FEBS 29372 FEBS Letters 579 (2005) 1911–1916

Similarities and differences in the transcriptional regulation of the leptin gene promoter in gastric and adipose cells

Hélène Goïot[a,1], Jean-Pierre Laigneau[a], Hélène Devaud[a], Iradj Sobhani[b], André Bado[a,*]

[a] *INSERM 683, Faculté de Médecine Xavier Bichat, 16 rue Henri Huchard, 75018 Paris, France*
[b] *CHU Henri Mondor, 51 Av. Du Gal. De Lattre de Tassigny, 94000 Créteil, France*

Received 14 December 2004; revised 26 January 2005; accepted 4 February 2005

Available online 24 February 2005

Edited by Robert Barouki

Abstract The stomach was reported to synthesize and secrete leptin mainly in the gastric lumen. Gastric leptin release is markedly increased after food intake, by vagal cholinergic stimulation and by cholecystokinin and secretin. Here we show that human gastric MKN-74 cells produce leptin that increases upon challenge with cholecystokinin, insulin, glucocorticoids and all-trans retinoic acid through activation of the leptin gene promoter. In addition, we demonstrate that forskolin and BRL37344 which increased cAMP levels, fail to affect the activity of leptin gene promoter in MKN74 expressing β_3-adrenoceptor cells but, induce a 2-fold decrease in this activity in adipose 3T3-L1 cells. These data described for the first time, similarities and more interestingly, differences in the regulation of the leptin gene promoter in gastric cells as compared to adipocytes.

Keywords: Stomach; Leptin; Transcriptional effect; cAMP, cholecystokinin; MKN-74 cell; Adipocyte cell

1. Introduction

Leptin, a 16 kDa protein that is mainly produced by adipose tissue [1], has been involved in the control of energy balance via its hypothalamic receptor (Lep-R) [2,3]. Actually, leptin can be considered as a multifunctional hormone that regulates not only body weight homeostasis, but also neuroendocrine function, fertility, immune function, and angiogenesis. This current status of leptin is consistent with its production by various tissues and organs such as the placenta [4], the skeletal muscles [5], the pituitary cells [6] and the stomach [7,8]. In the stomach, leptin is mainly secreted into the gastric lumen and some of the stomach-derived leptin reaches the intestine in an active form [9–11]. Thus, it was reported that gastric leptin entering the intestine can increase the absorption of oligopeptides through the brush-border transporter PepT1 [12], and enhance the uptake of butyrate via the monocarboxylate carrier MCT-1 [13]. These data argue for a physiological role of gastric leptin in the control of intestinal functions.

The regulation of the leptin gene expression in adipose cells has been extensively investigated and the molecular mechanisms underlying such a regulation defined [14,15]). Adipose leptin mRNA levels are increased by glucocorticoids [16,17] [18] and insulin [19–21] and decreased by β-adrenergic agonists [22–24]. Leptin gene expression is induced by the adipocyte transcription factor, C/EBPα [25–27] an effect consistent with the identification of C/EBP-binding site on the proximal region of the leptin promoter [28]. In contrast, ligands and activators of peroxisome proliferator-activated receptors gamma (PPARγ) that heterodimerize with retinoid X receptors, bind to PP responsive element of the leptin promoter and reduce expression of leptin [29]. Very few studies have reported that CCK stimulates gastric leptin secretion in vivo in the rat [7] and expression in isolated canine chief cells in vitro [30]. Moreover, leptin secretion was enhanced after infusion of secretin, an acid inhibitory hormone, [9] and after vagal stimulation [10] in humans. However, to our knowledge, there is no report on the regulation of the leptin gene promoter in the stomach.

The present study was undertaken to examine the hormonal regulation of leptin's gene promoter expression in gastric MKN-74 in comparison to adipose 3T3-L1 cells. The two nuclear receptors, retinoid acid receptors (RARs) and the X receptors (RXRs) [31,32] are well known to serve as active partners of peroxisome proliferator-activated receptors (PPARs) which have been involved in leptin expression. Thus, we determine whether an activation of these receptors by all-trans retinoic acid (ATRA) could affect the leptin promoter in gastric cells. The data reported in this study, constitute the first demonstration of the similarities and more interestingly the differences in the regulation of the leptin gene promoter in gastric cells as compared to adipocytes.

2. Materials and methods

2.1. Materials

Dulbecco's modified Eagle's medium with Glutamax-I, fetal calf serum, penicillin–streptomycin, EDTA-Trypsin, Lipofectamine and insulin were obtained from Life Technologies (Cergy-Pontoise, France). Cholecystokinin-octapeptide (CCK-8), all-trans retinoic acid (ATRA), dexamethasone (DXM), forskolin (FSK), bacitracin and 3-isobutyl-1-methylxanthine (IMBX) were purchased from Sigma Chemicals (Saint-Quentin Fallavier, France). The β_3-adrenoceptor agonist, BRL37344 was purchased from Interchim (Montluçon, France). Dual-luciferase Reporter® Assay System was purchased from Promega (Charbonnières, France).

*Corresponding author. Fax: +33 1 42 28 87 65.
E-mail address: bado@bichat.inserm.fr (A. Bado).

[1] Present address: Laboratoires BIOCODEX, Chemin d'Armacourt, 60200 Compiegne, France.

0014-5793/$30.00
doi:10.1016/j.febslet.2005.02.031

2.2. Cell culture

The human gastric cancer cell line MKN-74 has been established from a well-differentiated adenocarcinomas localized in the fundus [33,34]. MKN-74 cells were grown in Dulbecco's modified Eagle's medium with Glutamax-I plus 10% bovine calf serum, 100 IU/mL penicillin-Streptomycin at 37 °C in a humidified atmosphere (95% O_2/5% CO_2).

Preadipocytes 3T3-L1 cells were maintained in basal medium and induced to differentiate into adipocytes as described previously [35] in a medium consisting of basal medium supplemented with 500 nmol/L insulin, 250 nmol/L DXM, and 250 mmol/L isobutylmethylxanthine. Thereafter, fully differentiated 3T3-L1 adipocytes were maintained in basal medium and used for the experiments below.

2.3. RT-PCR analysis

Total RNA was isolated from MKN-74 and 3T3-L1 cells using the guanidium isothiocyanate-phenol-chloroform method as described by Chomczynski and Sacchi [36]. First-strand cDNA synthesis from 3 μg of total RNA was performed using a reverse transcriptase kit (Life Technologies, Inc), according to the manufacturer's instructions. The oligonucleotides primers used were: for LEP: f5′-CCCGGATTCCCT-GACCTTATCCAAGATGG-3′ and the r5′-CCCTCGAGGAGTAG-CCTGAAGCTTCCAG-3′ (Accession No. HUMFPOH2); for human CCK_1 receptor: f5′-CCCGAATTCTTGTACTCCTTGATATTCC-3′ and r5′- CCC CTC GAG TCA TGA TGG TAA AGG-3′ (Accession No. D85606); for human CCK_2 receptor: f5′-ATGCTCATCAT-CGTGGTCCTG-3′ and r5′-GTGCCCTACCCCGTGTACACT-3′ (Accession No. L07746); for human β_3-adrenoceptor: f5′-GCATGCT-CCGTGGCCTCACGAGAA-3′ and 5′-CTGGCTCATGATGGGC-GC-3′ (Accession No. X70812); for human insulin receptor: f5′-CAG-CGAGAAACTGCATGGT-3′ and r5′-CATTGGACATGGTAGAG-TCG-3′.

Samples were denatured at 95 °C for 3 min, then PCR was carried out for 35 cycles of 1 min denaturation at 95 °C, 1 min annealing at 60 °C, and 2 min extension at 72 °C. The amplification was terminated by a 10 min extension step at 72 °C. Reverse transcriptase was omitted from the controls to check that PCR amplification did not result from genomic DNA contamination. PCR products were separated by electrophoresis in a 2% agarose gel. The gel was stained with ethidium bromide and viewed under UV illumination. The expected sizes for PCR products were 241 bp for human *ob*, 398 bp for human CCK-1 receptor, 362 bp for human CCK-2 receptor; 525bp for β_3-adrenoceptor and 770 bp for human insulin receptor.

2.4. Immunocytochemistry

MKN-74 cells were cultured on Labtek® four compartments (Nunc, France) as described above for 48 h. The cells rinsed with PBS, fixed 10 min with 4% paraformaldehyde and treated 5 min with 3% H_2O_2 in distilled water to inhibit endogenous peroxidase activity. The cells were incubated overnight at 4 °C with rabbit polyclonal anti-human leptin antibody diluted at 1:50 (BioVendor GmH, Heidelberg, Germany). After washing, cells were further incubated with the affinity-purified biotinylated rabbit anti-goat IgG antibody (0.5%, Vector Laboratories Inc., CA, USA), for 40 min at room temperature, washed and then reacted with Vectastain® ABC Reagent (1%) for 30 min at RT. Immunohistochemical staining was performed using 3′,3′-diaminobenzidine hydrochloride as the peroxidase chromogen (Sigma, St Louis, MI, USA). Controls. No immunostaining was observed after prior incubation of leptin antiserum with the recombinant human leptin at 10 μg/mL overnight at 4 °C, and after omission of the primary antibody from the incubating medium.

2.5. Determination of leptin secretion

Gastric MKN-74 or adipose 3T3-L1 cells were cultured in 75 cm^2 culture flasks with 10 mL culture medium. At 80% confluence culture media were changed and cells were serum-deprived and incubated with or without various effectors for 12, 24, 48, and 96 h. The culture media were collected and stored at −80 °C. The cellular content was collected and washed with PBS. The cells were homogenized in a buffer consisting of HEPES 25 mM pH 7.8, 1% NP 40, PMSF 1 mM, Leupeptin, Pepstatin and Aprotinin at 1 μg/μL. Samples were centrifuged at 12000 × *g*, 10 min at 4 °C and supernatants were collected and stored at −20 °C until assayed. Leptin levels were determined using a human or mouse leptin radioimmunoassay (RIA) kit (Linco Research, St. Charles, MO, USA).

2.6. Transient transfections

The leptin reporter gene plasmid (pGL3-δOb2) contains 2400 bp of the human leptin gene 5′-flanking region ligand to the luciferase reporter gene [26] was used. Using Lipofectamine (Life Technologies, Inc), gastric MKN-74 and 3T3-L1 adipose cells (3×10^6) in 6-well culture plates, were co-transfected with 500 ng of a leptin reporter gene plasmid (pGL3-δOb2) and 1 ng of a dual luciferase reporter gene was used as a transfection control efficiency for 5 h in 2 mL/well of culture medium. Afterwards the cells were incubated with or without different drugs for 24 h. A Luciferase assay (Promega, Dual-Luciferase Reporter Assay System) was used to determine luciferase activity according to the manufacturer's instructions. This activity was then normalized to Dual Luciferase activity as an internal control. Transfection efficiency was monitored by evaluating the activity of the control vector pGL3-Basic. All transfections were performed in triplicate.

2.7. cAMP measurements

The levels of cAMP was measured as previously described in detail [37]. Briefly, MKN-74 cells were incubated for 1 h at 15 °C in phosphate-buffered saline (pH 7) containing 1.4% (w/v) bovine serum albumin, 0.1% bacitracin, and 0.2 mM 3-isobutyl-1-methylxanthine without or with 10 nM β_3-adrenoceptor agonist BRL37344 or 10 μM foskolin The reaction was stopped by addition of perchloric acid and centrifuged 10 min at 4000 × *g*. cAMP present in the supernatants were succinylated, and were measured by radioimmunoassay as described [37].

2.8. Statistical analysis

The data in each experiment were expressed as means ± S.E.M. They were analyzed by one-way analysis of variance (ANOVA) followed if significant, by Tukey–Kramer multiple comparison test. The differences were considered significant when P was <0.05.

3. Results

3.1. Gastric MKN-74 cells express leptin and CCK-receptors

By RT-PCR analysis, a 235 bp PCR product was amplified from MKN-74 total RNA extracts (Fig. 1A). cDNA sequencing (Genomexpress, Meylan, France) confirmed that this PCR product is 100% similar to the LEP gene.

Moreover, a 398 bp and a 362 bp PCR products (Fig. 1A) were amplified by RT-PCR. cDNA sequencing (Genom-express, Meylan, France) confirmed that these products were 100% similar to CCK-1 and CCK-2 receptor gene. Finally, PCR products of 525 and 770 bp were amplified from MKN-74 total RNA extracts which corresponded to human β_3-adrenoceptor and insulin receptor, respectively.

The leptin protein was evidenced by immunocytochemistry studies showing leptin immunoreactivity in the cytoplasm and on MKN-74 cell membrane (Fig. 1B). These cells secrete leptin in culture as a function of time (Fig. 1C) with a very low amount of leptin-immunoreactive protein at day 0 that increased by 2-fold and 4-fold after 12 and 48 h of cell culture.

Collectively, these data indicate that MKN-74 cells represent a suitable cell model for analyzing the regulation leptin gene's expression in response to various hormonal stimuli.

3.2. Regulation of leptin secretion from gastric MKN-74 and adipose 3T3-L1 cells

The basal secretion of leptin over 24 h was 0.17 ± 0.07 ng/mL which represented 2.3% of the total leptin content in gastric MKN-74 cells. In differentiated adipose 3T3-L1 cells, this basal secretion of leptin was 0.36 ± 0.1 ng/mL, also at very low levels. The addition of CCK-8 to gastric MKN-74 (Fig. 2A) and to differentiated adipose 3T3-L1 (Fig. 2B) cells induced

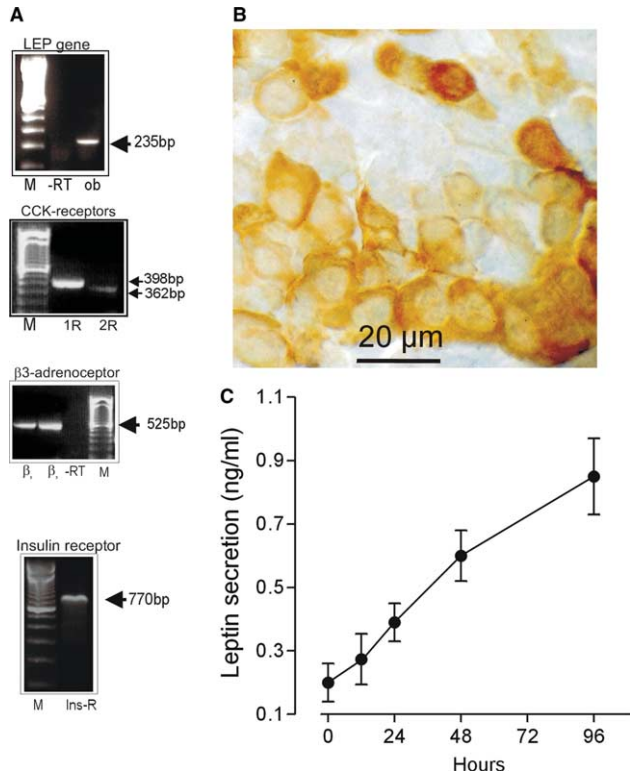

Fig. 1. MKN-74 cells expressed leptin and gut hormone receptors. (A) RT-PCR analysis from total RNA isolated from MKN-74 cells. *LEP gene:* -RT: omission of reverse transcriptase and PCR amplification with ob primers result in a PCR product of 241 bp. *CCK-receptors:* -RT: omission of reverse transcriptase; CCK_1-receptor (CCK-1R): 398 bp PCR product and CCK_2-receptor (CCK-2R): a 362 bp PCR product. *β_3-adrenoceptor*. lanes 1 and 2: PCR amplification with human β_3 primers result in a PCR product of 525 bp in two separate samples. *Insulin receptor:* PCR amplification with human insulin receptor primers result in a PCR product of 770 bp. (B) Immunodetection of leptin protein in human MKN-74 cells. Immunostaining was carried out with a specific antibody for leptin, followed by biotinylated second antibody, streptavidine-peroxydase and diaminobenzidine. Leptin immunoreactivity was visible in the intracytoplasmic compartments and on the cell membrane (bar = 20 µm). Note that there is no leptin immunostaining after preabsorption of anti-human leptin rabbit polyclonal antibody with the recombinant human leptin at 10 µg/mL overnight at 4 °C. (C) Leptin secretion by gastric MKN-74 cells. Cells were cultured as described in Section 2. The culture medium was sampled at the indicated period of time for leptin determination by radioimmunoassay (Linco Research, St. Charles, MO, USA). Each point represents the mean ± 1S.E.M. of four independent experiments performed in duplicate.

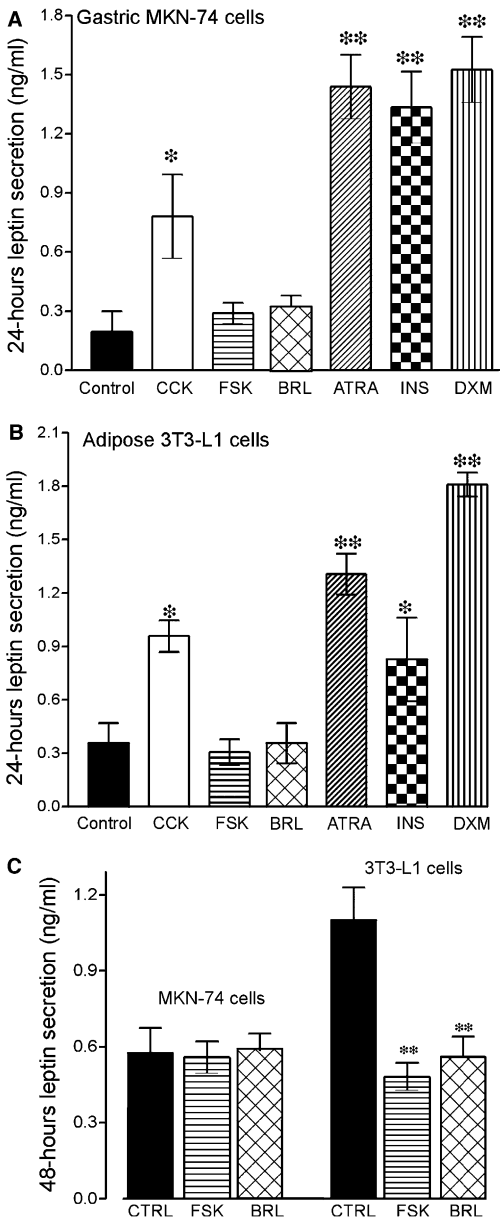

Fig. 2. Hormonal regulation of leptin secretion in gastric MKN-74 and in adipose 3T3-L1 cells. Gastric MKN-74 (A) and differentiated adipose 3T3-L1 cells (B) were treated for 24 h with or without 10 nM CCK8 (CCK), 10 nM BRL37344 (BRL), 20 nM insulin (INS), 100 nM dexamethasone (DXM), 10 µM forskolin (FSK) and 10 µM all-trans retinoic acid (ATRA). (C) The cells were treated for 48 h with or without 10 nM BRL37344 (BRL),or forskolin (FSK). 24-h (A, B) or 48-h (C) immunoreactive leptin release in the culture medium was determined by radioimmunoassay of leptin (RIA Kit fom Linco). Data represent means ± S.E.M. for five independent experiments performed in triplicate. $*P < 0.05$; $**P < 0.01$; $***P < 0.001$ vs. control.

a significant 3-fold increase in leptin secretion. In addition, insulin (20 nM), dexamethasone (DXM 100 nM) and ATRA (1 µM) induced a similar 4-fold increase in leptin secretion (Fig. 2A). However, in differentiated adipose 3T3-L1 cells, there was an hierarchy of efficiency with DXM, inducing a 6-fold, ATRA a 4-fold and insulin a 2-fold enhancement of basal secretion of leptin (Fig. 2B).

Finally, addition of forskolin or BRL-37344, the β_3-adrenoceptor agonist, did not show any significant effect on 24-h leptin secretion by gastric MKN-74 (Fig. 2A) and by differentiated adipose 3T3-L1 cells (Fig. 2B). However, after 48 h, forskolin or BRL-37344 did significantly reduced leptin secretion in differentiated 3T3-L1 cells but not, in gastric MKN-74 cells (Fig. 2C). We next examined whether these changes in the leptin's secretion in response to hormonal stimuli were associated with changes in the activity of the leptin gene promoter.

3.3. Activity of the leptin promoter in gastric MKN-74 and adipose 3T3-L1 cells

Transfection efficiency was monitored by evaluation of pGL3-Basic activity of, the control vector. In comparison to the promoterless parent vector, the LEP promoter pGL3-δOb2

increased up to 4-fold the luciferase expression in MKN-74 and to 1.5-fold in adipose 3T3-L1 cells.

In transfected gastric MKN-74 (Fig. 3A) or differentiated adipose 3T3-L1 (Fig. 3B) cells, 1 nM CCK-8 induced a 1.6- or 2.3-fold increase in luciferase activity. Dexamethasone (100 nM) was similarly efficient in increasing the luciferase activity (2.7-fold increase) in both MKN-74 and 3T3-L1 cells. In addition, upon challenge with 20 nM insulin, differentiated 3T3-L1 adipose as well as MKN-74 cells, exhibited a similar 1.7 fold increase in promoter activity. The addition of 1 μM ATRA induced a 2.5-fold increase in luciferase activity in gastric MKN-74 (Fig. 3A) and in differentiated adipose 3T3-L1 cells (Fig. 3B).

Neither the β_3-adrenergic receptor agonist BRL37344 nor the cAMP activator forskolin, had any effect on luciferase activity in gastric MKN-74 cells although they were able to increase cAMP levels (Fig. 3A). Indeed, basal levels of cAMP was 0.2 ± 0.7 pmol/mg protein in MKN-74 cells and these levels increased up to 18.2 ± 2.9 and 10.2 ± 3.7 pmol cAMP/mg protein ($P < 0.001$ vs CTRL) for 10 μM FSK and 10 nM BRL37344. On the other hand, luciferase activity in differentiated adipose 3T3-L1 cells were reduced by 2-fold by BRL37344 and by forskolin (Fig. 3B).

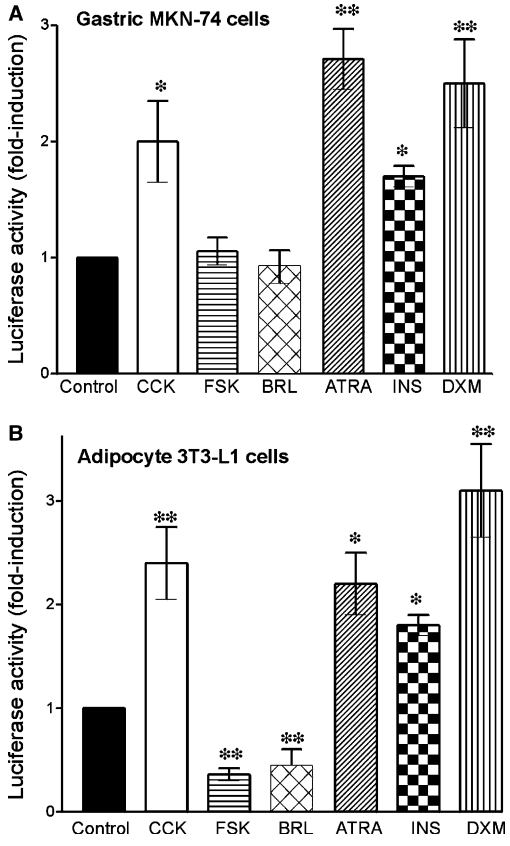

Fig. 3. Hormonal regulation of the human leptin promoter in gastric MKN-74 and in adipose 3T3-L1 cells. Luciferase reporter plasmid containing the leptin promoter or promoterless (basic) was transfected into gastric MKN-74 and differentiated adipose 3T3-L1 cells as described Section 2. The efficiency of the transfections studies monitored by evaluation of the control vector pGL3-Basic activity in comparison to the LEP promoter pGL3-δOb2 showed a 4-fold luciferase expression in MKN-74 and a 1.5-fold in adipose 3T3-L1 cells. The cells were treated with or without 10 nM CCK8 (CCK), 10 nM BRL37344 (BRL), 20 nM insulin (INS), 100 nM dexamethasone (DXM), 10 μM forskolin (FSK) and 10 μM all-trans retinoic acid (ATRA). Luciferase activity after 24 h was measured as described Section 2 and normalized to dual Luciferase activity as an internal control. The data calculated as ratio and expressed as means ± S.E.M. for five independent experiments performed in triplicate. *$P < 0.05$; **$P < 0.01$ vs. control.

4. Discussion

The human and rat stomach produce leptin that is mainly secreted in the gastric lumen [7,9]. The physiological role of this pool of leptin has been somewhat clarified by recent studies demonstrating that it regulates a number of intestinal functions (*for review* [38]) including the activation of the viscero-sensitive afferent neurones through leptin receptors on the vagus nerve [39,40]. By contrast to the adipocyte leptin gene, very few studies have concerned the regulation of stomach-derived leptin expression. Here, we show that gastric MKN-74 cells express not only leptin but also the intestinal hormone CCK-1 and CCK-2 receptors, the β_3-adrenoceptor and the insulin receptor. Therefore, this cell line represents a relevant and a suitable cellular model for analyzing the expression of the leptin gene in gastric epithelial cells in comparison to adipose cells.

First, we demonstrated that the intestinal food-inhibitory peptide CCK, activates LEP promoter and increases leptin secretion both in gastric MKN-74 and in differentiated adipose 3T3-L1 cells. These data are consistent with earlier reports showing that CCK increases leptin both in stomach and in the WAT [41] and, that meal-induced leptin secretion can be blocked by pre-treatment with CCK-receptors antagonists in rats [7,41]. These data are also in agreement with the only in vitro study showing that CCK can enhance leptin's expression via the high-affinity CCK-1 receptor in isolated canine gastric chief cells which are pepsinogen- and leptin-secreting cells [30]. Even though further studies are required to unravel the molecular mechanisms, CCK appears to be a key molecule in the control of the LEP promoter at least in the stomach.

Furthermore, we demonstrated that hormonal regulators of adipocyte leptin expression are also involved in the regulation of the stomach-derived leptin. Indeed, dexamethasone increases leptin secretion from gastric MKN-74 cells, an effect which is associated with an increase in the activity of LEP promoter. These data are consistent with in vivo as well as in vitro results, demonstrating that glucocorticoids increase leptin gene's expression and leptin secretion in humans or rats [42,43]. They fit well with the identification of a glucocorticoid response element on the leptin gene [44]. Since gastric mucosa expresses glucocorticoid receptors [45], it is likely that our data reflect a transcriptional mechanism. Similarly to adipocyte 3T3-L1 cells, insulin enhances leptin secretion in gastric MKN-74 cells through increased activity of the LEP promoter. The regulation of leptin gene's expression in adipose cells by insulin has been extensively investigated. It is generally assumed to have a stimulatory effect both in *vitro* and *in vivo* [21,46], through transcriptional mechanisms involving the insulin response sequence on the leptin promoter [47]. Our findings argue for a similar insulin regulatory pathway of leptin expression in gastric MKN-74 cells and by extension in the stomach.

Strong evidence exists to support a major role for retinoid receptors in the regulation of leptin expression in adipose tissue. The cellular response to retinoid acid is conferred by two distinct classes of nuclear receptors termed RARs and RXRs, with their transcriptional activity being reciprocally modulated by direct interactions of the two proteins [31]. On the other hand, peroxisome proliferator-activated receptors (PPARs) are well known to heterodimerize with nuclear retinoid receptors [48–50] and to display a ligand-dependent transcriptional activity through binding consensus DNA sequences or response elements within the regulatory regions of genes [44]. Previous studies demonstrated that the activation of peroxisome proliferator-activated receptors gamma (PPARγ), an adipocyte-specific and a lipid-activated transcription factor, potently reduced leptin gene's expression [29,51] through binding to PPRE within the leptin gene promoter [44]. In the present study, we clearly demonstrated that ATRA which binds RARs [52] potently increases the activity of the leptin gene promoter in gastric MKN-74 cells. These data may indicate that ATRA is actually operating as a functional inhibitor of RARs/PPARs binding to PPRE [53] as it was previously reported for RXR agonists such as LG100268 [54], 9-*cis* retinoic acid [52]. Our data are in line with the expression of PPARγ in these MKN cell lines [55] and, of both RARs and RXRs in the gastric mucosa [56]. Altogether, our data support a role of retinoic acid in leptin expression confirming in the stomach, the previous reports on adipocyte leptin.

One of the major finding in this study is that, increasing intracellular cAMP levels either by forskolin or by the β_3-adrenoceptor agonist BRL37344, affects neither LEP promoter activity nor protein secretion in gastric MKN-74 cells. On the other hand, the LEP promoter activity and leptin secretion is potently reduced in adipocyte 3T3-L1 cells. This result in adipose cells are consistent with earlier reports in which increased intracellular cAMP levels decreased expression of leptin mRNA and of leptin secretion in the rat [16,57]. Such a cAMP down-regulation of the leptin gene fits with the presence of the cAMP responsive element binding in the promoter of leptin gene [28]. The above results are also consistent with the reported β_3-adrenoceptor agonists powerful inhibitory effect on leptin gene expression and on leptin production in the rodent adipose tissue. The consensual inhibitory effect of cAMP on adipocyte leptin, contrasts however with its stimulatory effect reported in several non-adipose cells such as C6 glioma cells, GH3 pituitary tumor cells [58,59]; MCF-7 breast cancer cells [60] and placental choronic tissue [61]. Taken together, these results strongly suggest a cell-specific difference in the intracellular cAMP regulation of leptin gene expression. We have no clear explanation for the lack of response of gastric MKN-74 cells in terms of LEP promoter activity to a β_3-adrenoceptor agonist. However, this may probably suggest a negligible role of the sympathetic system in the regulation of leptin gene expression in the stomach. The molecular mechanisms underlying the current findings remained to be determined.

In summary, we described for the first time, similarities and more interestingly, differences in the regulation of the leptin gene promoter in gastric cells as compared to adipocytes. Further studies are required to determine the physiological relevance of such differences and to provide further insight into the mechanisms governing the leptin promoter in gastric epithelial cells.

Acknowledgement: Hélène Goïot was supported by ARC (Association pour la Recherche sur le Cancer).

References

[1] Zhang, Y., Proenca, R., Maffei, M., Barone, M., Leopold, L. and Friedman, J.M. (1994) Positional cloning of the mouse obese gene and its human homologue. Nature 372, 425–432.
[2] Tartaglia, L.A. (1997) The leptin receptor. J Biol Chem 272, 6093–6096.
[3] Tartaglia, L.A., et al. (1995) Identification and expression cloning of a leptin receptor, OB-R. Cell 83, 1263–1271.
[4] Masuzaki, H., et al. (1997) Nonadipose tissue production of leptin: leptin as a novel placenta-derived hormone in humans. Nat Med 3, 1029–1033.
[5] Wang, J., Liu, R., Hawkins, M., Barzilai, N. and Rossetti, L. (1998) A nutrient-sensing pathway regulates leptin gene expression in muscle and fat. Nature 393, 684–688.
[6] Jin, L., Zhang, S., Burguera, B.G., Couce, M.E., Osamura, R.Y., Kulig, E. and Lloyd, R.V. (2000) Leptin and leptin receptor expression in rat and mouse pituitary cells. Endocrinology 141, 333–339.
[7] Bado, A., et al. (1998) The stomach is a source of leptin. Nature 394, 790–793.
[8] Cinti, S., Matteis, R.D., Pico, C., Ceresi, E., Obrador, A., Maffeis, C., Oliver, J. and Palou, A. (2000) Secretory granules of endocrine and chief cells of human stomach mucosa contain leptin. Int J Obes Relat Metab Disord 24, 789–793.
[9] Sobhani, I., et al. (2000) Leptin secretion and leptin receptor in the human stomach. Gut 47, 178–183.
[10] Sobhani, I., Buyse, M., Go ot, H., Weber, N., Laigneau, J.P., Henin, D., Soul, J.C. and Bado, A. (2002) Vagal Stimulation Rapidly Increases Leptin Secretion in Human Stomach. Gastroenterology 122, 259–263.
[11] Groschl, M., et al. (2001) Identification of leptin in human saliva. J Clin Endocrinol Metab 86, 5234–5239.
[12] Buyse, M., et al. (2001) PepT1-mediated epithelial transport of dipeptides and cephalexin is enhanced by luminal leptin in the small intestine. J Clin Invest 108, 1483–1494.
[13] Buyse, M., Sitaraman, S.V., Liu, X., Bado, A. and Merlin, D. (2002) Luminal leptin enhances CD147/MCT-1-mediated uptake of butyrate in the human intestinal cell line Caco2-BBE. J Biol Chem 277, 28182–28190.
[14] Anthony, R.V., Limesand, S.W. and Jeckel, K.M. (2001) Transcriptional regulation in the placenta during normal and compromised fetal growth. Biochem Soc Trans 29, 42–48.
[15] Ashworth, C.J., Hoggard, N., Thomas, L., Mercer, J.G., Wallace, J.M. and Lea, R.G. (2000) Placental leptin. Rev Reprod 5, 18–24.
[16] Slieker, L.J., Sloop, K.W., Surface, P.L., Kriauciunas, A., LaQuier, F., Manetta, J., Bue-Valleskey, J. and Stephens, T.W. (1996) Regulation of expression of ob mRNA and protein by glucocorticoids and cAMP. J Biol Chem 271, 5301–5304.
[17] Kolaczynski, J.W., Goldstein, B.J. and Considine, R.V. (1997) Dexamethasone, OB gene, and leptin in humans; effect of exogenous hyperinsulinemia. J Clin Endocrinol Metab 82, 3895–3897.
[18] Wolf, G. (1999) The molecular mechanism of the stimulation of adipocyte differentiation by a glucocorticoid. Nutr Rev 57, 324–326.
[19] Leroy, P., Dessolin, S., Villageois, P., Moon, B.C., Friedman, J.M., Ailhaud, G. and Dani, C. (1996) Expression of ob gene in adipose cells. Regulation by insulin. J Biol Chem 271, 2365–2368.
[20] Saladin, R., De Vos, P., Guerre-Millo, M., Leturque, A., Girard, J., Staels, B. and Auwerx, J. (1995) Transient increase in obese gene expression after food intake or insulin administration. Nature 377, 527–529.
[21] Bradley, R.L. and Cheatham, B. (1999) Regulation of ob gene expression and leptin secretion by insulin and dexamethasone in rat adipocytes. Diabetes 48, 272–278.
[22] Trayhurn, P., Duncan, J.S. and Rayner, D.V. (1995) Acute cold-induced suppression of ob (obese) gene expression in white

adipose tissue of mice: mediation by the sympathetic system. Biochem J 311 (Pt 3), 729–733.
[23] Trayhurn, P., Duncan, J.S., Rayner, D.V. and Hardie, L.J. (1996) Rapid inhibition of ob gene expression and circulating leptin levels in lean mice by the beta 3-adrenoceptor agonists BRL 35135A and ZD2079. Biochem Biophys Res Commun 228, 605–610.
[24] Moinat, M., Deng, C., Muzzin, P., Assimacopoulos-Jeannet, F., Seydoux, J., Dulloo, A.G. and Giacobino, J.P. (1995) Modulation of obese gene expression in rat brown and white adipose tissues. FEBS Lett 373, 131–134.
[25] MacDougald, O.A., Cornelius, P., Lin, F.T., Chen, S.S. and Lane, M.D. (1994) Glucocorticoids reciprocally regulate expression of the CCAAT/enhancer-binding protein alpha and delta genes in3T3-L1 adipocytes and white adipose tissue. J Biol Chem 269, 19041–19047.
[26] Miller, S.G., De Vos, P., Guerre-Millo, M., Wong, K., Hermann, T., Staels, B., Briggs, M.R. and Auwerx, J. (1996) The adipocyte specific transcription factor C/EBPalpha modulates human ob gene expression. Proc Natl Acad Sci U S A 93, 5507–5511.
[27] Mason, M.M., He, Y., Chen, H., Quon, M.J. and Reitman, M. (1998) Regulation of leptin promoter function by Sp1, C/EBP, and a novel factor. Endocrinology 139, 1013–1022.
[28] He, Y., Chen, H., Quon, M.J. and Reitman, M. (1995) The mouse obese gene. Genomic organization, promoter activity, and activation by CCAAT/enhancer-binding protein alpha. J Biol Chem 270, 28887–28891.
[29] De Vos, P., et al. (1996) Thiazolidinediones repress ob gene expression in rodents via activation of peroxisome proliferator-activated receptor gamma. J Clin Invest 98, 1004–1009.
[30] Tsunoda, Y., Yao, H., Park, J. and Owyang, C. (2003) Cholecystokinin synthesizes and secretes leptin in isolated canine gastric chief cells. Biochem Biophys Res Commun 310, 681–684.
[31] Mangelsdorf, D.J., et al. (1995) The nuclear receptor superfamily: the second decade. Cell 83, 835–839.
[32] Mangelsdorf, D.J. and Evans, R.M. (1995) The RXR heterodimers and orphan receptors. Cell 83, 841–850.
[33] Sekiguchi, M., Sakakibara, K. and Fujii, G. (1978) Establishment of cultured cell lines derived from a human gastric carcinoma. Jpn J Exp Med 48, 61–68.
[34] Motoyama, T., Hojo, H. and Watanabe, H. (1986) Comparison of seven cell lines derived from human gastric carcinomas. Acta Pathol Jpn 36, 65–83.
[35] Student, A.K., Hsu, R.Y. and Lane, M.D. (1980) Induction of fatty acid synthetase synthesis in differentiating 3T3-L1 preadipocytes. J Biol Chem 255, 4745–4750.
[36] Chomczynski, P. and Sacchi, N. (1987) Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal Biochem 162, 156–159.
[37] Laburthe, M., Amiranoff, B. and Boissard, C. (1982) Alpha-Adrenergic inhibition of cyclic AMP accumulation in epithelial cells isolated from rat small intestine. Biochim Biophys Acta 721, 101–108.
[38] Buyse, M., Aparicio, T., Guilmeau, S., Goiot, H., Sobhani, I. and Bado, A. (2004) [Paracrine actions of the stomach-derived leptin]. Med Sci (Paris) 20, 183–188.
[39] Buyse, M., et al. (2001) Expression and regulation of leptin receptor proteins in afferent and efferent neurons of the vagus nerve. Eur J Neurosci 14, 64–72.
[40] Burdyga, G., et al. (2002) Expression of the leptin receptor in rat and human nodose ganglion neurones. Neuroscience 109, 339–347.
[41] Attoub, S., et al. (1999) Physiological role of cholecystokinin B/gastrin receptor in leptin secretion. Endocrinology 140, 4406–4410.
[42] Slieker, L.J., Sloop, K.W. and Surface, P.L. (1998) Differentiation method-dependent expression of leptin in adipocyte cell lines. Biochem Biophys Res Commun 251, 225–229.
[43] Zakrzewska, K.E., Cusin, I., Sainsbury, A., Rohner-Jeanrenaud, F. and Jeanrenaud, B. (1997) Glucocorticoids as counterregulatory hormones of leptin: toward an understanding of leptin resistance. Diabetes 46, 717–719.
[44] Gong, D.W., Bi, S., Pratley, R.E. and Weintraub, B.D. (1996) Genomic structure and promoter analysis of the human obese gene. J Biol Chem 271, 3971–3974.
[45] Kanemasa, H., et al. (1999) Distribution of glucocorticoid receptor immunoreactivity in gastric mucosa of normal and adrenalectomized rats. Dig Dis Sci 44, 2081–2087.
[46] Considine, R.V., Nyce, M.R., Kolaczynski, J.W., Zhang, P.L., Ohannesian, J.P., Moore, J.H., Jr, Fox, J.W. and Caro, J.F. (1997) Dexamethasone stimulates leptin release from human adipocytes: unexpected inhibition by insulin. J Cell Biochem 65, 254–258.
[47] Wang, F.N., Ma, C.G., Zhang, Y.L., Zhang, N.X., Chen, Y.M., Tang, Q.Q. and Song, H.Y. (2000) Identification of Glucose-responsive and Insulin-responsive Elements in Promoter of Mouse ob Gene. Sheng Wu Hua Xue Yu Sheng Wu Wu Li Xue Bao (Shanghai) 32, 541–544.
[48] Kliewer, S.A., Umesono, K., Mangelsdorf, D.J. and Evans, R.M. (1992) Retinoid X receptor interacts with nuclear receptors in retinoic acid, thyroid hormone and vitamin D3 signalling. Nature 355, 446–469.
[49] Lowell, B.B. (1999) PPARgamma: an essential regulator of adipogenesis and modulator of fat cell function. Cell 99, 239–242.
[50] Auwerx, J. (1999) PPARgamma, the ultimate thrifty gene. Diabetologia 42, 1033–1049.
[51] Kallen, C.B. and Lazar, M.A. (1996) Antidiabetic thiazolidinediones inhibit leptin (ob) gene expression in 3T3-L1 adipocytes. Proc Natl Acad Sci U S A 93, 5793–5796.
[52] Heyman, R.A., Mangelsdorf, D.J., Dyck, J.A., Stein, R.B., Eichele, G., Evans, R.M. and Thaller, C. (1992) 9-cis retinoic acid is a high affinity ligand for the retinoid X receptor. Cell 68, 397–406.
[53] Kliewer, S.A., Forman, B.M., Blumberg, B., Ong, E.S., Borgmeyer, U., Mangelsdorf, D.J., Umesono, K. and Evans, R.M. (1994) Differential expression and activation of a family of murine peroxisome proliferator-activated receptors. Proc Natl Acad Sci U S A 91, 7355–7359.
[54] Yamauchi, T., et al. (2001) Inhibition of RXR and PPARgamma ameliorates diet-induced obesity and type 2 diabetes. J Clin Invest 108, 1001–1013.
[55] Leung, W.K., et al. (2004) Effect of peroxisome proliferator activated receptor gamma ligands on growth and gene expression profiles of gastric cancer cells. Gut 53, 331–338.
[56] Jiang, S.Y., Shen, S.R., Shyu, R.Y., Yu, J.C., Harn, H.J., Yeh, M.Y., Lee, M.M. and Chang, Y.C. (1999) Expression of nuclear retinoid receptors in normal, premalignant and malignant gastric tissues determined by in situ hybridization. Br J Cancer 80, 206–214.
[57] Kosaki, A., Yamada, K. and Kuzuya, H. (1996) Reduced expression of the leptin gene (ob) by catecholamine through a G(S) protein-coupled pathway in 3T3-L1 adipocytes. Diabetes 45, 1744–1749.
[58] Li, A.W., Morash, B., Hollenberg, A.N., Ur, E., Wilkinson, M. and Murphy, P.R. (2001) Transcriptional regulation of the leptin gene promoter in rat GH3 pituitary and C6 glioma cells. Mol Cell Endocrinol 176, 57–65.
[59] Morash, B., Johnstone, J., Leopold, C., Li, A., Murphy, P., Ur, E. and Wilkinson, M. (2000) The regulation of leptin gene expression in the C6 glioblastoma cell line. Mol Cell Endocrinol 165, 97–105.
[60] O'Neil, J.S., Burow, M.E., Green, A.E., McLachlan, J.A. and Henson, M.C. (2001) Effects of estrogen on leptin gene promoter activation in MCF-7 breast cancer and JEG-3 choriocarcinoma cells: selective regulation via estrogen receptors alpha and beta. Mol Cell Endocrinol 176, 67–75.
[61] Yura, S., et al. (1998) Augmentation of leptin synthesis and secretion through activation of protein kinases A and C in cultured human trophoblastic cells. J Clin Endocrinol Metab 83, 3609–3614.