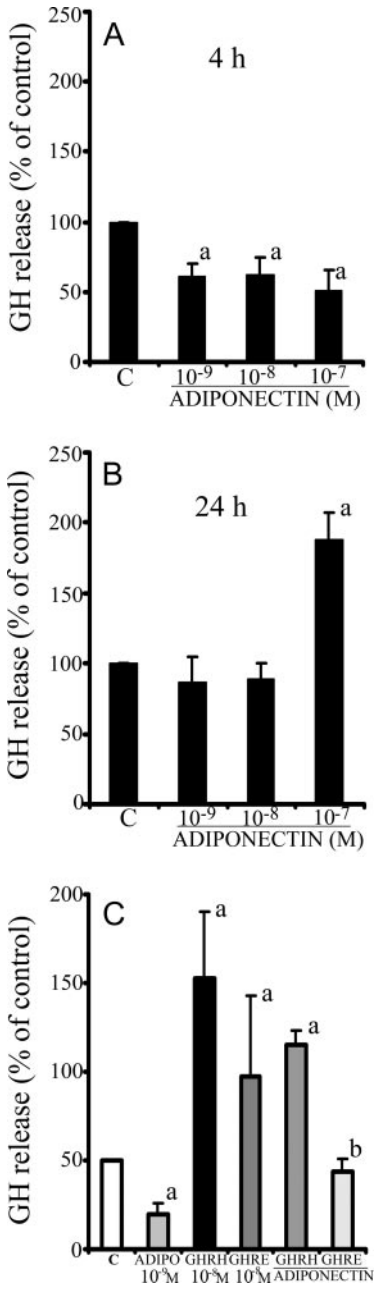

FIG. 1. Effect of adiponectin, alone (panels A and B) or in combination with GHRH or ghrelin (panel C), on GH release from cultured rat pituitary cells. After 3 d of culture in DMEM-FBS, cells were equilibrated for 3 h in serum-free DMEM and then incubated in the absence (C, Control) or presence of the corresponding test substances for the times indicated. A, Secretory response of rat pituitary cells to 4 h treatment with increasing doses (10^{-9}–10^{-7} M) of adiponectin. B, Secretory response of rat pituitary cells to 24 h treatment with increasing doses (10^{-9}–10^{-7} M) of adiponectin. C, Secretory response of rat pituitary cells to 4 h treatment with adiponectin (ADIPO; 10^{-7} M), GHRH (10^{-8} M), and ghrelin (GHRE; 10^{-8} M), alone or in combination. At the end of the incubations, culture media were recovered and GH release was evaluated by RIA. Data are expressed as a percentage of basal values in control cultures (100%, 27.5 ± 7.5 ng GH/ml, 5074.8 ± 163.0 ng GH/ml, and 332.1 ± 160.9 ng GH/ml for Fig. 1, A–C, respectively) and are the mean (±SEM) of three independent experiments. At least three replicate wells were evaluated per treatment in each experiment. a, $P < 0.05$ *vs.* corresponding control; b, $P < 0.05$ *vs.* ghrelin alone.

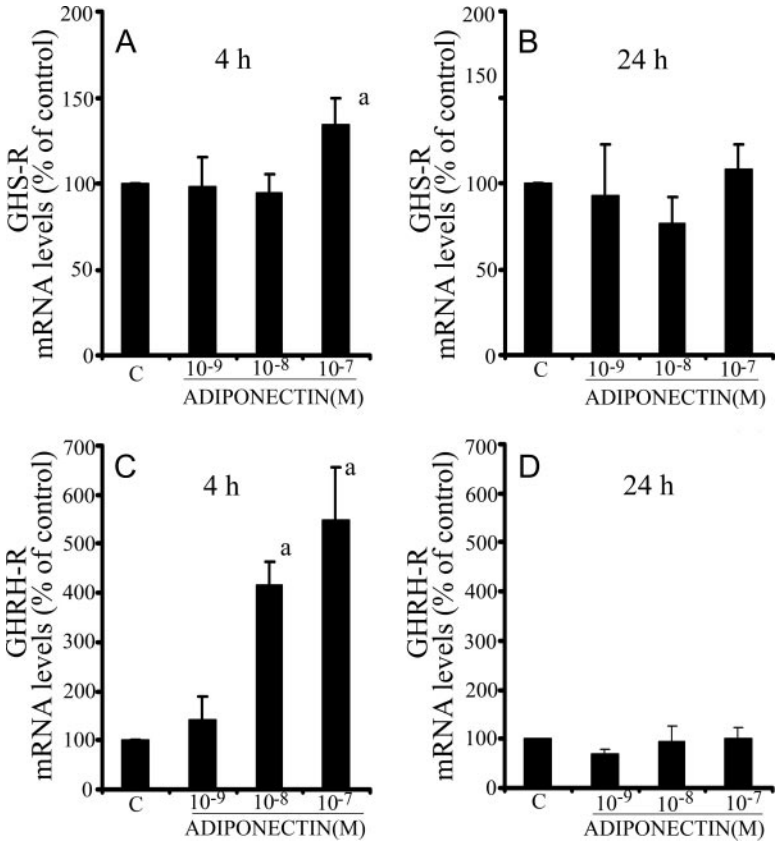

FIG. 2. Effect of adiponectin on mRNA levels of rat pituitary ghrelin/GHS-R (panels A and B) and GHRH-R (panels C and D) *in vitro*. After 3 d of culture, dispersed rat pituitary cells were incubated in medium alone (C, Control) or in the presence of 10^{-9}–10^{-7} M adiponectin for 4 h (panels A and C) or 24 h (panels B and D). After culture, cells were harvested and GHS-R and GHRH-R mRNA levels were determined by real-time RT-PCR. Receptor-specific band intensities were determined and adjusted by the signal intensity for HPRT. The averaged results were then calculated and expressed as a percentage of vehicle-treated control levels. Data are the mean (±SEM) of seven (panel A) or four (panels B–D) separate experiments. At least three replicate wells were evaluated per treatment in each experiment. a, $P < 0.05$ *vs.* corresponding control.

ing no consistent amplification. cDNAs were sequenced to ensure that the correct mRNA transcripts were quantified (Central Sequencing Service, University of Cordoba, Cordoba, Spain).

Calculation of relative expression levels of the different transcripts was performed based on the cycle threshold (C_T) method. Thus, the C_T value for each sample was calculated using the iCycler iQ real-time PCR detection system software with an automatic fluorescence threshold setting. Reactions were performed, at least, in triplicate. Standard curves were constructed for the receptors and HPRT (internal control) by plotting values of C_T (the cycle at which the fluorescence signal exceeds background) *vs.* log cDNA input (in nanograms). Accordingly, C_T values from each experimental sample were then used to calculate the amount of GHRH-R, GHS-R, GnRH-R, adiponectin, AdipoR1, and AdipoR2 mRNAs relative to the standard (HPRT).

Statistical analysis

Data are expressed as the mean ± SEM of the number of experiments indicated in each figure. A minimum of three replicate wells per treatment were tested in each experiment. To avoid variability between experiments, samples from each experiment were analyzed in the same assay and expressed as a percentage of the corresponding control value. To compare experimental treatments we applied a one-way ANOVA followed by a statistical test for multiple comparisons (Duncan's multiple range test and critical ranges) or, for nonparametric data, a Kruskal-Wallis Multiple Comparison Test followed by a Mann-Whitney U Test to compare pairs of data groups. Statistical analysis was assessed by the program Statistica for Windows (Statsoft Inc., Tulsa, OK). Differences were considered significant at $P < 0.05$.

Results

Effect of adiponectin on GH release in rat pituitary cell cultures

Exposure of dispersed anterior lobe cells to adiponectin at doses ranging between 10^{-9} and 10^{-7} M for 4 h induced a significant inhibition of GH release, an effect which was already noticeable at the lower concentration of the protein tested (Fig. 1A). Specifically, 10^{-9} M adiponectin reduced GH secretion by 34% with respect to control values. Although 10^{-7} M adiponectin evoked the highest numerical reduction in GH release (52%), this effect was not significantly higher than that induced by lower doses of the protein. In contrast to that found in cultures treated for 4 h with the adipokine, only the highest dose of adiponectin used modified basal GH release after long-term exposure (24 h) (Fig. 1B). Specifically, 10^{-7} M adiponectin induced a 2-fold increase in GH secretion when compared with the values observed in control cultures.

To investigate whether adiponectin modulates the secretory response of somatotrophs to the GH regulators GHRH and ghrelin, cultures of rat pituitary cells were treated for 4 h with 10^{-8} M of the peptides in the presence or absence of the adipokine. As shown in Fig. 1C, 10^{-7} M adiponectin inhibited the stimulatory effect of ghrelin on GH release but not that evoked by GHRH.

Effect of adiponectin on the expression of receptors for ghrelin/GHS and GHRH

PCR analysis of ghrelin/GHS-R and GHRH-R mRNA in cell cultures after adiponectin treatment revealed that this adipokine regulates the expression levels of both receptors in rat anterior pituitary cells. Thus, 10^{-7} M adiponectin significantly increased mRNA levels of both GHS-R (Fig. 2A) and GHRH-R (Fig. 2C) at 4 h. However, the stimulatory action of adiponectin on GHS-R mRNA content (34% over basal levels) was lower than that observed for GHRH-R (448% over

basal levels), whose transcript levels were also significantly enhanced by a lower dose of the adipokine (10^{-8} M). In contrast, 10^{-9} M had no effect on the expression levels of either GHS-R or GHRH-R.

Long-term (24 h) treatment of pituitary cell cultures with adiponectin caused no variations in basal mRNA content of either GHS-R (Fig. 2B) or GHRH-R (Fig. 2D) at any of the doses tested.

Effect of adiponectin on LH release in rat pituitary cell cultures

Similar to that found for GH, adiponectin significantly inhibited LH release in rat pituitary cell cultures exposed to doses ranging from 10^{-9} M to 10^{-7} M for 4 h (Fig. 3A). All the doses tested evoked similar reductions in LH secretion. In contrast, such an inhibitory effect was no longer evident after 24-h treatment with the adipokine (Fig. 3B).

As for the case of somatotrophs, we also investigated whether adiponectin may modify the response of gonadotrophs to their primary hypothalamic regulator, GnRH. Thus, we observed that the stimulatory effect induced by 10^{-8} M GnRH on LH release in 4 h-treated cultures was reduced by 74% in the presence of 10^{-7} M adiponectin (Fig. 3C).

Effect of adiponectin on the expression of GnRH receptor

In pituitary cell cultures exposed to any of the doses of adiponectin tested for 4 h, GnRH-R mRNA levels were reduced by 50% when compared with the corresponding values obtained in cultures exposed to medium alone (Fig. 4A). GnRH-R transcript content in adiponectin-treated cultures remained below basal levels after 24 h of exposure to the adipokine (Fig. 4B).

Expression of adiponectin, AdipoR1, and AdipoR2 in the pituitary

We also investigated the expression of the adiponectin/adiponectin receptor system in the pituitary. RT-PCR analysis demonstrated the expression of both the adipokine and its two receptors, AdipoR1 and AdipoR2, in rat pituitary extracts (Fig. 5A). Similar results were obtained in human pituitary tissue extracts, which also exhibited adiponectin as well as both AdipoR1 and AdipoR2 mRNA expression (Fig. 5B).

Regulation of pituitary adiponectin, AdipoR1, and AdipoR2 mRNA content by adiponectin

Results obtained in rat pituitary cell cultures exposed to increasing doses of adiponectin showed that this adipokine regulates its own expression. To be more specific, a 4-h treatment with 10^{-7} M adiponectin increased by 68,8% adiponectin transcript content with respect to that found in control cultures (Fig. 6A). Pituitary adiponectin mRNA content remained increased after 24 h of exposure to the protein (Fig. 6B). In particular, 10^{-8} M adiponectin induced a 4-fold increase in its mRNA levels in 24 h-treated cultures with respect to control values.

Analysis of the effect of adiponectin on the expression of

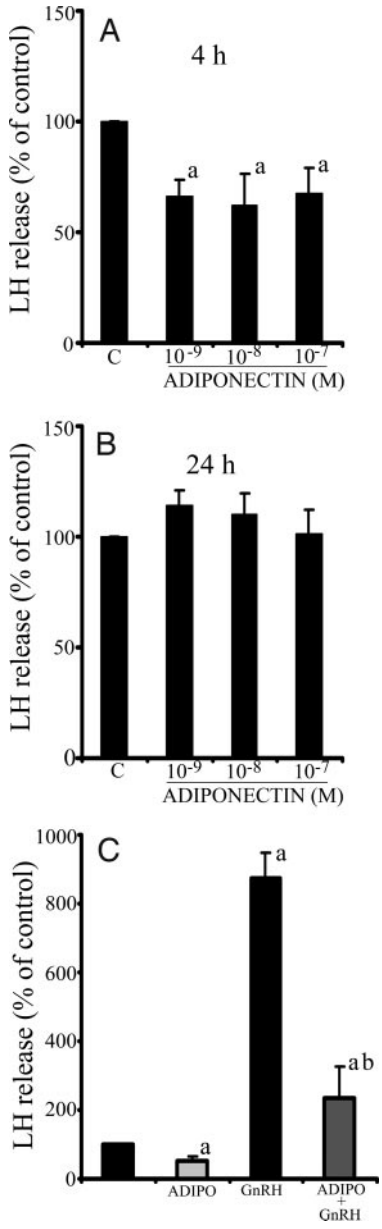

FIG. 3. Effect of adiponectin, alone (panels A and B) or in combination with GnRH (panel C), on LH release from cultured rat pituitary cells. After 3 d of culture in DMEM-FBS, cells were equilibrated for 3 h in serum-free DMEM and then incubated in the absence (C, Control) or presence of the corresponding test substances for the times indicated. A, Secretory response of rat pituitary cells to 4 h treatment with increasing doses (10^{-9}–10^{-7} M) of adiponectin. B, Secretory response of rat pituitary cells to 24 h treatment with increasing doses (10^{-9}–10^{-7} M) of adiponectin. C, Secretory response of rat pituitary cells to 4 h treatment with adiponectin (ADIPO; 10^{-7} M) and GnRH (10^{-8} M), alone or in combination. At the end of the incubations, culture media were recovered and LH release was evaluated by RIA. Data are expressed as a percentage of basal values in control cultures (100%, 25.1 ± 19 ng LH/ml, 34.3 ± 18 ng LH/ml, and 5.9 ± 1.4 ng LH/ml for Fig. 3, A–C, respectively) and are the mean (±SEM) of four (panel A) or three (panels B and C) separate experiments. a, $P < 0.05$ *vs.* corresponding control; b, $P < 0.05$ *vs.* GnRH alone.

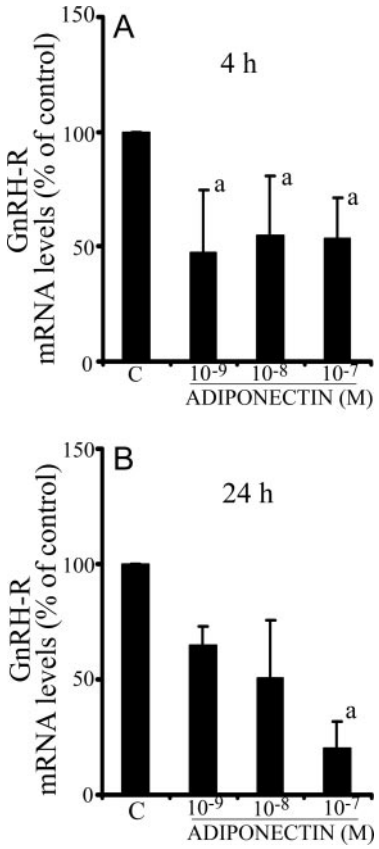

FIG. 4. Effect of adiponectin on mRNA levels of rat pituitary GnRH-R *in vitro*. After 3 d of culture, dispersed rat pituitary cells were incubated in medium alone (C, Control) or in the presence of 10^{-9}–10^{-7} M adiponectin for 4 h (panel A) or 24 h (panel B). Data are the mean (±SEM) of five (panel A) and three (panel B) independent experiments, each performed in triplicate. See Fig. 2 for further details. a, $P < 0.05$ *vs.* corresponding control.

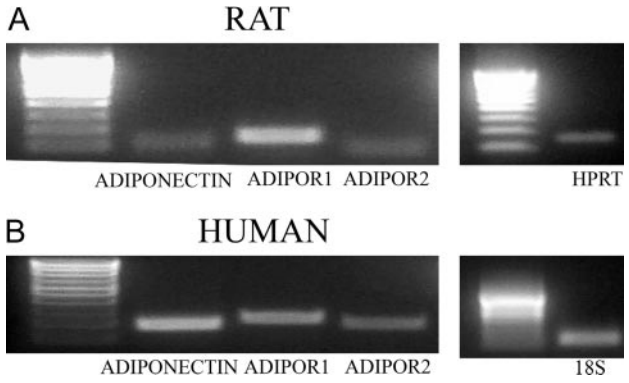

FIG. 5. Expression of adiponectin, AdipoR1 and AdipoR2 mRNA in rat (panel A) and human (panel B) pituitary extracts. Amplification products (110, 137, 109, and 150 bp, for rat adiponectin, AdipoR1, AdipoR2, and HPRT, respectively; 173, 210, 182, and 137 bp, for human adiponectin, AdipoR1, AdipoR2, and 18S, respectively) were sequenced to ensure correct amplification. Amplification of HPRT or 18S served as internal controls.

AdipoR1 and AdipoR2 showed that short-term (4 h) administration of the adipokine did not modify mRNA levels of either of the two receptors at any of the doses examined (Fig. 6, C and E). However, 24 h of exposure of pituitary cell cultures to adiponectin induced a significant decrease in AdipoR1 mRNA content when administered at 10^{-8} M (Fig. 6D). In contrast, AdipoR2 mRNA levels increased in cultures exposed to 10^{-7} M of the adipokine for 24 h (Fig. 6F).

Discussion

In the present study, we have shown that adiponectin regulates hormone secretion and gene expression in two endocrine cell types of the rat pituitary, somatotrophs and gonadotrophs, *in vitro*. In addition, we provide evidence demonstrating that both adiponectin and the two adiponectin receptors, AdipoR1 and AdipoR2, are expressed in the pituitary, thus indicating the existence of a local regulatory system for this adipokine at the pituitary level.

The effect of adiponectin on somatotrophs was assessed by analyzing both GH secretion and the expression of key somatotroph receptors, namely the GHRH receptor and the ghrelin/GHS receptor (39–41), in rat pituitary cell cultures after both short- and long-term exposure to the adipokine. Our results demonstrate that adiponectin inhibited basal GH release from the rat pituitary, an effect that was noticeable in short-term-treated cultures at adiponectin doses as low as 10^{-9} M. In contrast to its inhibitory action on GH secretion, adiponectin increased both GHS-R and GHRH-R mRNA levels. Taken together, these results strongly support the idea that adiponectin regulates somatotroph cell function. In particular, adiponectin would play a dual short-term action; thus, it decreases hormone secretion while up-regulating mRNA levels of the two main stimulatory receptors in somatotrophs. These results suggest that adiponectin, which would primarily act as an inhibitor of GH release, additionally and rapidly activates a compensatory mechanism in somatotrophs that might facilitate the response of the cells to future stimulation by GHRH and/or ghrelin. In line with these findings, the GH secreto-inhibitor somatostatin increased GHS-R levels in 4 h-treated porcine pituitary cell cultures, although the peptide did not modify GHRH-R expression (42). Interestingly, factors known to stimulate GH release such as GHRH, ghrelin and/or synthetic GHSs decrease pituitary GHRH-R and GHS-R transcript content, respectively, in the short-term (42–44).

Simultaneous treatment of rat pituitary cells with adiponectin and the somatotroph regulators ghrelin and GHRH revealed that, whereas the adipokine reduced the stimulatory effect of ghrelin on GH secretion, it did not modify GHRH-induced GH release. The discrepancy between adiponectin effects on ghrelin- and GHRH-induced GH secretion might be related to the selective activation of distinct signaling routes by the ghrelin/GHS-R system (*i.e.* phospholipase C/inositol phosphate/protein kinase C) and the GHRH/GHRH-R system (*i.e.* adenylate cyclase/cAMP/protein kinase A) (reviewed by Ref. 33) which, in turn, would be differentially counteracted by adiponectin. Additionally, the marked stimulatory effect of adiponectin on GHRH-R mRNA expression in short-term cultures, which was indeed considerably higher than that evoked by the adipokine on ghrelin/GHS-R mRNA levels, might also contribute to the differences observed in the interaction between adiponectin and the two peptides on GH release.

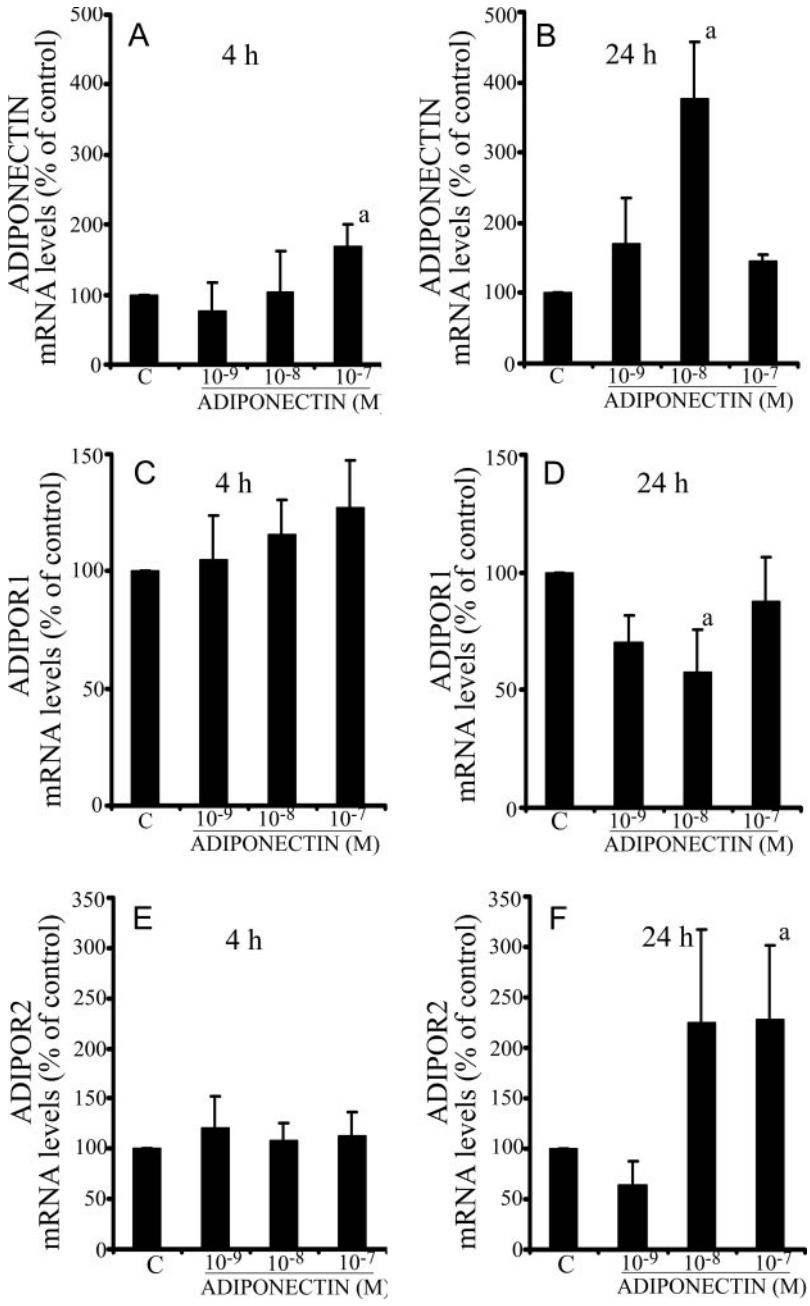

FIG. 6. Effect of adiponectin on mRNA levels of rat pituitary adiponectin (panels A and B), AdipoR1 (panels C and D), and AdipoR2 (panels E and F) *in vitro*. After 3 d of culture, dispersed rat pituitary cells were incubated in medium alone (C, Control) or in the presence of 10^{-9}–10^{-7} M adiponectin for 4 h (A, C, and E) or 24 h (panels B, D, and F). Data are the mean (±SEM) of four (panel A), three (panel B) or five (panels C–F) separate experiments. At least three replicate wells were evaluated per treatment in each experiment. See Fig. 2 for further details. a, $P < 0.05$ *vs.* corresponding control.

The stimulatory action of adiponectin on the expression of receptors that mediate positive cellular responses seems to be specific for somatotrophs because it was not observed for mRNA expression of the primary stimulatory receptor of gonadotrophs, the GnRH receptor (45, 46). Conversely, the adipokine caused a marked decrease in GnRH-R mRNA levels in rat pituitary cultures despite the fact that, similar to that found for GH release, it inhibited LH secretion. When viewed together, these results indicate that adiponectin acts on gonadotrophs by reducing hormone release as well as gonadotroph sensitivity to GnRH and can therefore be considered as a potent negative regulator of this cell type. In line with this idea are our results on the combined administration of adiponectin and GnRH demonstrating that the adipokine is also able to reduce significantly GnRH-induced LH release. It is important to note that, whereas a considerable number of peptides, including various neuropeptides and cytokines as well as the adipokine leptin, have been shown to exert direct stimulatory effects on gonadotropin release (47, 48), only certain opioid agonists have been reported so far to inhibit spontaneous and GnRH-induced LH release by the anterior pituitary *in vitro* (49), thus highlighting the relevance of our findings supporting a role for adiponectin as a novel inhibitor of gonadotroph cell function. Moreover, data from 24 h-treated cultures indicate that adiponectin induces long-acting inhibition of gonadotrophs, at least in terms of gene expression. In contrast, the effect of adiponectin on mRNA levels of somatotroph receptors disappeared in long-term-

treated cultures, wherein an increase in GH release in response to high-dose adiponectin was actually observed. In sum, our data demonstrate that adiponectin, by acting directly at the pituitary, regulates both somatotroph and gonadotroph cells and that this regulation is cell type specific and time dependent.

Further support for a regulatory role of adiponectin at the pituitary is provided by our findings that both human and rat pituitary gland express the two adiponectin receptors, AdipoR1 and AdipoR2, which confirm and extend recent observations from chicken pituitary (50). Likewise, in line with previous data obtained from chicken (29), we provide evidence showing that adiponectin is also expressed in the pituitary of both rat and human, thus suggesting that this gland may represent an important site of adiponectin production and action in vertebrates. Together, these findings demonstrate the coexistence of adiponectin and its cognate receptors in the pituitary, an observation that mirrors those found in other hormone-producing tissues including fat (24, 31) or placenta (25). Furthermore, taken as a whole, our data strongly support the existence of an autocrine/paracrine loop for adiponectin regulation in the pituitary. Consistent with this notion, we demonstrate that adiponectin not only controls GH and LH production but also regulates its own expression, as well as that of AdipoR1 and AdipoR2. To be more specific, adiponectin exerted a positive feedback on its mRNA content as well as on AdipoR2 transcript level, which likely reinforces the effects of this adipokine on the pituitary, whereas it decreased the expression of AdipoR1 in the long-term. Interestingly, a recent report has shown that adiponectin and AdipoR2 expression levels in human placenta exhibited parallel changes in response to *in vitro* treatment with several cytokines, whereas AdipoR1 expression followed an opposite pattern of response (25). When viewed together, these results indicate that AdipoR1 and AdipoR2 are differentially regulated in these tissues, thus suggesting that they may act on different targets and/or mediate distinct actions of adiponectin.

Previous studies have demonstrated that pituitary somatotrophs and gonadotrophs are also responsive to leptin (reviewed in Ref. 48) although, in contrast to that found herein for adiponectin, it stimulates LH and GH secretion (51) and decreases pituitary GHRH-R mRNA levels (52). In addition, leptin also indirectly regulates the somatotropic and gonadotropic systems through its action on the hypothalamus (reviewed in Refs. 53–55). Although some controversy exists on the ability of adiponectin to cross the blood-brain barrier (23, 56), the recent demonstration of the presence of adiponectin mRNA in the chicken diencephalon (29), together with the expression of the two adiponectin receptors in the hypothalamus in mouse (23), would support a central effect of adiponectin. Nevertheless, further studies are required to elucidate whether adiponectin, as leptin, acts at the hypothalamic level to regulate GH and LH production.

In addition to the potential autocrine/paracrine role of locally produced adiponectin, systemic adiponectin may also regulate pituitary cell function in an endocrine manner. Indeed, our results show that adiponectin exerts its effects on pituitary hormone release *in vitro* at doses equal or $<10^{-7}$ M, which fits well within the concentration range of circulating adiponectin [1.5–3 $\times$ 10^{-7} M in rodents (7)]. In the case of somatotrophs, these observations suggest that a functional link is in place between adiponectin from fat depots and the somatotropic axis that may be relevant for the control of metabolism and growth. Likewise, some evidence exists supporting an interaction between adiponectin and the gonadotropic axis, including the observation that transgenic female mice expressing high circulating adiponectin levels are infertile (57). In view of our data on the inhibitory action of adiponectin on gonadotrophs from male rat, it is tempting to speculate that the adverse effect of adiponectin overexpression on fertility observed in transgenic female mice might be due, at least in part, to a direct action of this adipokine on the pituitary. In line with this notion, the reduction in LH secretion (58) as well as in GH pulses (59) seen during fasting in rats might be explained by the increase in adiponectin serum levels observed in response to weight loss (8), which would support a role for adiponectin as a mediator in the adaptation of pituitary function to fasting. In all, these data strongly suggest that adiponectin may serve as a signal that links metabolic status and endocrine control of reproduction and growth, as has been proposed for leptin (48). Interestingly, circulating levels of leptin are inversely correlated to those of adiponectin in obese and lean animals (6, 7, 48). This, together with the divergent actions of these adipokines on both somatotrophs and gonadotrophs, supports the view that these two adipokines convey different metabolic signals to the somatotropic and reproductive axes.

In summary, our results demonstrate that the pituitary expresses the components of the adiponectin/AdipoR system and that this expression is under the regulation of adiponectin itself. Furthermore, our data show that adiponectin inhibits both GH and LH release from the rat pituitary *in vitro* and modulates the response of somatotrophs and gonadotrophs to their primary stimulatory factors. Taken together, these results suggest that the pituitary constitutes a relevant site of action for adiponectin and support a role for this adipokine as a link in the regulation of metabolism, growth, and reproduction. Current studies in our laboratory on the identification of the cellular distribution of adiponectin receptors as well as of the source of adiponectin in the pituitary will likely help to understand the role of this adipokine in pituitary hormone regulation.

Acknowledgments

Received July 28, 2006. Accepted October 5, 2006.

Address all correspondence and requests for reprints to: Dr. María M. Malagón, Department of Cell Biology, Physiology and Immunology, Edificio Severo Ochoa, Pl. 3, Campus de Rabanales, University of Córdoba, E-14014 Córdoba, Spain. E-mail: bc1mapom@uco.es.

This work was supported by CVI-0139 (Plan Andaluz de Investigación, Junta de Andalucía, Spain), BFU2004-03883 (Ministerio de Educación y Ciencia, Spain), and Centro de Investigación Biomédica en Red Obesity and Nutrition (Instituto de Salud Carlos III, Spain).

Disclosure Statement: The authors have nothing to disclose

References

1. **Rajala MW, Scherer PE** 2003 Minireview: the adipocyte—at the crossroads of energy homeostasis, inflammation, and atherosclerosis. Endocrinology 144: 3765–3773

2. **Trayhurn P** 2005 Endocrine and signalling role of adipose tissue: new perspectives on fat. Acta Physiol Scand 184:285–293
3. **Scherer PE, Williams S, Fogliano M, Baldini G, Lodish HF** 1995 A novel serum protein similar to C1q, produced exclusively in adipocytes. J Biol Chem 270:26746–26749
4. **Maeda K, Okubo K, Shimomura I, Funahashi T, Matsuzawa Y, Matsubara K** 1996 cDNA cloning and expression of a novel adipose specific collagen-like factor, apM1 (adiPose most abundant gene transcript 1). Biochem Biophys Res Commun 221:286–289
5. **Nakano Y, Tobe T, Choi-Miura NH, Mazda T, Tomita M** 1996 Isolation and characterization of GBP28, a novel gelatin-binding protein purified from human plasma. J Biochem (Tokyo) 120:803–812
6. **Hu E, Liang P, Spiegelman BM** 1996 AdipoQ is a novel adipose-specific gene dysregulated in obesity. J Biol Chem 271:10697–10703
7. **Arita Y, Kihara S, Ouchi N, Takahashi M, Maeda K, Miyagawa J, Hotta K, Shimomura I, Nakamura T, Miyaoka K, Kuriyama H, Nishida M, Yamashita S, Okubo K, Matsubara K, Muraguchi M, Ohmoto Y, Funahashi T, Matsuzawa Y** 1999 Paradoxical decrease of an adipose-specific protein, adiponectin, in obesity. Biochem Biophys Res Commun 257:79–83
8. **Hotta K, Funahashi T, Arita Y, Takahashi M, Matsuda M, Okamoto Y, Iwahashi H, Kuriyama H, Ouchi N, Maeda K, Nishida M, Kihara S, Sakai N, Nakajima T, Hasegawa K, Muraguchi M, Ohmoto Y, Nakamura T, Yamashita S, Hanafusa T, Matsuzawa Y** 2000 Plasma concentrations of a novel, adipose-specific protein, adiponectin, in type 2 diabetic patients. Arterioscler Thromb Vasc Biol 20:1595–1599
9. **Berg AH, Combs TP, Du X, Brownlee M, Scherer PE** 2001 The adipocyte-secreted protein Acrp30 enhances hepatic insulin action. Nat Med 7:947–953
10. **Combs TP, Berg AH, Obici S, Scherer PE, Rossetti L** 2001 Endogenous glucose production is inhibited by the adipose-derived protein Acrp30. J Clin Invest 108:1875–881
11. **Wu X, Motoshima H, Mahadev K, Stalker TJ, Scalia R, Goldstein BJ** 2003 Involvement of AMP-activated protein kinase in glucose uptake stimulated by the globular domain of adiponectin in primary rat adipocytes. Diabetes 52: 1355–1363
12. **Tomas E, Tsao TS, Saha AK, Murrey HE, Zhang Cc C, Itani SI, Lodish HF, Ruderman NB** 2002 Enhanced muscle fat oxidation and glucose transport by ACRP30 globular domain: acetyl-CoA carboxylase inhibition and AMP-activated protein kinase activation. Proc Natl Acad Sci USA 99:16309–16313
13. **Yamauchi T, Kamon J, Minokoshi Y, Ito Y, Waki H, Uchida S, Yamashita S, Noda M, Kita S, Ueki K, Eto K, Akanuma Y, Froguel P, Foufelle F, Ferre P, Carling D, Kimura S, Nagai R, Kahn BB, Kadowaki T** 2002 Adiponectin stimulates glucose utilization and fatty-acid oxidation by activating AMP-activated protein kinase. Nat Med 8:1288–1295
14. **Ouchi N, Shibata R, Walsh K** 2006 Cardioprotection by adiponectin. Trends Cardiovasc Med 16:141–146
15. **Gable DR, Hurel SJ, Humphries SE** 2006 Adiponectin and its gene variants as risk factors for insulin resistance, the metabolic syndrome and cardiovascular disease. Atherosclerosis 188:231–244
16. **Brakenhielm E, Veitonmaki N, Cao R, Kihara S, Matsuzawa Y, Zhivotovsky B, Funahashi T, Cao Y** 2004 Adiponectin-induced antiangiogenesis and antitumor activity involve caspase-mediated endothelial cell apoptosis. Proc Natl Acad Sci USA 101:2476–2481
17. **Kelesidis I, Kelesidis T, Mantzoros CS** 2006 Adiponectin and cancer: a systematic review. Br J Cancer 94:1221–1225
18. **Luo XH, Guo LJ, Yuan LQ, Xie H, Zhou HD, Wu XP, Liao EY** 2005 Adiponectin stimulates human osteoblasts proliferation and differentiation via the MAPK signaling pathway. Exp Cell Res 309:99–109
19. **Yamauchi T, Kamon J, Ito Y, Tsuchida A, Yokomizo T, Kita S, Sugiyama T, Miyagishi M, Hara K, Tsunoda M, Murakami K, Ohteki T, Uchida S, Takekawa S, Waki H, Tsuno NH, Shibata Y, Terauchi Y, Froguel P, Tobe K, Koyasu S, Taira K, Kitamura T, Shimizu T, Nagai R, Kadowaki T** 2003 Cloning of adiponectin receptors that mediate antidiabetic metabolic effects. Nature 423:762–769
20. **Kharroubi I, Rasschaert J, Eizirik DL, Cnop M** 2003 Expression of adiponectin receptors in pancreatic β cells. Biochem Biophys Res Commun 312:1118–1122
21. **Motoshima H, Wu X, Mahadev K, Goldstein BJ** 2004 Adiponectin suppresses proliferation and superoxide generation and enhances eNOS activity in endothelial cells treated with oxidized LDL. Biochem Biophys Res Commun 315:264–271
22. **Berner HS, Lyngstadaas SP, Spahr A, Monjo M, Thommesen L, Drevon CA, Syversen U, Reseland JE** 2004 Adiponectin and its receptors are expressed in bone-forming cells. Bone 35:842–849
23. **Qi Y, Takahashi N, Hileman SM, Patel HR, Berg AH, Pajvani UB, Scherer PE, Ahima RS** 2004 Adiponectin acts in the brain to decrease body weight. Nat Med 10:524–529
24. **Nilsson L, Binart N, Bohlooly YM, Bramnert M, Egecioglu E, Kindblom J, Kelly PA, Kopchick JJ, Ormandy CJ, Ling C, Billig H** 2005 Prolactin and growth hormone regulate adiponectin secretion and receptor expression in adipose tissue. Biochem Biophys Res Commun 331:1120–1126
25. **Chen J, Tan B, Karteris E, Zervou S, Digby J, Hillhouse EW, Vatish M, Randeva HS** 2006 Secretion of adiponectin by human placenta: differential modulation of adiponectin and its receptors by cytokines. Diabetologia 49: 1292–1302
26. **Ding ST, Liu BH, Ko YH** 2004 Cloning and expression of porcine adiponectin and adiponectin receptor 1 and 2 genes in pigs. J Anim Sci 82:3162–3174
27. **Punyadeera C, Zorenc AH, Koopman R, McAinch AJ, Smit E, Manders R, Keizer HA, Cameron-Smith D, van Loon LJ** 2005 The effects of exercise and adipose tissue lipolysis on plasma adiponectin concentration and adiponectin receptor expression in human skeletal muscle. Eur J Endocrinol 152:427–436
28. **Pineiro R, Iglesias MJ, Gallego R, Raghay K, Eiras S, Rubio J, Dieguez C, Gualillo O, Gonzalez-Juanatey JR, Lago F** 2005 Adiponectin is synthesized and secreted by human and murine cardiomyocytes. FEBS Lett 579:5163–5169
29. **Maddineni S, Metzger S, Ocon O, Hendricks 3rd G, Ramachandran R** 2005 Adiponectin gene is expressed in multiple tissues in the chicken: food deprivation influences adiponectin messenger ribonucleic acid expression. Endocrinology 146:4250–4256
30. **Jorgensen JO, Vestergaard E, Gormsen L, Jessen N, Norrelund H, Christiansen JS, Moller N** 2005 Metabolic consequences of GH deficiency. J Endocrinol Invest 28:47–51
31. **Fasshauer M, Klein J, Kralisch S, Klier M, Lossner U, Bluher M, Paschke R** 2004 Growth hormone is a positive regulator of adiponectin receptor 2 in 3T3–L1 adipocytes. FEBS Lett 558:27–32
32. **Brann DW, Wade MF, Dhandapani KM, Mahesh VB, Buchanan CD** 2002 Leptin and reproduction. Steroids 67:95–104
33. **Pombo M, Pombo CM, Garcia A, Caminos E, Gualillo O, Alvarez CV, Casanueva FF, Dieguez C** 2001 Hormonal control of growth hormone secretion. Horm Res 55(Suppl 1):11–16
34. **Millar RP** 2003 GnRH II and type II GnRH receptors. Trends Endocrinol Metab 14:35–43
35. **Dobado-Berrios PM, Ruiz-Navarro A, Almaden Y, Malagon MM, Garrido JC, Ramirez-Gutierrez JL, Gracia-Navarro F** 1996 Heterogeneity of growth hormone (GH)-producing cells in aging male rats: ultrastructure and GH gene expression in somatotrope subpopulations. Mol Cell Endocrinol 118:181–191
36. **Kojima M, Hosoda H, Date Y, Nakazato M, Matsuo H, Kangawa K** 1999 Ghrelin is a growth-hormone-releasing acylated peptide from stomach. Nature 402:656–660
37. **Tena-Sempere M, Pinilla L, Aguilar E** 1996 In vitro pituitary GH secretion after GHRH, forskolin, dibutyryl cyclic-adenosine 3′,5′-monophosphate and phorbol 12-myristate 13-acetate stimulation in long-term orchidectomized rats. J Mol Endocrinol 16:81–88
38. **Tsujii T, Ishizaka K, Winters SJ** 1994 Effects of pituitary adenylate cyclase-activating polypeptide on gonadotropin secretion and subunit messenger ribonucleic acids in perifused rat pituitary cells. Endocrinology 135:826–833
39. **Howard AD, Feighner SD, Cully DF, Arena JP, Liberator PA, Rosenblum CI, Hamelin M, Hreniuk DL, Palyha OC, Anderson J, Paress PS, Diaz C, Chou M, Liu KK, McKee KK, Pong SS, Chaung LY, Elbrecht A, Dashkevicz M, Heavens R, Rigby M, Sirinathsinghji DJ, Dean DC, Melillo DG, Patchett AA, Nargund R, Griffin PR, DeMartino JA, Gupta SK, Schaeffer JM, Smith RG, Van der Ploeg LH** 1996 A receptor in pituitary and hypothalamus that functions in growth hormone release. Science 273:974–977
40. **Muller EE, Locatelli V, Cocchi D** 1999 Neuroendocrine control of growth hormone secretion. Physiol Rev 79:511–607
41. **Petersenn S, Schulte HM** 2000 Structure and function of the growth-hormone-releasing hormone receptor. Vitam Horm 59:35–69
42. **Luque RM, Kineman RD, Park S, Peng XD, Gracia-Navarro F, Castano JP, Malagon MM** 2004 Homologous and heterologous regulation of pituitary receptors for ghrelin and growth hormone-releasing hormone. Endocrinology 145:3182–3189
43. **Aleppo G, Moskal 2nd SF, De Grandis PA, Kineman RD, Frohman LA** 1997 Homologous down-regulation of growth hormone-releasing hormone receptor messenger ribonucleic acid levels. Endocrinology 138:1058–1065
44. **Kineman RD, Kamegai J, Frohman LA** 1999 Growth hormone (GH)-releasing hormone (GHRH) and the GH secretagogue (GHS), L692,585, differentially modulate rat pituitary GHS receptor and GHRH receptor messenger ribonucleic acid levels. Endocrinology 140:3581–3586
45. **Rispoli LA, Nett TM** 2005 Pituitary gonadotropin-releasing hormone (GnRH) receptor: structure, distribution and regulation of expression. Anim Reprod Sci 88:57–74
46. **Cheng CK, Leung PC** 2005 Molecular biology of gonadotropin-releasing hormone (GnRH)-I, GnRH-II, and their receptors in humans. Endocr Rev 26:283–306
47. **Evans JJ, Janmohamed S** 1999 In vitro basal and GnRH-stimulated secretion of gonadotrophins reflects long-lasting modulatory effects, and peripheral levels are not predicted by pituitary responsiveness to GnRH. Eur J Endocrinol 141:512–520
48. **Zieba DA, Amstalden M, Williams GL** 2005 Regulatory roles of leptin in reproduction and metabolism: a comparative review. Domest Anim Endocrinol 29:166–185
49. **Dragatsis I, Papazafiri P, Zioudrou C, Gerozissis K** 1995 Opioids modify the release of LH at the pituitary level: in vitro studies with entire rat pituitaries. J Endocrinol 145:263–270
50. **Ramachandran R, Ocon-Grove OM, Metzger SL** 2 May 2006 Molecular clon-

ing and tissue expression of chicken AdipoR1 and AdipoR2 complementary deoxyribonucleic acids. Domest Anim Endocrinol, 16697136
51. **Mizuno I, Okimura Y, Takahashi Y, Kaji H, Abe H, Chihara K** 1999 Leptin stimulates basal and GHRH-induced GH release from cultured rat anterior pituitary cells in vitro. Kobe J Med Sci 45:221–227
52. **Roh SG, Nie GY, Loneragan K, Gertler A, Chen C** 2001 Direct modification of somatotrope function by long-term leptin treatment of primary cultured ovine pituitary cells. Endocrinology 142:5167–5171
53. **Dieguez C, Carro E, Seoane LM, Garcia M, Camina JP, Senaris R, Popovic V, Casanueva FF** 2000 Regulation of somatotroph cell function by the adipose tissue. Int J Obes Relat Metab Disord 24(Suppl 2):S100–S103
54. **Chan JL, Mantzoros CS** 2001 Leptin and the hypothalamic-pituitary regulation of the gonadotropin-gonadal axis. Pituitary 4:87–92
55. **Barb CR, Kraeling RR** 2004 Role of leptin in the regulation of gonadotropin secretion in farm animals. Anim Reprod Sci 82–83:155–167
56. **Spranger J, Verma S, Gohring I, Bobbert T, Seifert J, Sindler AL, Pfeiffer A, Hileman SM, Tschop M, Banks WA** 2006 Adiponectin does not cross the blood-brain barrier but modifies cytokine expression of brain endothelial cells. Diabetes 55:141–147
57. **Combs TP, Pajvani UB, Berg AH, Lin Y, Jelicks LA, Laplante M, Nawrocki AR, Rajala MW, Parlow AF, Cheeseboro L, Ding YY, Russell RG, Lindemann D, Hartley A, Baker GR, Obici S, Deshaies Y, Ludgate M, Rossetti L, Scherer PE** 2004 A transgenic mouse with a deletion in the collagenous domain of adiponectin displays elevated circulating adiponectin and improved insulin sensitivity. Endocrinology 145:367–383
58. **Castellano JM, Navarro VM, Fernandez-Fernandez R, Nogueiras R, Tovar S, Roa J, Vazquez MJ, Vigo E, Casanueva FF, Aguilar E, Pinilla L, Dieguez C, Tena-Sempere M** 2005 Changes in hypothalamic KiSS-1 system and restoration of pubertal activation of the reproductive axis by kisspeptin in undernutrition. Endocrinology 146:3917–3925
59. **Carro E, Senaris R, Considine RV, Casanueva FF, Dieguez C** 1997 Regulation of *in vivo* growth hormone secretion by leptin. Endocrinology 138:2203–2206

***Endocrinology* is published monthly by The Endocrine Society (http://www.endo-society.org), the foremost professional society serving the endocrine community.**