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Human Adipose Angiotensinogen Gene Expression and Secretion Are Stimulated by Cyclic AMP via Increased DNA Cyclic AMP Responsive Element Binding Activity

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Components of the adipose renin–angiotensin system (RAS) have been suggested as providing a potential pathway linking obesity to hypertension. In adipose cells, the biological responses to β-adrenergic stimulation are mediated by an increase in intracellular cAMP. Because an association exists among body fat mass, hypertension, and increased sympathetic stimulation, we examined the influence of cAMP on angiotensinogen (ATG) expression and secretion in human adipose tissue and in parallel we studied the DNA binding activity of CRE transcriptional factors. A 24 h exposure to the cAMP analog 8Br-cAMP resulted in significant increases in ATG mRNA levels (+176 ± 60%) and protein secretion (+40 ± 27%). The ability of 8Br-cAMP to promote ATG gene expression was unaltered by H89, a protein kinase A inhibitor, because H89 per se was found to stimulate ATG mRNA levels and protein secretion. Moreover, 8Br-cAMP stimulated the specific CRE DNA binding activity (+115 ± 14%) in human adipocyte nuclear extracts as assessed by electrophoretic mobility shift assays. These results indicate that cAMP upregulates in vitro ATG expression and secretion in human adipose tissue and that the induction in ATG mRNA levels appears to result, at least in part, from positive effects on the DNA binding activity of CRE transcription factors. Further studies are required to determine whether this regulatory pathway is activated in human obesity and to elucidate the importance of adipose ATG to the elevated blood pressure observed in this pathological state.

Key Words: Angiotensinogen expression; adipocytes; cyclic AMP.

Introduction

Because abdominal obesity is an important risk factor for the development of metabolic alterations, such as cardiovascular disease, hypertension, type-2 diabetes, and hyperlipidemia, it significantly contributes to cardiovascular morbidity and mortality *(1,2)*.

In the past few years, adipose tissue has proven to be a real endocrine organ expressing and secreting many signals that act at different levels as autocrine/paracrine or endocrine factors *(3)*. In particular, various studies suggest the existence of a functional renin–angiotensin system (RAS) in human and rodent adipose tissues *(4–6)*, as already identified in a number of other organs *(7)*, making white adipose tissue the second most abundant source of angiotensinogen (ATG) *(7)*, the precursor of angiotensin II (ANG II) *(8)*.

In animal models, RAS has been implicated in the positive regulation of adipose tissue processes such as differentiation of preadipocytes into adipocytes *(9,10)* and induction of key genes for lipogenesis *(11)*. Moreover, overfeeding in the rat enhances the expression and the secretion of ATG from mature adipocytes, which is accompanied by an increase in blood pressure *(12)*. In spontaneously hypertensive rats, ATG gene expression in adipose tissue is increased *(13)*, whereas the ATG knock-out mice model demonstrated that ATG secreted from adipose tissue is released into the circulation and contributes to blood pressure regulation *(14)*.

In humans, there is a well-established association between hypertension and obesity *(15)*, although the causal mechanisms for their coexistence are poorly understood. The discovery that adipose tissue expresses and secretes ATG together with the finding that obese patients have abnormally high circulating ATG levels *(16)* suggest the possible involvement of the adipocyte-derived ATG in the pathogenesis of obesity-related hypertension *(17)*. Evidence was provided that the increased sympathetic tone observed in obese subjects could be responsible in part for obesity-related hypertension *(18)*. Increased sympathetic activity leads to enhanced secretion of catecholamines, which are known to stimulate (via β-adrenoceptors) or reduce (via α-adrenoceptors) the intracellular cyclic AMP (cAMP) levels in adipose cells *(19)*.

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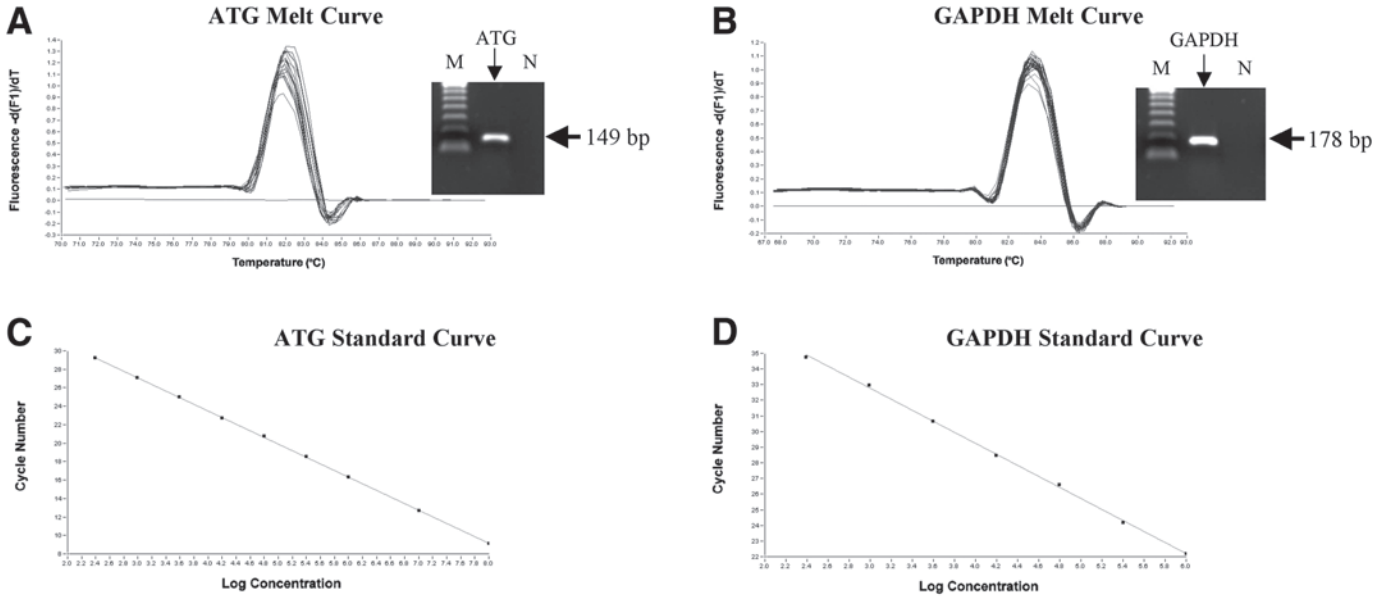

Fig. 1. ATG and GAPDH quantitative real-time PCR. Data show (**A**) a representative PCR melt curve of ATG and (**B**) GAPDH PCR products indicating single amplicons in each reaction and the corresponding agarose gel analysis of PCRs, indicating products with the expected sizes for angiotensinogen (149 bp) and GAPDH (178 bp). Lane M represents the molecular weight marker; lane N is control without cDNA (negative control). (**C**) and (**D**) represent standard curves of input mRNA concentration vs. number of amplification cycles required to yield a signal above threshold for ATG and GAPDH, respectively, demonstrating linearity.

Cyclic AMP stimulates target gene expressions via a conserved cAMP responsive element (CRE). A composite CRE has been identified in the promoter of the human ATG gene *(20)*, located around 840 bases upstream from the transcriptional initiation site. This element is recognized by members of the CRE binding protein (CREB) family of transcription factors.

We have previously reported that cAMP and isoproterenol (a β-adrenergic receptor agonist) in vitro upregulates ATG expression and secretion in rat adipose tissue *(21)*. Because opposite results have been reported between the rat and the human models concerning the influence of RAS on adipogenesis *(9,22)*, the aim of the present study was to establish whether cAMP regulates in vitro the expression and secretion of human adipocyte ATG and whether such a regulation involves changes in the DNA binding activity of CRE transcriptional factors.

Results

Quantitative Real-Time RT-PCR Optimization

After extensive optimization of primers, reaction temperature, and times, we developed a highly specific, sensitive, and reproducible real-time RT-PCR assay to quantify human ATG and GAPDH mRNA. The selected primers produced a single product of the expected size without observable primer dimers (Figs. 1A,B). The specific melting curves of ATG and GAPDH PCR products showed single peaks with a T_m of 81.89 ± 0.13°C and 83.66 ± 0.05°C, respectively (Figs. 1A,B). The sensitivity of the PCR method was determined from the crossing point (Cp) values obtained with known quantities of cDNA. The calibration curves for human ATG and GAPDH cDNAs (each diluted fourfold from 10^6 to 250 copies of "estimated" mRNA per reaction mixture) showed linearity over the entire quantification range with correlation coefficients >0.99, indicating a precise log-linear relationship (Figs. 1C,D). Slopes for the dilution series of the two calibrators were 3.46–3.47, indicating comparable PCR amplification efficiencies. The intrarun variability was calculated from duplicate samples for the two targets, and the difference in absolute Cp values for each set of duplicates was < 0.55 cycle. The interrun variability was assessed by comparing the results of three different runs using calibrator dilution duplicates and the difference in Cp values never exceeded 1.2 cycles.

Effect of cAMP on ATG Expression and Secretion in Human Adipose Tissue

The effect of cAMP on the steady-state level of ATG mRNA was examined in human adipose fragments maintained in culture for 24 h in the absence or presence of the cAMP analog 8-Bromo-cyclic AMP (8Br-cAMP) (Sigma, St. Louis, MO). A 24 h exposure was chosen because preliminary kinetic experiments revealed that this time gave optimal results for the ATG mRNA levels and protein secretion determinations compared with those obtained after 6 or 12 h (data not shown). The validity of our experimental conditions was warranted by positive response of ATG expression to glucocorticoids *(23)*.

Table 1
Influence of 8Br-cAMP and/or a PKA Inhibitor (H89)
on ATG Gene Expression (Real-Time PCR) and ATG Secretion in Human Adipose Tissue Fragments

	Control	8Br-cAMP	H89	8Br-cAMP + H89	Dexamethasone
Expression					
ATG copy/10^3 GAPDH copies	0.017 ± 0.002	0.047 ± 0.010*	0.080 ± 0.025*	0.066 ± 0.017*	0.148 ± 0.015**
% of control	100 ± 9	276 ± 60	468 ± 146	385 ± 97	863 ± 85
Secretion					
ng/g tissue/24h	0.30 ± 0.04	0.42 ± 0.08*	0.56 ± 0.03**	0.66 ± 0.16**	0.59 ± 0.07**
% of control	100 ± 13	140 ± 27	187 ± 10	220 ± 53	197 ± 23

Values are means of four separate experiments ± SEM.
*$p \leq 0.05$ vs control.
**$p \leq 0.005$ vs control.

As shown in Table 1, exposure to 8Br-cAMP (0.25 m*M*) for 24 h resulted in a significant increase (+ 176 ± 60%) in ATG mRNA levels (Table 1). A 24 h exposure to dexamethasone (Dex) (10 n*M*) led to a larger increase (+ 763 ± 85%) in ATG mRNA. At the same time, ATG secretion was also slightly but significantly enhanced by 8Br-cAMP (+ 40 ± 27%) and almost doubled by Dex (Table 1). Furthermore, increases in ATG mRNA and protein secretion caused by 8Br-cAMP, rather than to be suppressed by the protein kinase A (PKA) inhibitor H89 (Sigma, St. Louis, MO), were maintained or even enhanced due to a positive effect of H89 per se (Table 1).

Effect of 8Br-cAMP on CRE DNA Binding Activity

Gel mobility shift assays were next performed to determine whether the cAMP modulation of ATG expression is associated to a variation in the DNA binding activity of CRE transcriptional factors. First, the specificity of the CRE DNA binding was verified. As previously described in other cell types *(20,24)*, nuclear extracts of human adipose tissue fragments formed two complexes with labeled CRE, and these complexes were blocked by excess amounts of a cold CRE probe but not by the nonspecific competitor (Oct-1) (Fig. 2A). Moreover, data in Fig 2B show that the CRE binding activity was significantly increased (+115 ± 14%, $p < 0.05$, $n = 3$) following human adipose tissue exposure to 8Br-cAMP. This effect was rapid (2 h 30 min) and short because it disappeared after 6 h of treament (data not shown).

Discussion

From the recent literature, regulation of ATG gene expression appears to be species and tissue specific *(25–27)*. In fact, ATG gene expression decreases in rat hepatocytes after exposure to catecholamines and in the 3T3-L1 adipocyte model after beta-adrenergic stimulation *(27,28)*. In contrast, DNA binding and transient transfection experiments in HepG2 cells have revealed that cAMP increases the expression of human ATG gene through a combination of CREB and a liver-specific transcription factor (member of the C/EBP family), which bind to the pseudo-symmetrical CRE of the human ATG gene promoter *(20)*. Our present results on human adipose tissue are consistent with the latter study, because we also found that cAMP analogs increase the expression and the secretion of ATG and, at the same time, enhance DNA binding activity toward CRE transcription factors.

In rat adipose tissue, we have recently demonstrated that cAMP and isoproterenol treatment increases ATG mRNA and protein secretion as well, via the PKA-dependent pathway *(21)*. Others have shown the same positive effect in mouse hepatoma cells and in rat hepatocytes *(29,30)*. In contrast, the present study indicates that the addition of H89 alone, an inhibitor of cAMP-dependent PKA, increases ATG mRNA levels and protein secretion in human adipose tissue. This increase in ATG mRNA due to H89, which was not found in rat fat cells *(21)*, may result from a cAMP-independent action of H89 on the transcriptional or post-transcriptional regulation of ATG expression in human adipocytes specifically. However, it has been described for a number of proteins that the rate of their synthesis is regulated by post-transcriptional stabilization or destabilization of their mRNA *(31)*. Therefore, it appears possible that H89 may regulate ATG, by stabilizing proteins whose effect depends on post-translational modification, such as phosphorylation or dephosphorylation reactions. Such a mechanism, i.e., phosphorylation and dephosphorylation of some mRNA-binding proteins, has been found to be responsible for the stabilization of mRNA coding for cytokines *(32,33)*. In addition, tissue-specific factors have been identified that bind to unphosphorylated CREB and increase promoter activity through a CRE *(34)*. Further studies, such as adipocyte transient transfection with a reporter construct containing human ATG promoter and gel mobility shift assay, will be necessary to characterize fully the relationship among H89, cAMP, and ATG gene expression in human adipose tissue.

The adrenoreceptors play a major role in the regulation of white fat cell function. Catecholamines stimulate the

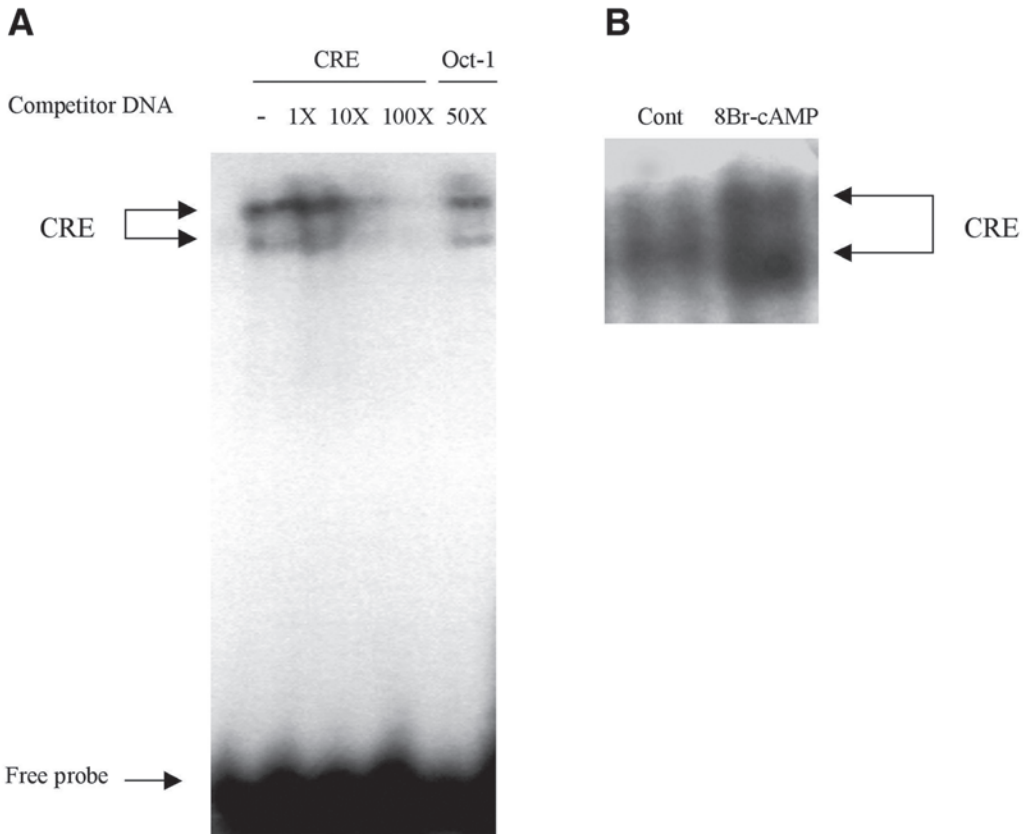

Fig. 2. Influence of 8Br-cAMP on CRE DNA binding activity. (**A**) Adipose nuclear extracts were incubated with a radiolabeled CRE probe in the presence of 1-, 10-, and 100-fold molar excesses of unlabeled CRE or of 50-fold molar excess of unlabeled Oct-1 DNA competitor. (**B**) Adipose tissue fragments were incubated for 2 h 30 min with 8Br-cAMP (0.25 m*M*). Nuclear extracts prepared from these fragments were then incubated with the radiolabeled CRE probe as described in the Materials and Methods section. This figure is representative of three independent experiments. The two arrows denote specific binding complexes.

membrane adenylyl cyclase and increase intracellular cAMP in adipose cells via β-adrenergic receptors. An increased activation of the sympathetic nervous system is commonly observed in obese subjects *(18)* and is believed to be involved in the pathogenesis of obesity-related hypertension *(35)*. Indeed, arterial hypertension is a condition that often occurs in obese patients *(36)* and that adversely affects their prognosis so that weight loss is an efficacious part of the treatment for the hypertensive state *(37)*. It thus appears important to get a better understanding on how ATG production is regulated in thus adipocytes, all the more so because several observations point to a potential role to the adipose tissue RAS in obesity-associated hypertension. In fact, (i) a positive relation between ATG plasma levels and blood pressure was first described in humans *(38)*, and also in rat models of hypertension *(13,39)* and (ii) various studies reported a positive correlation between plasma ATG levels and body mass index (BMI) in different human populations *(16,40,41)*. Moreover, a number of studies have found increased adipose ATG gene expression in obesity, in both humans and rodents *(28,42–44)*. In contrast, (i) in one study, ATG expression was found unaltered in adipose tissue of obese hypertensive subjects compared with those normotensive and controls *(45)* and (ii) previous studies, reported either positive *(42)* or negative *(46)* correlation between ATG expression in adipose tissue and BMI. On the other hand, the ATG-deficient hypotensive mouse model *(14)* has demonstrated that adipose tissue is a source of circulating ATG, although the exact contribution of the adipose to the whole blood ATG was not assessed in this study. However, once released into the circulation, adipose ATG seems involved in blood pressure regulation, because mice whose ATG expression is restricted to adipose tissue have ATG circulating in the blood stream and are normotensive compared with the hypotensive ATG-deficient mice *(14)*. However, further studies, performed under more physiological conditions, are required to firmly establish whether variations of adipose ATG expression and secretion can influence plasma ATG and, also, blood pressure.

The increased adipocyte ATG expression and secretion by cAMP suggests that the sympathetic nervous system may have a regulatory role in the activation of the local RAS.

Hence, we speculate that the activation of the sympathetic nervous system during obesity may enhance the release of adipocyte ATG into the circulation, which could then intervene in blood pressure regulation.

In summary, we have demonstrated that cAMP increases the expression and secretion of human ATG in adipose tissue and that the induction in ATG mRNA levels appears to result, at least in part, from positive effects on the DNA binding activity of CRE transcription factors. Our data may be useful for a better understanding of the link among sympathetic tone, elevated plasma ATG levels, and hypertension, all of which characterize the obese state.

Materials and Methods

Subjects

Adipose tissue samples (10–20 g) were obtained from subcutaneous fat depots of men (57 ± 8.5 yr). The tissue donor group was composed of four men (BMI: 26.2 ± 3.2 kg/m^2). None of these patients suffered from endocrine malignant or chronic inflammatory diseases. All patients were undergoing elective surgery in accordance with the local ethical committee.

Adipose Tissue Culture Conditions

The adipose tissue samples were collected in saline (150 m*M* NaCl) and immediately transferred to the laboratory. After removing blood vessels and connective tissue, adipose tissue was rinsed in saline containing antibiotics (100 U/mL penicillin and 0.1 mg/mL streptomycin) and cut into small pieces. Tissue fragments (0.3–1 g) were placed in cell culture dishes and incubated for 24 h at 37°C under 5% CO_2 and 95% air atmosphere in 6 mL of Dulbecco's modified Eagle's medium (DMEM)–Ham's F12 medium containing bovine serum albumin (BSA) (1.5%) (Sigma, St. Louis, MO), penicillin (100 U/mL), streptomycin (0.1 mg/mL), antiproteolytic agents [10 µg/mL 4-(2-amoniethyl)-benzenesulfonylfluoride hydrochloride (AEBSF) (Interchim, Montluçon, France), 2 µ*M* leupeptin, and 25 µg/mL aprotinin (Sigma, St. Louis, MO)], and vitamin E (4 mg/mL). At the end of incubation, tissue fragments were collected in a standard acid guanidinium isothiocyanate solution *(47)*, homogenized, and centrifuged at 3000*g* for 3 min at 4°C to remove lipids and kept at –80°C. Culture medium was collected 24 h later in the presence of 10 µ*M* captopril and stored at –80°C until they were used for ATG assay. To check possible cellular lysis and unviability, lactate dehydrogenase (LDH) activity released into the culture medium and cellular glucose uptake were determined in parallel, but no differences between each incubation set could be recorded.

Total RNA Extraction and cDNA Synthesis

Total RNA was extracted and purified following the guanidinium isothiocyanate procedure described by Chomczynski and Sacchi *(47)*. The yield and quality of extracted RNA were assessed by the 260/280 nm optical density ratio and by electrophoresis under denaturing conditions on 1% agarose gel. The RNA was stored at –80°C until use.

The reverse transcription was carried out in a final volume of 10 µL as previously described *(48)* by use of the Superscript™ II RNase H- reverse transcriptase (Life Technologies, Grand Island, NY) with random hexamers and 0.1 µg of total RNA. Controls without reverse transcriptase were systematically performed to detect genomic DNA contamination. The cDNA was stored at –20°C until use.

Quantitative Real-Time PCR

Human ATG mRNA levels were analyzed with a Light Cycler® instrument (Roche Diagnostics, Mannheim, Germany) using QuantiTect SYBR Green PCR Master Mix (Qiagen, Hilden, Germany).

Primers

On the basis of the published genomic sequence of human ATG mRNA (GenBank accession no. K02215), one gene-specific primer set was designed using specific primer analysis software (Oligo 4.0), and these sequences were analyzed by FASTA in the EMBL database. The primer set used in the analysis of human ATG gene expression was $ATGH_U$ (5' TGG GGG AGG TGC TGA ACA 3') and $ATGH_L$ (5' AGT GGC GCT TTG ATC ATA CA 3') which produces a 149 bp amplicon. The $ATGH_U$ primer spanned two exons to avoid contamination by genomic DNA. The glyceraldehyde phosphate dehydrogenase (GAPDH) primer set was G_U (5' ACC CAC TCC TCC ACC TTT G 3') and G_L (5' CTC TTG TGC TCT TGC TGG G 3'), which produces a 178 bp amplicon *(49)*.

Preparation of cDNA Calibrators for Quantitative Real-Time PCR and Calibration Curve Construction

cDNA calibrators were prepared by PCR amplification run to saturation (35 cycles) with the appropriate primers. The resulting cDNAs were purified by QIAquick PCR Purification Kit (Qiagen, Hilden, Germany) and eluted with Tris–EDTA (pH 8.0) buffer. The samples showed a unique band in agarose electrophoresis. The copy numbers of cDNA were calculated from the absorbance at 260 nm. Calibrators were defined to contain arbitrary units of angiotensinogen and GAPDH mRNA, and all calculated concentrations are relative to these concentrations.

Separate calibration curves for human ATG and GAPDH were constructed from serial dilutions from 10^8 copies to 250 copies of cDNA calibrators.

PCR Conditions

Real-time PCR was performed in a total reaction volume of 20 µL per capillary for the LightCycler format. Each provided cDNA preparation (± 10 ng/µL) was diluted 1:10 in water. The reaction buffer contained 10 µL of 2X Quanti Tect SYBR Green PCR Master Mix (including HotStar *Taq*

DNA polymerase, reaction buffer, desoxynucleotide triphosphate mixture, and SYBR Green I), 0.5 μ*M* each primer and 4 μL of diluted cDNA or calibrator. The cycling conditions were as follows: initial denaturation at 94°C for 15 min, followed by 50 cycles of denaturation at 94°C for 15 s, annealing at 58°C for human ATG or at 55°C for GAPDH for 20 s and extension at 72°C for 10 s. The temperature transition rate was set at 20°C/s.

Melting Curve

After PCR, a melting curve was constructed by increasing the temperature from 65°C to 95°C with a transition rate of 0.1°C/s to verify the specificity of the desired PCR products and the absence of primer–dimers. To verify the melting curve results, representative samples of PCR products were separated by 1.5% agarose gel electrophoresis.

Normalization and Quantification

The Fit Points Method was used to determine the crossing point (Cp) (the virtual cycle number where all the standards and unknowns would have had the same amount of PCR product) automatically for the individual samples. The LightCycler software 3.1 constructed the calibration curve by plotting the Cp vs the logarithm of the number of copies for each calibrator. The copy numbers for unknown samples were determined by LightCycler software 3.1, according to the calibration curve. To correct the differences in both RNA quality and quantity between samples, data were normalized by dividing the copy number of ATG mRNA by the copy of GAPDH mRNA.

Assay for ATG Protein Secretion

ATG protein was indirectly determined by a radioimmunoassay of angiotensin I generated by an excess of hog renin (Sigma, St. Louis, MO) using the REN-CT2 RIA kit (CIS bio International, Gif sur Yvette, France), as previously described *(48)*. The inter- and intraassay variation coefficients were less than 10%. The anti-angiotensin I antibody cross-reactivity was less than 0.01% for angiotensin II and III. The sensitivity of the assay was 0.15 ng/mL.

Nuclear Extracts Preparation

At the end of incubation, human adipose tissue fragments were rapidly rinsed in cold PBS. They were then homogenized in 3 vol (wt/vol) of cold buffer A (10 m*M* HEPES, pH 7.9, 1.5 m*M* $MgCl_2$, 10 m*M* KCl, 0.05 m*M* dithiothreitol (DTT), 0.57 m*M* AEBSF, 0.5 m*M* sodium deoxycholate, 1 m*M* orthovanadate, 30 m*M* β-glycerophosphate, 5 μg/mL aprotinin, and 5 μg/mL leupeptin). The homogenates were centrifuged at 7000*g* for 10 min at 4°C. The nuclear extract was prepared as previously described *(50)*, with some modifications. Briefly, the supernatant was decanted and the resulting pellet was resuspended in 5 vol of buffer A and centrifuged a second time at 25,000*g* for 20 min at 4°C. Crude nuclei were resuspended in cold buffer B [20 m*M* HEPES, pH 7.9, 25% (vol/vol) glycerol, 0.42 *M* NaCl, 1 m*M* EDTA, 0.05 m*M* DTT, 0.57 m*M* AEBSF, 0.5 m*M* sodium deoxycholate, 1 m*M* orthovanadate, 30 m*M* β-glycerophosphate, 5 μg/mL aprotinin, and 5 μg/mL leupeptin]. The suspension was vigourously shaken at 4°C for 30 min, followed by centrifugation at 25,000*g* for 30 min at 4°C. The supernatant containing the nuclear extracts was aliquoted and stored at −80°C until use for electrophoretic mobility shift assay (EMSA).

EMSA

Protein–DNA complexes were formed by incubating 10 μg of nuclear proteins in a buffer containing 10 m*M* HEPES, pH 7.9, 50 m*M* KCl, 5 m*M* $MgCl_2$, and 1 m*M* DTT in the presence of 1 μg of nonspecific competitor DNA poly-(deoxyinosine-deoxycytidine) for 15 min at 4°C. The ^{32}P-labeled probe was then added and the incubation was conducted for a further 15 min at room temperature. The resulting DNA–protein complexes were separated from the unbound probe by electrophoresis on a native 6% polyacrylamide gel in 0.5X TBE (Tris/borate/EDTA buffer). The gels were then dried and subjected to autoradiography. The probe for gel mobility shift analysis was chemically synthetized. After annealing, the resultant CRE double-stranded oligonucleotides (5'- CTC ACC CAC TGC GTC ACT TGT GAT CAC TG -3') were labeled with [γ-^{32}P]ATP (3000 Ci/mmol) using T4 polynucleotide kinase kit (Promega Corp.). Unincorporated nucleotides were removed by chromatography in a G25 column. Signals were quantified by densitometry.

Parallel gels were performed and stained to ensure that the amount of nuclear extracts loaded was identical whatever the experimental conditions.

In competition experiments, 1- 10-, and 100-fold molar excesses of unlabeled CRE double-stranded oligonucleotides or a 50-fold molar excess of unlabeled heterologue Oct-1 double-stranded oligonucleotides (5'-TGT CGA ATG CAA ATC ACT AGA A-3') were included in the binding reaction mixture.

Other Determinations

LDH activity and glucose concentrations in the incubation medium were assessed as previously described *(48)*.

Statistical Analysis

Results are expressed as mean ± SEM of at least three individual experiments. Statistics were performed using Excel 2000 computer software (Microsoft Inc.). Statistical analysis of comparisons among groups was undertaken using unpaired *t* tests.

Acknowledgments

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References

1. Kissebah, A. H. and Krakower, G. R. (1994). *Physiol. Rev.* **74,** 761–811.
2. Bjorntorp, P. (1997). *Lancet* **350,** 423–426.
3. Ailhaud, G. (2000). *Int. J. Obes. Relat. Metab. Disord.* **24 (Suppl 2),** S1–3.
4. Saye, J. A., Ragsdale, N. V., Carey, R. M., and Peach, M. J. (1993). *Am. J. Physiol.* **264,** C1570–1576.
5. Pinterova, L., Krizanova, O., and Zorad, S. (2000). *Gen. Physiol. Biophys.* **19,** 329–334.
6. Engeli, S., Gorzelniak, K., Kreutz, R., Runkel, N., Distler, A., and Sharma, A. M. (1999). *J. Hypertens.* **17,** 555–560.
7. Phillips, M. I., Speakman, E. A., and Kimura, B. (1993). *Regul. Pept.* **43,** 1–20.
8. Reid, I. A., Morris, B. J., and Ganong, W. F. (1978). *Annu. Rev. Physiol.* **40,** 377–410.
9. Darimont, C., Vassaux, G., Ailhaud, G., and Negrel, R. (1994). *Endocrinology* **135,** 2030–2036.
10. Saint-Marc, P., Kozak, L. P., Ailhaud, G., Darimont, C., and Negrel, R. (2001). *Endocrinology* **142,** 487–492.
11. Jones, B. H., Standridge, M. K., and Moustaid, N. (1997). *Endocrinology* **138,** 1512–1519.
12. Frederich, R. C., Jr., Kahn, B. B., Peach, M. J., and Flier, J. S. (1992). *Hypertension* **19,** 339–344.
13. Tamura, K., Umemura, S., Nyui, N., et al. (1996). *Hypertension* **27,** 1216–1223.
14. Massiera, F., Bloch-Faure, M., Ceiler, D., et al. (2001). *FASEB J.* **15,** 2727–2729.
15. Faloia, E., Giacchetti, G., and Mantero, F. (2000). *J. Endocrinol. Invest.* **23,** 54–62.
16. Umemura, S., Nyui, N., Tamura, K., et al. (1997). *Am. J. Hypertens.* **10,** 629–633.
17. Engeli, S., Negrel, R., and Sharma, A. M. (2000). *Hypertension* **35,** 1270–1277.
18. Berglund, G. and Andersson, O. (1981). *Int. J. Obes.* **5(Suppl 1),** 143–150.
19. Rodbell, M. (1964). *J. Biol. Chem.* **239,** 375–380.
20. Narayanan, C. S., Cui, Y., Kumar, S., and Kumar, A. (2000). *Mol. Cell. Biochem.* **212,** 81–90.
21. Serazin, V., Dieudonne, M. N., Morot, M., de Mazancourt, P., and Giudicelli, Y. (2004). *Am. J. Physiol. Endocrinol. Metab.* **286,** E434–438.
22. Janke, J., Engeli, S., Gorzelniak, K., Luft, F. C., and Sharma, A. M. (2002). *Diabetes* **51,** 1699–1707.
23. Aubert, J., Darimont, C., Safonova, I., Ailhaud, G., and Negrel, R. (1997). *Biochem. J.* **328(Pt 2),** 701–706.
24. Yang, S., Fang, Z., Suzuki, T., et al. (2002). *J. Clin. Endocrinol. Metab.* **87,** 2336–2345.
25. Wang, T. T., Chen, M., Lachance, S., Delalandre, A., Carriere, S., and Chan, J. S. (1994). *Kidney Int.* **46,** 703–710.
26. Coezy, E. D., Corvol, P., and Howlett, A. C. (1990). *Cell Signal.* **2,** 67–76.
27. Klett, C., Nobiling, R., Gierschik, P., and Hackenthal, E. (1993). *J. Biol. Chem.* **268,** 25095–25107.
28. Jones, B. H., Standridge, M. K., Taylor, J. W., and Moustaid, N. (1997). *Am. J. Physiol.* **273,** R236–242.
29. Ming, M., Wang, T. T., Lachance, S., Delalandre, A., Carriere, S., and Chan, J. S. (1995). *Am. J. Physiol.* **268,** R105–111.
30. Ohtani, R., Yayama, K., Takano, M., Itoh, N., and Okamoto, H. (1992). *Endocrinology* **130,** 1331–1338.
31. Nielsen, D. A. and Shapiro, D. J. (1990). *Mol. Endocrinol.* **4,** 953–957.
32. Gillis, P. and Malter, J. S. (1991). *J. Biol. Chem.* **266,** 3172–3177.
33. Malter, J. S. and Hong, Y. (1991). *J. Biol. Chem.* **266,** 3167–3171.
34. Fimia, G. M., De Cesare, D., and Sassone-Corsi, P. (1999). *Nature* **398,** 165–169.
35. Anderson, E. A., Sinkey, C. A., Lawton, W. J., and Mark, A. L. (1989). *Hypertension* **14,** 177–183.
36. Kannel, W. B., Brand, N., Skinner, J. J., Jr., Dawber, T. R., and McNamara, P. M. (1967). *Ann. Intern. Med.* **67,** 48–59.
37. Eliahou, H. E., Iaina, A., Gaon, T., Shochat, J., and Modan, M. (1981). *Int. J. Obes.* **5(Suppl 1),** 157–163.
38. Caulfield, M., Lavender, P., Newell-Price, J., Kamdar, S., Farrall, M., and Clark, A. J. (1996). *Hypertension* **28,** 1123–1125.
39. Nyui, N., Tamura, K., Yamaguchi, S., et al. (1997). *Hypertension* **30,** 859–867.
40. Cooper, R., McFarlane-Anderson, N., Bennett, F. I., et al. (1997). *J. Hum. Hypertens.* **11,** 107–111.
41. Cooper, R., Forrester, T., Ogunbiyi, O., and Muffinda, J. (1998). *J. Hypertens.* **16**, 571–575.
42. Van Harmelen, V., Ariapart, P., Hoffstedt, J., Lundkvist, I., Bringman, S., and Arner, P. (2000). *Obes. Res.* **8,** 337–341.
43. Hainault, I., Nebout, G., Turban, S., Ardouin, B., Ferre, P., and Quignard-Boulange, A. (2002). *Am. J. Physiol. Endocrinol. Metab.* **282,** E59–66.
44. Giacchetti, G., Faloia, E., Sardu, C., et al. (2000). *Int. J. Obes. Relat. Metab. Disord.* **24(Suppl 2)**, S142–143.
45. Faloia, E., Gatti, C., Camilloni, M. A., et al. (2002). *J. Endocrinol. Invest.* **25,** 309–314.
46. Gorzelniak, K., Engeli, S., Janke, J., Luft, F. C., and Sharma, A. M. (2002). *J. Hypertens.* **20**, 965–973.
47. Chomczynski, P. and Sacchi, N. (1987). *Anal. Biochem.* **162,** 156–159.
48. Serazin-Leroy, V., Morot, M., de Mazancourt, P., and Giudicelli, Y. (2000). *Am. J. Physiol. Endocrinol. Metab.* **279,** E1398–1405.
49. Dotzlaw, H., Leygue, E., Watson, P. H., and Murphy, L. C. (1997). *J. Clin. Endocrinol. Metab.* **82,** 2371–2374.
50. Dignam, J. D., Lebovitz, R. M., and Roeder, R. G. (1983). *Nucleic Acids Res.* **11,** 1475–1489.