Journal of Cellular Biochemistry 82:666–673 (2001)

Molecular and Cellular Mechanisms of Adipose Secretion: Comparison of Leptin and Angiotensinogen

S. Turban, I. Hainault, J. André, P. Ferré, A. Quignard-Boulangé, and M. Guerre-Millo*

U 465 INSERM, 15 rue de l'Ecole de Médecine, 75006 Paris, France

Abstract Besides their function of lipid storage, the adipose cells secrete a number of proteins of physiopathological importance. To get further insights into this function, which remains poorly characterized, we sought to compare the mechanisms and regulation of secretion of two individual proteins in the same cells. Leptin and angiotensinogen were chosen and assessed by radioimmunoassay and quantitative immunoblotting, respectively, in primary culture of epididymal adipose cells from young obese Zucker rats. Leptin was secreted at a steady rate of 4 ng/10^6 cells/h over 2–6 h. Despite secretion, leptin cellular content remained stable at 3 ng/10^6 cells. In contrast, the rate of angiotensinogen secretion decreased regularly from 45 arbitrary units/10^6 cells/h at 2 h, to half this value at 6 h, although cell content remained constant at 100 arbitrary units/10^6 cells. Inhibition of protein synthesis by cycloheximide depleted the cells from leptin, but not from angiotensinogen for up to 6 h. Insulin increased leptin secretion (+75%) and cell content (+70 %), without affecting angiotensinogen. Secretion of both proteins was inhibited by Golgi-disturbing agents, brefeldin A and monensin. The presence of brefeldin A led to a specific rise in leptin cell content, an effect inhibited by cycloheximide and enhanced by insulin (+80%). These data show that leptin and angiotensinogen are both secreted through Golgi-dependent pathways and that their respective intracellular pool exhibit distinct turn-over rate and insulin sensitivity. These characteristics might account for the differential response of these adipose proteins to variations in the systemic environment. J. Cell. Biochem. 82: 666–673, 2001. © 2001 Wiley-Liss, Inc.

Key words: adipose cell; obesity; brefeldin A; monensin; Golgi apparatus; insulin

Besides their well known function of lipid storage and mobilization, the adipose cells secrete several proteins. Most of them are dysregulated in the obese state and may be involved in obesity-linked complications. This emphasizes the physiopathological importance of this function, which remains less well characterized than in other secretory cells. The adipose cells are a major source of leptin, a cytokine crucial to body weight homeostasis [Zhang et al., 1994]. Genetic alteration of leptin results in obesity in rodents and humans, and exogenous administration reduces feeding and body fat [review in Ahima and Flier, 2000]. Nevertheless, leptin production is actually increased in obese state, leading to the hypothesis of obesity-induced leptin resistance. The adipose cells are also the main, if not unique, source of proteins thought to participate to the complement pathways: adipsin [Cook et al., 1987; Rosen et al., 1989], adipoQ/Acrp 30 [Scherer et al., 1995; Hu et al., 1996], and adiponectin [Arita et al., 1999; Yokota et al., 2000], the likely human homologue of adipoQ/Acrp 30. At variance with leptin, these proteins are decreased in obesity, suggesting a link with alteration of immune response in the obese. Other secreted proteins are produced both in adipose tissue and in liver, including plasminogen activator inhibitor 1 (PAI 1) [Eriksson et al., 1998; Samad et al., 1999], tumor necrosis factor α (TNFα) [Hotamisligil et al., 1993, 1995], angiotensinogen (AGE) [Frederich et al., 1992] and a member of the angiopoietin family, termed PGAR or FIAF [Kersten et al., 2000; Yoon et al., 2000]. Increased circulating levels in obesity points to adipose tissue as an important source of these factors, which are thought to be part of the molecular links between obesity and cardiovascular diseases [Samad et al., 1999], hypertension [Frederich et al., 1992], and insulin resistance [Hotamisligil et al., 1993].

*Correspondence to: M. Guerre-Millo, U 465 INSERM, 15 rue de l'Ecole de Médecine, 75006 Paris, France.
E-mail: mguerre@bhdc.jussieu.fr

Received 6 November 2000; Accepted 19 March 2001

©2001 Wiley-Liss, Inc.

Production of secreted proteins involves multiple steps, including gene transcription, de novo synthesis, vesicular trafficking, and release. Each step has the potential to be affected by variations in the cellular and/or systemic environment. Serum insulin levels are of major importance in the regulation of various processes in the adipose cells. Therefore, numerous studies have addressed the question of the effect of this hormone on adipose secreted proteins. Although extensively studied in rodent and human cultured adipose cells, the effect of insulin on leptin release remains controversial. Indeed, both an increase [Hardie et al., 1996; Leroy et al., 1996; Slieker et al., 1996; Wabitsch et al., 1996; Russell et al., 1998] and no change [Kolaczynski et al., 1996; Halleux et al., 1998; Mueller et al., 1998] in leptin production have been reported after 24 h in presence of the hormone. The effect of insulin has also been tested over shorter period of time, demonstrating more consistently enhanced leptin production [Barr et al., 1997; Bradley and Cheatham, 1999; Levy et al., 2000]. Interestingly, increased leptin release in culture medium is not always accompanied by coordinated changes in gene expression [Russell et al., 1998; Bradley and Cheatham, 1999], suggesting that insulin may affect post-transcriptionnal steps in the secretory route of leptin. A stimulatory effect of insulin has also been reported on adipsin and Acrp 30 release, which is increased in a time frame of minutes in presence of the hormone [Kitagawa et al., 1989; Scherer et al., 1995; Bogan and Lodish, 1999]. These observations have raised the hypothesis that adipose cells may possess an insulin-regulated pathway of exocytosis for certain secreted proteins. In contrast, insulin down-regulates AGE gene expression and release in mouse adipose cells [Aubert et al., 1998].

In the present study, we sought to get new insights into the mechanisms and regulation of adipose secretion by assessing two secreted proteins, leptin and AGE in the same cells. We present evidences that differential sensitivity of these adipose proteins to variations in the systemic environment could be accounted for by distinct cellular mechanisms of secretion.

MATERIALS AND METHODS

Materials

Dulbecco's modified Eagle's medium (DMEM) was purchased from Life Technologies SARL (Cergy-Pontoise, France). Human insulin (Actrapid) was from Novo-Nordisk (Copenhagen, Denmark). Collagenase A was from Boehringer Mannheim (Meylan, France). Fraction V bovine serum albumin (BSA), cycloheximide (CHX), brefeldin A (BFA), and monensin were from Sigma Aldrich (St Quentin Fallavier, France).

Animals

Animal studies were conducted according to the French Guidelines for the Care and Use of Experimental Animals. Obese *fa*/*fa* male Zucker rats were bred at the laboratory from pairs originally provided by the Harriet G. Bird Memorial Hospital (Stow, MA). Animals were housed at 22±2°C under alternating 12 h light/dark cycle and allowed ad libitum access to food and water.

Primary Cultured Rat Adipocytes

Adipocytes were isolated by collagenase digestion [Rodbell, 1965] from the epididymal adipose tissue of 6-week-old obese Zucker rats. The cells were pre-incubated for 2 h in Eppendorf tubes in 0.4 ml DMEM supplemented with 3% BSA, at a density of $0.2–0.4 \times 10^6$ cells/ml. The medium was changed and the cells were then incubated in quadruplicate under various conditions as indicated in the figure legends. Culture was conducted at 37°C, under 5% CO_2 and 95% air atmosphere. At the end of incubation, infranatants and cells were separately frozen until use. The medium was assayed for leptin, AGE, and lactate. The cells were homogenized in cell lysis buffer (Promega, Charbonnière, France). A clear supernatant, obtained by centrifugation at 10,000*g* for 10 min, was used for measurement of leptin and AGE. The number of cells per tube was determined by dividing the lipid content of an aliquot of each cell suspension by fat cell weight calculated from the mean cell diameter. The measurement of cell diameter was performed by a procedure derived from a microphotographic method [Lavau et al., 1977], using an image analysis software (Perfect-Image Numeris, Nanterre, France).

Western Blotting

Total proteins from medium and cells homogenate were electrophoresed concomitantly on 12% SDS–polyacrylamide gel and electroblotted onto nitrocellulose membrane (Hybond

ECL, Amersham Pharma Biotech, Inc., Piscataway, NJ). The low range Mr marker from BioRad (Richmond, CA) was used as standard. Following blockage with low fat milk, membranes were incubated for 1 h with 1/5,000 dilution of anti-AGE antibody (rabbit antibody against rat AGE [Bouhnik et al., 1982], kind gift from P. Corvol). The blots were washed and exposed for 1 h to horse-radish peroxydase conjugated anti-rabbit IgG. The immune complex was detected by luminescent visualization (ECL, Amersham Pharma Biotech, Inc., Piscataway, NJ) on X-ray film (Kodak Biomax MR). The antibody used in this study revealed three bands of slightly different Mr (56, 53, and 48 kDa), which were quantified individually by image analysis (Perfect-Image, Numeris, Nanterre, France). Quantification of total AGE is shown as arbitrary units (AU), including the three signals.

Protein, Leptin, and Lactate Determination

Proteins from medium and adipose cells homogenate were assayed by the BioRad protein assay procedure (Richmond, CA). Leptin was measured with a specific rat leptin RIA kit (Linco Research, St Charles, MO) as described by the manufacturer. Lactate was measured spectrophotometrically, as previously described [Burnol et al., 1986].

Statistical Analysis

Results are expressed as mean±SEM. Statistical analysis was performed with Student's *t*-test for paired data, except for the data presented in Figure 4, where unpaired *t*-test was used. $P < 0.05$ was considered statistically significant.

RESULTS

A first series of experiments was designed to compare the time course of leptin and AGE secretion in rat adipose cells. The cells were cultured for 2–6 h. Lactate concentration in the culture medium was routinely determined, as an index of glucose utilization. Significant amounts of lactate were released at a constant rate (0.4±0.05 μmol/10^6 cells/h; n = 12), indicating adequate and stable glucose metabolism in these cells. Moreover, insulin responsiveness was demonstrated by a 4-fold increase in lactate production. The rate of leptin secretion averaged 4 ng/10^6 cells/h and was remarkably stable over time (Fig. 1A). Despite secretion, the size of leptin intracellular pool remained constant at 3 ng/10^6 cells and in the same range as in freshly isolated adipose cells (2.5±0.3 ng/10^6 cells; n = 7). Thus, the amount of leptin secreted over the whole period of culture was equivalent to 8-fold the initial leptin cellular content. This indicates that leptin was actively synthesized and that its intracellular pool was turned over more than once per hour. In addition, insulin increased slightly but significantly leptin secretion rate (+75%) and intracellular content (+70%). AGE was measured by immunoblotting in a subset of these kinetics experiments. Quantification of AGE is presented as arbitrary units (AU), including three specific signals revealed on immunoblots. The rate of AGE secretion decreased regularly with culture time, from 45 AU/10^6 cells/h after 2 h, to half this value at 6 h (Fig. 1B). Nevertheless, AGE cellular content (100 AU/10^6 cells)

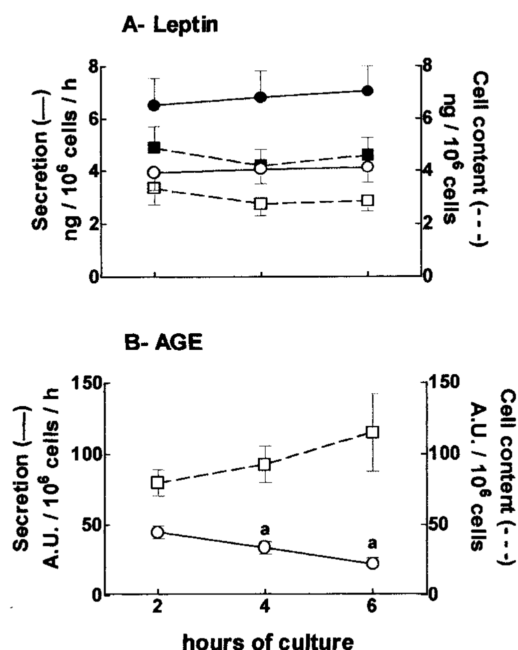

Fig. 1. Time course of leptin and AGE secretion rate and cell content. Obese Zucker rats adipose cells were isolated by collagenase digestion of the epididymal adipose tissue. Cells were pre-incubated for 2 h in basal conditions. The medium was changed. Incubations were then performed for the indicated period of time, without (open symbols) or with 30 nM insulin (closed symbols). Leptin (**A**) and AGE (**B**) were assayed in the medium (circles, solid lines) and within the cells (squares, dotted lines). Data are the means±SEM from 12 and 5 separate experiments for leptin and AGE, respectively. Insulin effect on leptin secretion rate and cell content was statistically significant at each time point ($P < 0.05$ vs. basal). a: $P < 0.05$ vs. 2 h.

remained stable. From these data, it can be calculated that a total amount of 130 AU/10^6 cells of AGE was released over 6 h of culture. Thus, the secreted pool was roughly similar in size with the cellular pool, indicating that the latter was turned over once in 6 h at the most. When the insulin effect was tested on AGE, neither secretion rate (+29±25.7%, NS, n = 4) nor cellular amounts (−10±4.93%, NS, n = 3) were significantly changed after 2 h in presence of the hormone.

The data from kinetics experiments suggest strikingly different turn-over rate for leptin and AGE in the adipose cells. This prompted us to assess directly the effect of protein synthesis inhibition by cycloheximide (CHX), in conditions where basal and insulin-stimulated lactate production was not altered (data not shown). The CHX-induced change in the amounts of protein recovered in medium and cells was expressed as per cent of inhibition vs. cells cultured without CHX (Fig. 2). AGE secretion and cell content were unchanged by inhibition of protein synthesis, at least for 6 h. Thus, it is likely that the initial intracellular pool of AGE, although not determined, was actually large enough to ensure secretion over this period of time. In sharp contrast, the cells became rapidly depleted of leptin, resulting in virtually no secretion by 2 h, whatever the presence of insulin (Fig. 2, data not shown).

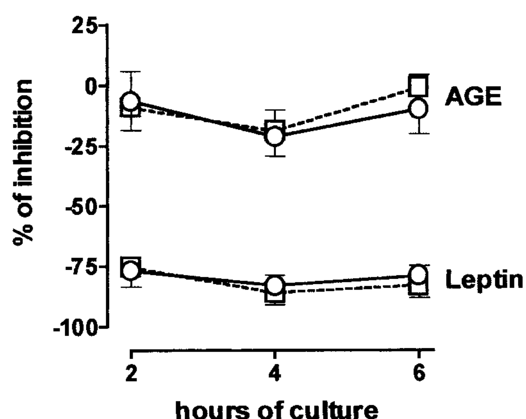

Fig. 2. Effect of cycloheximide (CHX) on leptin and AGE secretion and cell content. Cells were obtained as described in Figure 1. Incubations were performed for the indicated period of time, without or with CHX (15 μM). The effect of CHX, expressed as percent of inhibition, was assessed on leptin and AGE recovered in the medium (circles, solid lines) and within the cells (squares, dotted lines). Data are means±SEM from 10 and 5 separate experiments for leptin and AGE, respectively.

These data demonstrate directly that leptin secretion was highly dependent on ongoing protein synthesis, in agreement with the results from kinetics experiments.

The above observations clearly show that leptin and AGE intracellular pool exhibit distinct properties. Therefore, it was of interest to determine whether or not the two proteins were secreted through the same exocytotic pathway. The adipose cells were incubated with two Golgi-disturbing agents, brefeldin A (BFA), which blocks the anterograde flow of proteins from endoplasmic reticulum to the Golgi apparatus and monensin, known to induce osmotic swelling of Golgi cisterns. The presence of BFA for 4 h did not alter basal and insulin-stimulated lactate production (data not shown). In sharp contrast, leptin secretion was markedly inhibited (Fig. 3A, + BFA) and this was accompanied by accumulation of leptin within the cells (Fig. 3B, + BFA). Upon BFA removal (see bars labeled: ±BFA), secretion resumed at pre-blockage rate. The cell leptin content decreased accordingly, but remained significantly higher than in cells incubated without BFA (−BFA). In addition, insulin increased leptin secretion and cell content in presence of BFA, indicating that the hormone acted upstream of the drug in the secretory route of leptin.

Similar to leptin, AGE secretion was markedly inhibited by BFA. This was demonstrated by a marked decrease in the most abundant 56 kDa signal detected in the culture medium (Fig. 4A; Medium). In cell homogenates (Fig. 4A; Cells), two bands of 56 and 53 kDa were of roughly similar intensity, and the presence of BFA induced a decrease in the 56 kDa, concomitant with an increase in the 53 kDa signal. This resulted in no change in the total cellular amounts of AGE. Assuming that these bands represent multiple glycosylation forms of the protein [Campbell et al., 1984], the switch towards a lower Mr signal suggests that AGE was not fully glycosylated in presence of BFA. When tested on leptin (data not shown) and AGE secretion (Fig. 4), the effect of monensin, although less pronounced, confirmed the effect of BFA. Taken together, these observations indicate that leptin and AGE are both secreted through Golgi-dependent pathway.

To verify whether the effect of BFA to induce leptin accumulation within cells was dependent on protein synthesis, cells were cultured for 4 h in presence of BFA with CHX added or not, after

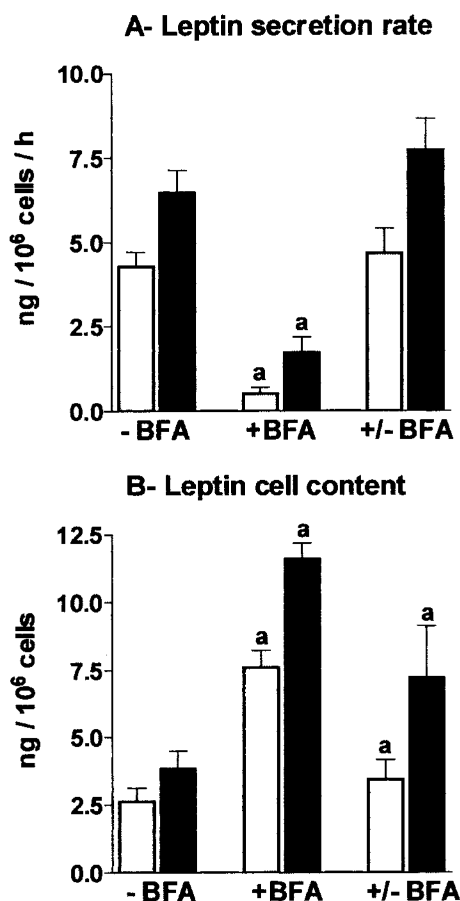

Fig. 3. Effect of BFA on leptin secretion rate and cell content. Cells, obtained as described in Figure 1, were incubated without or with 0.5 μg/ml BFA for 2 h and the medium was changed. For the next 2 h of culture, cells were either kept without BFA (−BFA), kept with BFA (+BFA) or relieved from the drug (±BFA). Insulin 30 nM was present (closed bars) or not (open bars) during the whole culture. Medium (**A**) and cells (**B**) were collected and assayed for leptin by RIA. Data are the means±SEM from nine separate experiments. The effect of insulin was always statistically significant ($P < 0.05$ vs. minus insulin). a: $P < 0.05$ vs. cells incubated minus BFA.

2 h. The cellular amounts of leptin increased linearly with time in the presence of BFA alone (data not shown) and this increase was abolished by the addition of CHX. This allowed us to estimate a rate of leptin synthesis derived from the difference in leptin cell content in cells cultured without and with CHX (Table I). In presence of insulin, enhanced intracellular leptin accumulation was fully abolished by CHX. From these experiments, we estimated that leptin was synthesized at a rate amounting 2.0 and 3.6 ng/10^6 cells/h in basal and insulin-stimulated conditions, respectively. Thus, insulin increased leptin synthesis by 80%, an effect strikingly similar in magnitude to its stimulatory effect on leptin secretion.

TABLE I. Rate of Leptin Synthesis

Conditions	Cellular leptin content (ng/10^6 cells)	Rate of leptin synthesis (ng/10^6 cells/h)
BFA	8.6±0.2[a]	2.0
BFA+CHX	4.7±1.5	
BFA+ins	11.6±0.6[a,b]	3.6
BFA+CHX+ins	4.5±1.3	

Cells, obtained as described in Figure 1, were incubated with 0.5 μg/ml BFA for 4 h. After 2 h, CHX (15 μM) and insulin (ins, 30 nM) were added or not, as indicated. Leptin cellular content was determined at the end of the total period (4 h). The rate of leptin synthesis was estimated by the difference of cellular leptin content in cells cultured without and with CHX, divided by the culture time in presence of the drugs (2 h). [a]$P < 0.5$ vs., cells with CHX; [b]$P < 0.05$ vs. basal conditions.

DISCUSSION

The secretory function of the adipose cells is less well characterized than in "classical" endocrine or exocrine cells. In the present study, we compared the mechanisms and regulation of secretion of two individual proteins in the same cells. This experimental design allowed to assess protein-specific mechanisms of secretion, independent of potential confounding factors linked to cell type.

Secretion of leptin and AGE exhibits distinct insulin responsiveness. Consistent with previous studies [Barr et al., 1997; Bradley and Cheatham, 1999; Levy et al., 2000], we found that insulin increased the rate of leptin secretion within a time frame of hours. The magnitude of this effect is moderate (<2-fold), although insulin was fully potent to increase lactate production by a 4-fold factor in the same cells. In contrast, the hormone failed to alter AGE secretion, in line with observations showing that the release of this adipose protein is marginally decreased by insulin in mouse Ob 1771 cells [Aubert et al., 1998]. Our data confirm the differential effect of the hormone on leptin and AGE secretion in rat adipose cells. This could, at least in part, account for distinct sensitivity of circulating leptin and AGE to nutritional status. Indeed, while serum leptin levels are markedly decreased by fasting and increased by refeeding in rodents [Ahima et al., 1996], serum AGE is unaffected [Frederich et al., 1992].

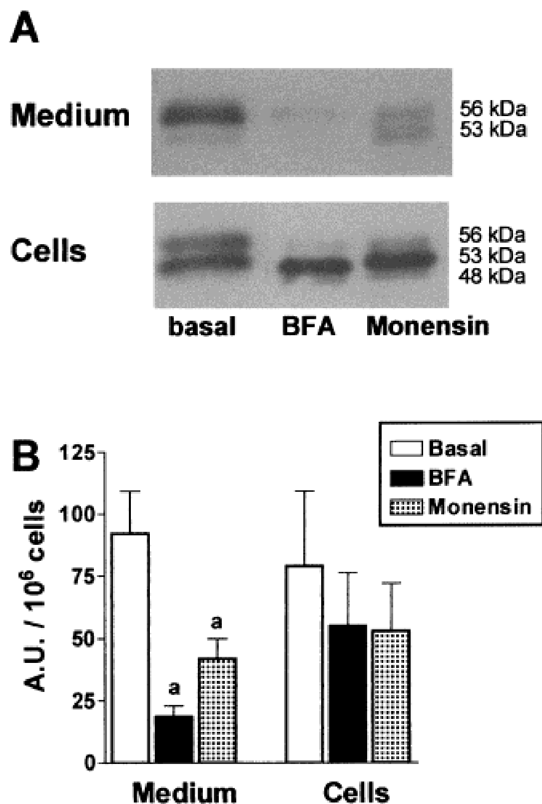

Fig. 4. Effect of Golgi-disturbing agents on AGE secretion and cell content. Cells were obtained as described in Figure 1. Incubations were performed for 2 h, in absence (basal) or in presence of 0.5 μg/ml BFA (BFA) or 1 μM monensin (Monensin). Medium and cells were collected and assayed for AGE by Western blotting. **A:** AGE signals arising from equal volumes of medium (Medium) or cell homogenate (Cells). **B:** Quantification of AGE specific signals, including the three bands, in medium and cells, as indicated. The data show the means±SEM of three experiments. a: $P < 0.05$ vs. basal.

Comparison of leptin and AGE suggests that their respective intracellular pools exhibit distinct characteristics. AGE storage appears to be sufficient to sustain secretion for at least 6 h in vitro. In sharp contrast, leptin secretion is highly dependent on ongoing protein synthesis. Due to virtually total depletion of the cellular pool, leptin release is abolished when protein synthesis is inhibited. These observations, coupled with the kinetics experiment data, indicate that the turn-over rate of leptin intracellular pool is markedly faster than that for AGE in rat adipose cells.

Despite these distinct characteristics, leptin and AGE are both secreted through Golgi-dependent routes of exocytosis. This conclusion is supported by the effect of two Golgi-disturbing agents, BFA and monensin, which markedly inhibit the release of the two proteins. An inhibitory effect of BFA on leptin secretion has been previously observed in human embryonic kidney cells 293 transfected with a leptin expression vector [MacDougald et al., 1995] and in 3T3-L1 adipose cells [Kirchgessner et al., 1997]. Our data extend these observations to primary rat adipose cells and demonstrate that the effect of BFA occurs within hours, is reversible and leads to leptin intracellular accumulation in a protein synthesis-dependent manner. In contrast, no detectable increase was observed in the total cellular amounts of AGE, as expected if AGE is not actively synthesized during culture. Nevertheless, an alteration in glycosylation is likely to occur in presence of BFA, since the protein is detected at a lower Mr. This supports the hypothesis that AGE has been sequestered in a pre-Golgi compartment.

By virtue of the effect of BFA, we were able to determine the rate of leptin synthesis in conditions where secretion is suppressed. The BFA-induced increase in leptin intracellular pool relies on ongoing protein synthesis and is enhanced by insulin. Moreover, this effect of insulin was identical in magnitude with the stimulation of leptin secretion, as assessed independently in a different experimental setting. These data strongly argue for a stimulatory effect of insulin on leptin de novo synthesis which, in turn, leads to increased secretion. It is noteworthy, however, that the absolute rate of leptin synthesis (Table I) is twice lower than the absolute rate of leptin secretion, whatever the presence of insulin (Fig. 1). This raises the hypothesis that intracellular accumulation of leptin exerts a feed-back negative regulation on its own synthesis by a mechanism which remains to be determined. Previous studies have shown that insulin treatment for 2 h did not alter [Bradley and Cheatham, 1999] or marginally increased [Saladin et al., 1995] ob mRNA in rat adipose cells, when we clearly show an effect of the hormone on leptin synthesis. This suggests that insulin might increase leptin synthesis and secretion via a post-transcriptional mechanism. In fitting with this hypothesis, a constitutively active version of the Ser/Thr kinase Akt, an enzyme known to participate to the insulin signaling cascade, markedly increased leptin release in 3T3-L1 adipocytes without altering ob mRNA levels [Barthel et al., 1997].

Recent observations indicate that insulin increases leptin secretion within a time frame when protein synthesis is unlikely to have occurred [Barr et al., 1997] and even, at least partially, in presence of CHX [Bradley and Cheatham, 1999]. From these data, the hypothesis has been raised that insulin could mobilize a pre-existing pool of leptin or stimulate export of leptin directly from the endoplasmic reticulum. Such possibilities have been suggested for other adipose proteins, adipsin and adipoQ/Acrp 30, whose secretion is acutely regulated by insulin [Kitagawa et al., 1989; Scherer et al., 1995; Bogan and Lodish, 1999]. Our data rather suggest that insulin acts mainly on leptin synthesis. Nevertheless, a rapid effect of insulin on leptin release, which could involve a minor fraction of newly synthesized leptin, cannot be totally excluded. A recent study suggests that the adipose cells could secrete leptin via a specific regulated pathway [Roh et al., 2000]. Comparison of the subcellular localization of leptin with AGE, whose secretion is insensitive to the hormone, could help to better define this potential secretory route.

In conclusion, the present study points out to distinct cellular mechanisms and regulation of secretion in the adipose cells, which could account, at least in part, for differential release of adipose proteins under physiopathological conditions. Further studies are needed to extend these observations to other adipose secreted proteins.

REFERENCES

Ahima RS, Flier JS. 2000. Leptin. Annu Rev Physiol 62:413–437.

Ahima RS, Prabakaran D, Mantzoros C, Qu D, Lowell B, Maratos-Flier E, Flier JS. 1996. Role of leptin in the neuroendocrine response to fasting. Nature 382:250–252.

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.

Aubert J, Safonova I, Negrel R, Ailhaud G. 1998. Insulin down-regulates angiotensinogen gene expression and angiotensinogen secretion in cultured adipose cells. Biochem Biophys Res Commun 250:77–82.

Barr VA, Malide D, Zarnowski MJ, Taylor SI, Cushman SW. 1997. Insulin stimulates both leptin secretion and production by rat white adipose tissue. Endocrinology 138:4463–4472.

Barthel A, Kohn AD, Luo Y, Roth RA. 1997. A constitutively active version of the Ser/Thr kinase Akt induces production of the ob gene product, leptin, in 3T3-L1 adipocytes. Endocrinology 138:3559–3562.

Bogan JS, Lodish HF. 1999. Two compartments for insulin-stimulated exocytosis in 3T3-L1 adipocytes defined by endogenous ACRP30 and GLUT4. J Cell Biol 146:609–620.

Bouhnik J, Clauser E, Gardes J, Corvol P, Menard J. 1982. Direct radioimmunoassay of rat angiotensinogen and its application to rats in various endocrine states. Clin Sci 62:355–360.

Bradley RL, Cheatham B. 1999. Regulation of ob gene expression and leptin secretion by insulin and dexamethasone in rat adipocytes. Diabetes 48:272–278.

Burnol AF, Guerre-Millo M, Lavau M, Girard J. 1986. Effect of lactation on insulin sensitivity of glucose metabolism in rat adipocytes. FEBS Lett 194:292–296.

Campbell DJ, Bouhnik J, Coezy E, Pinet F, Clauser E, Menard J, Corvol P. 1984. Characterization of precursor and secreted forms of rat angiotensinogen. Endocrinology 114:776–785.

Cook KS, Min HY, Johnson D, Chaplinsky RJ, Flier JS, Hunt CR, Spiegelman BM. 1987. Adipsin: a circulating serine protease homolog secreted by adipose tissue and sciatic nerve. Science 237:402–405.

Eriksson P, Reynisdottir S, Lonnqvist F, Stemme V, Hamsten A, Arner P. 1998. Adipose tissue secretion of plasminogen activator inhibitor-1 in non-obese and obese individuals. Diabetologia 41:65–71.

Frederich RCJ, Kahn BB, Peach MJ, Flier JS. 1992. Tissue-specific nutritional regulation of angiotensinogen in adipose tissue. Hypertension 19:339–344.

Halleux CM, Servais I, Reul BA, Detry R, Brichard SM. 1998. Multihormonal control of ob gene expression and leptin secretion from cultured human visceral adipose tissue: increased responsiveness to glucocorticoids in obesity. J Clin Endocrinol Metab 83:902–910.

Hardie LJ, Guilhot N, Trayhurn P. 1996. Regulation of leptin production in cultured mature white adipocytes. Horm Metab Res 28:685–689.

Hotamisligil GS, Shargill NS, Spiegelman BM. 1993. Adipose expression of tumor necrosis factor-alpha: direct role in obesity-linked insulin resistance. Science 259:87–91.

Hotamisligil GS, Arner P, Caro JF, Atkinson RL, Spiegelman BM. 1995. Increased adipose tissue expression of tumor necrosis factor-alpha in human obesity and insulin resistance. J Clin Invest 95:2409–2415.

Hu E, Liang P, Spiegelman BM. 1996. AdipoQ is a novel adipose-specific gene dysregulated in obesity. J Biol Chem 271:10697–10703.

Kersten S, Mandard S, Tan NS, Escher P, Metzger D, Chambon P, Gonzalez FJ, Desvergne B, Wahli W. 2000. Characterization of the fasting induced adipose factor FIAF, a novel PPAR target gene. J Biol Chem 275: 28488–28493.

Kirchgessner TG, Uysal KT, Wiesbrock SM, Marino MW, Hotamisligil GS. 1997. Tumor necrosis factor-alpha contributes to obesity-related hyperleptinemia by regulating leptin release from adipocytes. J Clin Invest 100:2777–2782.

Kitagawa K, Rosen BS, Spiegelman BM, Lienhard GE, Tanner LI. 1989. Insulin stimulates the acute release of

adipsin from 3T3-L1 adipocytes. Biochim Biophys Acta 1014:83–89.

Kolaczynski JW, Nyce MR, Considine RV, Boden G, Nolan JJ, Henry R, Mudaliar SR, Olefsky J, Caro JF. 1996. Acute and chronic effects of insulin on leptin production in humans: studies in vivo and in vitro. Diabetes 45:699–701.

Lavau M, Susini C, Knittle J, Blanchet-Hirst S, Greenwood MR. 1977. A reliable photomicrographic method to determining fat cell size and number: application to dietary obesity. Proc Soc Exp Biol Med 156:251–256.

Leroy P, Dessolin S, Villageois P, Moon BC, Friedman JM, Ailhaud G, Dani C. 1996. Expression of ob gene in adipose cells. Regulation by insulin. J Biol Chem 271:2365–2368.

Levy JR, Gyarmati J, Lesko JM, Adler RA, Stevens W. 2000. Dual regulation of leptin secretion: intracellular energy and calcium dependence of regulated pathway. Am J Physiol Endocrinol Metab 278:E892–E901.

MacDougald OA, Hwang CS, Fan H, Lane MD. 1995. Regulated expression of the obese gene product (leptin) in white adipose tissue and 3T3-L1 adipocytes. Proc Natl Acad Sci USA 92:9034–9037.

Mueller WM, Gregoire FM, Stanhope KL, Mobbs CV, Mizuno TM, Warden CH, Stern JS, Havel PJ. 1998. Evidence that glucose metabolism regulates leptin secretion from cultured rat adipocytes. Endocrinology 139:551–558.

Rodbell M. 1965. Modulation of lipolysis in adipose tissue by fatty acid concentration in fat cell. Ann NY Acad Sci 131:302–314.

Roh C, Thoidis G, Farmer SR, Kandror KV. 2000. Identification and characterization of leptin-containing intracellular compartment in rat adipose cells. Am J Physiol Endocrinol Metab 279:E893–E899.

Rosen BS, Cook KS, Yaglom J, Groves DL, Volanakis JE, Damm D, White T, Spiegelman BM. 1989. Adipsin and complement factor D activity: an immune-related defect in obesity. Science 244:1483–1487.

Russell CD, Petersen RN, Rao SP, Ricci MR, Prasad A, Zhang Y, Brolin RE, Fried SK. 1998. Leptin expression in adipose tissue from obese humans: depot-specific regulation by insulin and dexamethasone. Am J Physiol 275:E507–E515.

Saladin R, De Vos P, Guerre-Millo M, Leturque A, Girard J, Staels B, Auwerx J. 1995. Transient increase in obese gene expression after food intake or insulin administration. Nature 377:527–529.

Samad F, Uysal KT, Wiesbrock SM, Pandey M, Hotamisligil GS, Loskutoff DJ. 1999. Tumor necrosis factor alpha is a key component in the obesity-linked elevation of plasminogen activator inhibitor 1. Proc Natl Acad Sci USA 96:6902–6907.

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.

Slieker LJ, Sloop KW, Surface PL, Kriauciunas A, LaQuier F, Manetta J, Bue-Valleskey J, Stephens TW. 1996. Regulation of expression of ob mRNA and protein by glucocorticoids and cAMP. J Biol Chem 271:5301–5304.

Wabitsch M, Jensen PB, Blum WF, Christoffersen CT, Englaro P, Heinze E, Rascher W, Teller W, Tornqvist H, Hauner H. 1996. Insulin and cortisol promote leptin production in cultured human fat cells. Diabetes 45:1435–1438.

Yokota T, Oritani K, Takahashi I, Ishikawa J, Matsuyama A, Ouchi N, Kihara S, Funahashi T, Tenner AJ, Tomiyama Y, Matsuzawa Y. 2000. Adiponectin, a new member of the family of soluble defense collagens, negatively regulates the growth of myelomonocytic progenitors and the functions of macrophages. Blood 96:1723–1732.

Yoon JC, Chickering TW, Rosen ED, Dussault B, Qin Y, Soukas A, Friedman JM, Holmes WE, Spiegelman BM. 2000. Peroxisome proliferator-activated receptor gamma target gene encoding a novel angiopoietin-related protein associated with adipose differentiation. Mol Cell Biol 20:5343–5349.

Zhang Y, Proenca R, Maffei M, Barone M, Leopold L, Friedman JM. 1994. Positional cloning of the mouse obese gene and its human homologue. Nature 372:425–432.