Regulation by glucocorticoids of angiotensinogen gene expression and secretion in adipose cells

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Adipose cells are an important source of angiotensinogen (AT). Its activation product, angiotensin II, stimulates *in vitro* and *in vivo* the production and release of prostacyclin which acts as a potent adipogenic signal in promoting the terminal differentiation of preadipocytes to adipocytes. Since glucocorticoids are known to promote adipose cell differentiation *in vitro* as well as *in vivo*, their role in the regulation of AT gene expression and secretion has been investigated in cultured Ob1771 mouse adipose cells. In contrast with liver cells, which are the major source of AT and the target of several hormones for the regulation of its expression, adipose cells are only responsive to glucocorticoids, which are

able to up-regulate AT gene expression and AT secretion rapidly and dose-dependently. On exposure to glucocorticoids, accumulation of AT mRNA appears primarily to be due to transcriptional activation of the gene and is paralleled by secretion of the protein. Similar results on AT mRNA expression and AT secretion were obtained using explants of rat adipose tissue *ex vivo* demonstrating a major if not exclusive mechanism of regulation of AT production by glucocorticoids in mature adipose cells. Together these results provide a potential link between glucocorticoids, AT, the growth of adipose tissue and increased blood pressure.

INTRODUCTION

Angiotensinogen (AT) is the unique substrate of renin and the precursor of angiotensin II (angII) in the renin–angiotensin system (RAS), which plays a major role in the regulation of blood pressure as well as in the Na⁺ and extracellular fluid balance [1]. Additional roles for AT have been suggested since the following pieces of evidence support the existence of local RASs in various tissues [2–4], including the expression of AT in white and brown adipose tissue [4–6]. First, the involvement of RAS in organogenesis has been proposed, as angII receptor of the AT2 subtype is transiently expressed at high levels in rodent, primate and human fetuses [7,8]. Secondly, with respect to adipose tissue, which is the major source of AT after the liver [2], the characterization in adult rodents of renin-like and angiotensin I-converting enzyme activities has suggested the occurrence of a functional RAS in adipose tissue development [9–11]. From *in vitro* and *in vivo* studies, it has been hypothesized that AT is the first partner in a paracrine/autocrine mechanism in which only mature adipocytes secrete AT [12]. When preadipose and adipose cells are co-cultured and exposed to angII, this peptide binds to specific receptors in adipocytes, which respond by a specific increase in prostacyclin production [12]. Since prostacyclin behaves as an adipogenic effector able to trigger preadipocytes to differentiate to adipocytes, this process leads to the formation of new fat-cells [12–15]. This mechanism may operate *in vivo*, since angII triggers adipose-tissue prostacyclin production [16] and prostacyclin perfusion by means of *in situ* microdialysis enhances the formation of new fat-cells (C. Darimont, P. Saint-Marc, G. Ailhaud and R. Négrel, unpublished work). The fact that adipose tissue is the second largest producer of AT after the liver is also of interest since epidemiological studies have shown a tight

correlation between accumulation of intra-abdominal fat and high blood pressure [17], although the contribution of AT produced by adipose tissue to this pathophysiological situation is not well understood. As a first step to gaining some insight into this phenomenon, we studied in adipose cells the effect of glucocorticoids on AT gene expression and AT secretion, as the relative enrichment of glucocorticoid receptors has been consistently implicated over the last decade in the selective development of human visceral fat [18]. Moreover the role of glucocorticoids and the importance of the hypothalamic–pituitary–adrenal axis in excessive development of adipose tissue have been documented in two models of transgenic mice [19,20]. In both cases, the observed increase in corticotrophin-releasing factor and corticosterone levels was accompanied by an increase in adipose-tissue mass, with the development of Cushing's syndrome in mice overexpressing corticotrophin-releasing factor [20]. The results presented here show that, in contrast with liver cells which have been reported to respond positively to glucocorticoids, oestrogens, tri-iodothyronine (T₃), growth hormone and angII, Ob1771 adipocytes were only responsive to glucocorticoids for up-regulation of AT gene expression and AT secretion.

MATERIALS AND METHODS

Cell culture

Ob1771 cells [21] were plated at a density of 2×10^3 /per cm² and grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 8% fetal bovine serum (FBS), 200 units/ml penicillin, 50 µg/ml streptomycin, 33 µM biotin and 17 µM pantothenate. When cells reached confluence 5 days later, they

Abbreviations used: angl, angiotensin I; angII, angiotensin II; AT, angiotensinogen; Dex, dexamethasone; DMEM, Dulbecco's modified Eagle's medium; FBS, fetal bovine serum; IBMX, isobutylmethylxanthine; IT medium, DMEM supplemented with 5 µg/ml insulin (850 nM) and 10 µg/ml transferrin; RAS, renin–angiotensin system; T₃, tri-iodothyronine.

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were fed with this culture medium enriched with 17 nM insulin and 2 nM T_3 . This medium, referred to as standard differentiation medium, was usually supplemented with 10 nM dexamethasone (Dex) and 100 μ M isobutylmethylxanthine (IBMX) during the first 3 days only, to trigger differentiation. Cells were then fed with standard differentiation medium renewed every other day until day 16 or 18 after confluence, at which time more than 80 % of the cell population appeared under the microscope as lipid-filled differentiated adipose cells. Unless otherwise stated, experiments were performed using differentiated adipose cells after the shift to a serum-free chemically defined medium composed of DMEM supplemented with 5 μ g/ml insulin (850 nM) and 10 μ g/ml transferrin (referred to as IT medium) for 24 h before exposure to various effectors in the same medium.

Culture of adipose-tissue explants

Dissected periepididymal fat pads from 8-week-old Wistar rats were rinsed in PBS, pH 7.4, chopped into approx. 150 mm³ pieces, and incubated in IT medium enriched with 200 pM T_3 , 20 nM sodium selenite and 100 μ M sodium ascorbate, in the presence or absence of 10^{-7} M Dex. The culture medium was collected 24 h later for assay of AT as described below, and adipose-tissue fragments were then used for RNA analysis either on the whole tissue or after digestion with collagenase and separation by centrifugation of the stromal-vascular and adipocyte fractions, as described previously [22].

RNA analysis

RNA was extracted and purified by the guanidinium thiocyanate technique [23]. For Northern-blot analysis, 20 μ g of total RNA was separated by electrophoresis on 1.2 % (w/v) agarose gel containing 1.1 M formaldehyde and transferred to a Hybond N⁺ membrane (Amersham, Les Ulis, France). Hybridizations were performed for 24 h at 65 °C in 0.5 M phosphate buffer, pH 7.2, containing 5 % SDS and 10^6 c.p.m./ml randomly primed ³²P-labelled cDNA probes. The autoradiographs were quantified by densitometry with an LKB-XL (Rockville, MD, U.S.A.) laser photodensitometer or a Fujix phosphorImager. All results were normalized to β -actin signals.

AT protein assay

Ob1771 cells cultured in standard differentiation medium in 24 multiwell culture plates were washed (twice for 30 s and once for 1 h) with IT medium at the indicated times. IT medium (1 ml) supplemented or not with other effectors was then added to the culture wells. After various times of exposure, the culture medium was collected in the presence of a cocktail of BSA and anti-proteolytic agents giving final concentrations of 1 % BSA, 1 mM PMSF, 5 mM EDTA, 1 mM hydroxyquinoline and 10 μ M captopril. Cells were then scraped into 50 mM sodium phosphate buffer, pH 7.4, containing anti-proteolytic agent and BSA as above and homogenized by sonication. AT was determined after its total conversion in the presence of exogenous mouse renin to angI, which was measured by RIA as described by Sealy and Laragh [24]. Mouse renin was prepared from submandibular glands using the protocol of Jacobsen and Poulsen [25].

Materials

Culture media were obtained from Gibco (Cergy-Pontoise, France). FBS was a product of Seromed (Berlin, Germany). All other cell culture products were obtained as previously reported [13–15,21]. Random-priming kits, [α -³²P]dCTP, Hybond mem-

branes and products used for the RIA of AT were purchased from Amersham, except the angI antibody which was from ICN (Orsay, France). Restriction enzymes were from Eurogentec (Seraing, Belgium). Actinomycin D, cycloheximide, angII, forskolin, Dex and other steroid hormones were purchased from Sigma Chimie (Saint Quentin Fallavier, France). RU 38486 was a gift from Roussel Uclaf. The source of the various cDNAs used in this study is acknowledged in the appropriate section.

RESULTS

Regulation of AT mRNA in adipose cells in response to various effectors: specific effect of glucocorticoids

As previously reported [26–29], accumulation of AT mRNA appears as a late phenomenon of adipose-cell differentiation and thus maximal expression of this gene occurs in differentiated cells. To investigate positive modulations of AT gene expression in differentiated cells, a culture medium that allowed a low steady-state level of expression of the gene had first to be set up. A serum-free chemically defined medium was selected since it (i) avoided undesirable effects of ill-defined serum components and (ii) facilitated the determination of AT secreted into the culture medium, in the absence of serum proteins such as AT, angI, renin and other proteases. As shown in Figure 1, preincubation of differentiated Ob1771 cells for 24 or 48 h in such a serum-free chemically defined medium, composed of DMEM supplemented with 5 μ g/ml insulin (850 nM) and 10 μ g/ml transferrin (IT medium), led to a marked decrease in the steady-state level of AT mRNA. Under these conditions no morphological change in the cells was observed up to 48 h. Similarly, the specific activity of lactate dehydrogenase, indicative of cell viability, and glycerol-3-phosphate dehydrogenase, indicative of terminal differentiation, did not change significantly (not shown). In addition, the level of mRNA encoding the adipose fatty acid-binding protein, another late marker of differentiation, and β -actin mRNA, taken as an internal standard for normalization of RNA in Northern-blot

Figure 1 Modulation of AT mRNA expression in a chemically defined serum-free medium (IT medium)

Differentiated Ob1771 cells were previously grown and differentiated under the conditions described in the Materials and methods section. At day 18 after confluence (time zero, first lane), cells were washed and shifted to IT medium for 24 or 48 h (second and third lanes respectively) or preincubated in IT medium for 24 h and then maintained for 24 h in IT medium supplemented with 10^{-7} M Dex (fourth lane) or DMEM supplemented with 8 % FBS (fifth lane). RNA was extracted and analysed as described in the Materials and methods section for AT mRNA (1.8 kb), adipose fatty acid-binding protein mRNA (a-FABP) (0.6 kb) and β -actin mRNA (2.2 kb) content. The results are representative of at least two separate experiments for the conditions in lanes 1, 3 and 5 and more than ten for the conditions in lanes 2 and 4.

Table 1 Hormonal control of AT mRNA accumulation in Ob1771 adipose cells

Differentiated Ob1771 cells, previously grown and differentiated under the conditions described in the Materials and methods section, were washed, shifted to and maintained for 24 h in IT medium. Cells were then incubated for 16 h in IT medium supplemented with various effectors at the concentrations indicated. RNA was extracted and analysed as described in the Materials and methods section. The values expressed as fold increase above the control (taken as 1 unit) were normalized to β -actin signals. The data are means $\pm$ S.E.M. from three separate experiments.

Addition to IT medium	AT mRNA (fold increase)
Dex (10^{-7} M)	12.00 $\pm$ 2.0
T ₃ (10^{-9} M)	0.97 $\pm$ 0.6
Growth hormone (10^{-9} M)	1.60 $\pm$ 0.6
Oestradiol (10^{-9} M)	0.70 $\pm$ 0.3
AngII (10^{-7} M)	0.65 $\pm$ 0.4
Forskolin (10^{-5} M)	1.20 $\pm$ 0.5

analysis, remained almost constant during the 48 h in IT medium (Figure 1). Maintenance of viability and the differentiated state was expected since insulin, present at high concentration (850 nM) in IT medium, is known to act as an insulin-like growth factor, involved in the control of both viability and differentiation of preadipose cells [30,31]. Therefore, in the experiments reported below, differentiated Ob1771 cells were preincubated for 24 h in IT medium before addition of the various effectors. Under these conditions, exposure to 10^{-7} M Dex for 24 h dramatically enhanced AT mRNA to a higher level than that observed when cells were shifted back to the initial culture conditions, i.e. 8% FBS in DMEM (Figure 1).

Of the hormones and effectors reported to induce AT gene expression in liver cells [32–35], only the synthetic glucocorticoid Dex produced a spectacular increase ($\sim$ 12-fold) in AT mRNA levels after a 16 h exposure (Table 1). Under the same conditions, T₃, growth hormone, oestradiol and angII, all present at con-

centrations reported to induce maximal stimulation of both AT gene expression in liver cells [32–35] and other specific biological effects in Ob1771 adipocytes [12,30], did not cause any change in AT mRNA levels. Similarly, forskolin, a direct activator of adenylate cyclase which increases cAMP levels enabling it to regulate AT secretion in hepatoma cells positively [36], failed to induce any change in AT gene expression, although this agent was clearly lipolytic in these cells [15].

Therefore regulation of AT mRNA accumulation by glucocorticoid in Ob1771 adipocytes was further investigated, and, to determine whether increased levels of AT mRNA were accompanied by increased parallel rates of AT synthesis and secretion, AT was also measured (after its total conversion into angI in the presence of exogenous mouse renin) in cell homogenates and/or conditioned medium of Ob1771 cells.

Regulation by glucocorticoids of AT gene expression and AT production and secretion in preadipose and adipose cells

In agreement with the fact that accumulation of AT mRNA is a late phenomenon of adipose-cell differentiation, the results in Figure 2 clearly show that an increase in AT synthesis and secretion (note the difference in scales) occurred as a function of differentiation, mainly between day 7 and day 11. Moreover, from day 7 to day 18, exposure of the cells to 10^{-7} M Dex led to a striking increase in both cellular and secreted AT. Under these conditions, more than 90% of AT produced by the cells was recovered as secreted protein.

It is worth pointing out that AT mRNA accumulation was strongly stimulated ($\sim$ 20-fold, not shown) by 10^{-7} M Dex in preadipocytes as early as day 1. However, very low amounts of cellular and secreted AT protein were observed whether or not preadipocytes were exposed to this glucocorticoid. This may be related to some impairment of the translational and/or post-translational processes. However, later on, the rate of AT secretion became well correlated with mRNA levels.

In agreement with the increase in AT mRNA observed after 16–24 h of exposure to 10^{-7} M Dex (Figure 1 and Table 1), AT

Figure 2 Time course of stimulated AT synthesis and secretion during adipose-cell differentiation

Ob1771 cells were grown in standard medium to confluence (time zero) and then cultured in standard differentiation medium as described in the Materials and methods section (i.e. without the usual 3 day exposure to Dex and IBMX). At the indicated times after confluence, cells were washed and incubated for 24 h in IT medium in the absence (○) or presence (●) of 10^{-7} M Dex. The amounts of AT present in cell homogenates (A) and culture media (B) were measured as described in the Materials and methods section. The data are means $\pm$ S.E.M. from three separate experiments.

Figure 3 Time course of AT secretion stimulated by Dex in Ob1771 adipose cells

Differentiated cells, obtained and treated as described in the Materials and methods section, were exposed (●) or not (○) to 10^{-7} M Dex for various time periods. At the times indicated, AT was measured in the culture medium as described in the Materials and methods section. The data are means $\pm$ S.E.M. from three separate experiments.

Table 2 Dose-response relationships of Dex concentration to AT mRNA accumulation and AT production and secretion in Ob1771 adipose cells

After being washed and preincubated for 24 h in IT medium, differentiated cells were exposed or not to increasing concentrations of Dex for 24 h. RNA was then extracted and analysed by Northern blot using AT and β -actin probes. The AT mRNA signals were quantified by densitometry and normalized to those of β -actin. The amounts of AT present in cell homogenates and culture media were determined as described in the Materials and methods section. Data are means $\pm$ S.E.M. from three separate experiments.

Dex (M)	AT mRNA (arbitrary units)	AT production (fmol/culture well)	
		Cell homogenates	Culture media
0	1.00 $\pm$ 0.0	38 $\pm$ 12	84 $\pm$ 20
10^{-10}	1.35 $\pm$ 0.5	30 $\pm$ 14	79 $\pm$ 34
10^{-9}	9.00 $\pm$ 1.2	54 $\pm$ 22	259 $\pm$ 61
10^{-8}	22.00 $\pm$ 2.0	77 $\pm$ 22	736 $\pm$ 205
10^{-7}	28.00 $\pm$ 3.8	112 $\pm$ 32	1060 $\pm$ 213

secretion was readily detectable 16 h after exposure to this concentration of the hormone (Figure 3). The results presented in Table 2, illustrating the parallelism of the dose-dependence of Dex with respect to both AT mRNA accumulation and AT protein secretion in Ob1771 adipocytes, show that the EC_{50} was in the nanomolar range (~ 5 nM), i.e. in good agreement with the affinity of Dex for the glucocorticoid receptor [18,37].

As illustrated in Table 3, a study of the effect on AT secretion of various steroid hormones present for 24 h at a maximally effective concentration (10^{-7} M) showed that it was confined to glucocorticoids, i.e. Dex, cortisone, cortisol and corticosterone. Other steroid hormones, such as progesterone, testosterone and 17α - or 17β -oestradiol, did not produce significant secretion, except for aldosterone. As expected, the receptor antagonist RU 38486 counteracted the ability of the glucocorticoids to induce AT secretion. It also counteracted the response induced by aldosterone, suggesting that this steroid, as previously observed

Table 3 Secretion of AT from Ob1771 adipocytes on exposure to various steroid hormones: effect of the glucocorticoid antagonist RU 38486

Differentiated cells, obtained and treated as described in the Materials and methods section, were exposed or not (control) in IT medium to various steroid hormones at a concentration of 10^{-7} M either alone (first column) or in the presence of 10^{-7} M RU 38486 (second column, + RU). Levels of AT present in the culture medium were measured 24 h later. Data are means $\pm$ S.E.M. from three separate experiments.

	AT secretion (fmol/well)	
		+ RU
Control	74 $\pm$ 10	65 $\pm$ 11
Dex	1368 $\pm$ 286	90 $\pm$ 10
Cortisone	1387 $\pm$ 97	61 $\pm$ 24
Cortisol	1661 $\pm$ 340	72 $\pm$ 5
Corticosterone	640 $\pm$ 720	59 $\pm$ 11
Aldosterone	383 $\pm$ 98	70 $\pm$ 10
Progesterone	100 $\pm$ 24	45 $\pm$ 2
Testosterone	150 $\pm$ 74	55 $\pm$ 4
17α -Oestradiol	70 $\pm$ 24	40 $\pm$ 10
17β -Oestradiol	128 $\pm$ 53	46 $\pm$ 9

for other biological effects, was able to act via the glucocorticoid receptor. A similar regulation of AT gene expression and AT secretion by Dex was observed in differentiated 3T3-F442A adipose cells (not shown), indicating that this regulation is not restricted to a particular preadipocyte clonal line.

Regulation of AT gene expression and secretion by Dex in rat adipose tissue *ex vivo*

To provide more physiological support for the results obtained *in vitro*, experiments were carried out *ex vivo* using explants of rat periepididymal adipose tissue incubated for 24 h under similar conditions as above, i.e. in IT medium supplemented with T_3 , ascorbate and selenium [22], in the absence or presence of 10^{-7} M Dex. Such serum-free culture conditions have previously been used to study the regulation of gene expression in adipose tissue [38] because they are known to support terminal differentiation of adipose precursor cells for 1 week [22] and to maintain intact morphology and hormonal responsiveness of mature adipocytes for several days [39]. RNA extracted from these adipose-tissue explants was analysed to determine AT mRNA content (Table 4). Although the signal obtained for AT mRNA was decreased in explants maintained for 24 h in the absence of Dex, the presence of the hormone led to a significant enhancement (5-fold and 2.5-fold as compared with time 24 h and time zero respectively). Hence Dex did not appear to produce a less rapid decrease in AT mRNA as a function of time, but actually behaved as a stimulator of AT gene expression in adipose tissue. This enhancement of AT mRNA accumulation caused by Dex was corroborated by a mild but significant increase ($P < 0.05$) in AT in the conditioned medium after 24 h exposure to this hormone (Table 4). Furthermore separation of adipocytes from cells present in the stromal-vascular fraction and known to contain preadipocytes able to express early markers of differentiation only [13] provided evidence that the regulation of AT gene expression by Dex in adipose tissue takes place mainly if not exclusively in mature fat cells (Table 4). The mean increase in AT mRNA induced by the presence of Dex in the adipocyte fraction measured after normalization to the β -actin signal was 5-fold in three separate experiments. Although a very slight

Table 4 AT gene expression and secretion in rat adipose tissue exposed to Dex

Explants of periepididymal fat pads were prepared and incubated or not (time zero) as described in the Materials and methods section for 24 h in the absence or presence of 10^{-7} M Dex. RNA was extracted from the respective tissue fragments and analysed by Northern blot for AT mRNA and β -actin mRNA, used as internal standard for normalization. The values obtained by using a phosphorimager (means $\pm$ S.E.M. from five separate experiments) are expressed as a percentage of the maximum observed in Dex-treated tissue (first column). The amounts of AT measured in the respective culture media are shown in the second column. According to analysis of variance (Stat View software), the absolute values in the presence of Dex are statistically different from those obtained in its absence ($P < 0.05$). Northern blots prepared with RNA extracted from the stromal-vascular and adipocyte fractions isolated from rat adipose-tissue explants exposed or not to Dex are presented in the right hand side of the Table.

Time	Addition	Whole adipose tissue		AT mRNA (% of maximum)	
		AT mRNA (% of maximum)	AT secreted (pmol/g of tissue)	Stromal-vascular fraction	Adipocyte fraction
0	None	40 $\pm$ 2	—	—	—
24 h	None	18 $\pm$ 1	15 $\pm$ 0.4	0.28 $\pm$ 0.02	17 $\pm$ 5
24 h	Dex 10^{-7} M	100	23 $\pm$ 0.5	0.82 $\pm$ 0.01	100

increase in AT mRNA accumulation was also observed in cells of the stromal-vascular fraction on exposure to Dex, this contribution was very small compared with that observed in the adipocyte fraction.

DISCUSSION

Since the characterization of RAS components in various tissues, its possible involvement in adipose-tissue development [11,12] and in the aetiology of hypertension in obese patients [17,40], to our knowledge no detailed study has been performed on the hormonal control of AT gene expression in adipocytes and adipose tissue, except those carried out with preadipose 3T3 cells during their differentiation [26,27,41,42]. The existence of nutritional regulation of AT resulting in the control of local blood supply and thereby fatty acid efflux from adipose tissue has been reported [43]. In this context, a recent report from our laboratory has given evidence of transcriptional regulation of AT gene expression in preadipose cells by fatty acids [29], which may implicate nuclear receptors of the peroxisome proliferator-activated receptor family [44]. In agreement with this hypothesis, we have recently reported that, in both preadipose and adipose cells, in a way similar to that elicited by fatty acids, the expression of AT and adipose fatty acid-binding protein genes was also up-regulated by carbaprostacyclin, a stable analogue of prostacyclin arising from arachidonate metabolism [45].

The present study demonstrates in adipose tissue that, in addition to fatty acids and metabolites, glucocorticoids are the only steroid hormones able to modulate AT gene expression. This modulation is probably exerted at the transcriptional level since AT mRNA accumulation was abolished in the presence of 2 μ g/ml actinomycin D. Although an indirect mechanism cannot definitely be ruled out, it is interesting to recall that two glucocorticoid-response elements have been characterized in the promoter of the AT gene [46,47].

The subsequent enhancement in AT mRNA levels is accompanied by a parallel increase in AT synthesis and secretion from adipose cells. This situation is strikingly different from that observed in liver cells in which numerous hormones, e.g. glucocorticoids and also T_3 , growth hormone and angII, have been shown to enhance both AT mRNA levels and AT secretion.

The regulation of AT gene transcription by glucocorticoids in late differentiated cells gives rise via the glucocorticoid receptor, on a time and concentration basis, to parallel increases in AT secreted into the culture medium. It is interesting to note that this regulation was observed *in vitro* in mouse Ob1771 cells as well as in 3T3-F442A cells and extended *ex vivo* using explants of rat periepididymal adipose tissue. Taken together, the present results support the hypothesis that glucocorticoids are critical hormones in the regulation of AT gene expression in adipose tissue.

Although previous studies have shown specific accumulation of AT mRNA in brain and liver on treatment of rats with Dex [4], a similar study has not been reported to our knowledge for adipose tissue. It should be stressed that mRNA levels in periepididymal and retroperitoneal adipose depots are 60 and 80 % respectively of those in liver (J. Aubert, G. Ailhaud and R. Négrel, unpublished work). Thus it is assumed that the ability of periepididymal adipose tissue explants *ex vivo* to respond to Dex gives support to a physiological role for glucocorticoids in AT gene expression and AT secretion.

The control of AT gene expression by glucocorticoids is an addition to the multiple regulations already described for these steroid hormones in adipose cells. Glucocorticoids have in particular been shown *in vitro* to induce, on a long-term basis, the terminal differentiation of Ob1771 preadipose cells as well as that of rodent and human adipose precursor cells [48,49]. Consequently, glucocorticoids have been implicated not only in the hypertrophic but also in the hyperplastic growth of adipose tissue. This involvement could explain the excessive development of intra-abdominal adipose tissue in Cushing's syndrome associated in humans with hypercortisolism [18,50] and in transgenic mice overexpressing corticotrophin-releasing factor with high corticosterone levels [20]. Taken together, our previous and present results suggest that glucocorticoids and the RAS play a key role in overgrowth of adipose tissue which may in turn lead to increased blood pressure.

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REFERENCES

- Campbell, D. J. (1987) *J. Clin. Invest.* **79**, 1–6
- Campbell, D. J. (1985) *J. Hypertens.* **3**, 199–207
- Lynch, K. R. and Peach, M. J. (1991) *Hypertension* **17**, 263–269
- Campbell, D. J. and Habener, J. (1987) *Endocrinology* **121**, 1616–1626
- Cassis, L. A., Lynch, K. R. and Peach, M. J. (1988) *Circ. Res.* **62**, 1259–1262
- Cassis, L. A., Saye, J. A. and Peach, M. J. (1988) *Hypertension* **11**, 591–596
- Grady, E. F., Sechi, L. A., Griffin, C. A., Schambelan, M. and Kalinyak, J. E. (1991) *J. Clin. Invest.* **88**, 921–933
- Johnson, M. C. and Aguilera, G. (1991) *Endocrinology* **129**, 1266–1274
- Zorad, S., Fickova, M., Zelezna, B., Macho, L. and Kral, J. G. (1995) *Gen. Physiol. Biophys.* **14**, 383–391
- Saye, J. A., Ragsdale, V., Carey, R. M. and Peach, M. J. (1993) *Am. J. Physiol.* **264**, C1570–C1576
- Harp, J. B. and Di Girolamo, M. (1995) *J. Geront.* **50A**, B270–B276
- Darimont, C., Vassaux, G., Ailhaud, G. and Négrel, R. (1994) *Endocrinology* **135**, 2030–2036
- Négrel, R., Gaillard, D. and Ailhaud, G. (1989) *Biochem. J.* **257**, 399–405
- Catalioto, R. M., Gaillard, D., Macclouf, J., Ailhaud, G. and Négrel, R. (1991) *Biochim. Biophys. Acta* **1091**, 364–369
- Vassaux, G., Gaillard, D., Ailhaud, G. and Négrel, R. (1992) *J. Biol. Chem.* **267**, 11092–11097
- Darimont, C., Vassaux, G., Gaillard, D., Ailhaud, G. and Négrel, R. (1994) *Int. J. Obes.* **18**, 783–788

- 17 Rocchini, A. P. (1992) *Hypertension* **19**, (suppl. 1), 56–60
- 18 Rebuffé-Scrive, M., Brönnegard, M., Nillson, A., Eldh, J., Gustafsson, J. A. and Björntorp, P. (1990) *J. Clin. Endocrinol. Metab.* **71**, 1215–1219
- 19 Pepin, M. C., Pothier, F. and Barden, N. (1992) *Nature (London)* **355**, 725–728
- 20 Stenzel-Poore, M. P., Cameron, V. A., Vaughan, J., Sawchenko, P. E. and Vale, W. (1992) *Endocrinology* **130**, 3378–3386
- 21 Négrel, R., Grimaldi, P. and Ailhaud, G. (1978) *Proc. Natl. Acad. Sci. U.S.A.* **75**, 6054–6058
- 22 Deslex, S., Négrel, R. and Ailhaud, G. (1987) *Exp. Cell Res.* **168**, 15–30
- 23 Chomczynski, P. and Sacchi, N. (1987) *Anal. Biochem.* **162**, 156–159
- 24 Sealy, J. and Laragh, J. (1977) *Cardiovasc. Med.* **2**, 1079–1092
- 25 Jacobsen, J. and Poulsen, K. (1985) *J. Hypertens.* **3**, 155–157
- 26 Saye, J. A., Cassis, L. A., Sturgill, T. W., Lynch, K. R. and Peach, M. J. (1989) *Am. J. Physiol.* **256**, C448–C451
- 27 Saye, J. A., Lynch, K. R. and Peach, M. J. (1990) *Hypertension* **15**, 867–871
- 28 Tamura, K., Umemura, S., Iwamoto, T., Yamaguchi, S., Kobayashi, S. I., Takeda, K., Tokita, Y., Tagaki, N., Murakami, K., Fukamizu, A. and Ishii, M. (1994) *Hypertension* **23**, 364–368
- 29 Safonova, I., Aubert, J., Négrel, R. and Ailhaud, G. (1997) *Biochem. J.* **322**, 235–239
- 30 Catalioto, R. M., Gaillard, D., Ailhaud, G. and Négrel, R. (1992) *Growth Factors* **6**, 255–264
- 31 Smith, P. J., Wise, L. S., Berkowitz, R., Wan, C. and Rubin, C. S. (1988) *J. Biol. Chem.* **263**, 9402–9408
- 32 Chang, C. and Perlman, A. J. (1987) *Endocrinology* **121**, 513–519
- 33 Clauser, E., Bouhnik, J., Coezy, E., Corvol, P. and Menard, J. (1983) *Endocrinology* **112**, 1188–1193
- 34 Hong-Brown, L. Q. and Deschepper, C. F. (1992) *Endocrinology* **130**, 1231–1237
- 35 Klett, C., Ganten, D., Hellmann, W., Kaling, M., Ryffel, G. U., Weimar-Ehl, T. and Hackenthal, E. (1992) *Endocrinology* **130**, 3660–3668
- 36 Sernia, C., Zeng, T., Shinkel, T., Kerr, D. and Raizada, M. (1994) *Kidney Int.* **46**, 1574–1576
- 37 Baxter, J. and Tomkins, M. (1971) *Proc. Natl. Acad. Sci. U.S.A.* **68**, 932–937
- 38 Ibrahim, A., Teboul, L., Gaillard, D., Amri, E., Ailhaud, G., Young, P., Cawthorne, M. A. and Grimaldi, P. A. (1994) *Mol. Pharmacol.* **46**, 1070–1076
- 39 Ottosson, M., Vikman-Adolfsson, K., Enerbäck, S., Olivecrona, G. and Björntorp, P. (1994) *J. Clin. Endocrinol. Metab.* **79**, 820–825
- 40 Hall, J. E., Zappe, D. H., Alonso-Galicia, M., Granger, J. P., Brands, M. W. and Kassab, S. E. (1996) *News Physiol. Sci.* **11**, 255–261
- 41 McGehee, R. E., Ron, D., Brasier, R. B. and Habener, J. F. (1993) *Mol. Endocrinol.* **7**, 551–560
- 42 McGehee, R. E. and Habener, J. F. (1995) *Mol. Endocrinol.* **9**, 487–501
- 43 Frederich, Jr., R. C., Kahn, B. B., Peach, M. J. and Flier, J. S. (1992) *Hypertension* **19**, 339–344
- 44 Mandrup, S. and Lane, M. D. (1997) *J. Biol. Chem.* **272**, 5367–5370
- 45 Aubert, J., Ailhaud, G. and Négrel, R. (1996) *FEBS Lett.* **397**, 117–121
- 46 Brasier, A. R., Li, J. and Copland, A. (1994) *Kidney Int.* **46**, 1564–1566
- 47 Brasier, A. R. and Li, J. (1996) *Hypertension* **27**, 465–475
- 48 Gaillard, D., Wabitsch, M., Pipy, B. and Négrel, R. (1991) *J. Lipid Res.* **32**, 569–579
- 49 Hauner, H., Entenmann, G., Wabitsch, M., Gaillard, D., Ailhaud, G., Négrel, R. and Pfeiffer, E. F. (1989) *J. Clin. Invest.* **84**, 1663–1670
- 50 Rebuffé-Scrive, M., Krotkiewski, M., Elfverson, J. and Björntorp, P. (1988) *J. Clin. Endocrinol. Metab.* **67**, 1122–1128