Angiotensin II-responsive element is the insulin-responsive element in the adipocyte fatty acid synthase gene: role of adipocyte determination and differentiation factor 1/sterol-regulatory-element-binding protein 1c

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We have previously shown that angiotensin II (Ang II) increases the expression of the gene encoding adipocyte fatty acid synthase (FAS). Here we investigate the mechanism responsible for increased FAS gene transcription by Ang II. We demonstrate that Ang II increased luciferase activity by 3-fold in 3T3-L1 adipocytes transfected with fusion constructs linking the FAS promoter to the luciferase reporter gene. Interestingly, we mapped the Ang II regulatory sequences to the insulin-responsive region (E box) in the proximal FAS promoter. The E box alone was able to mediate Ang II responsiveness when linked to a heterologous promoter. However, this response was lost when mutations that abolished the binding of the E box to its transcription factors were introduced. Using adenoviral over-expression of a dominant-negative form of adipocyte deter-

mination and differentiation factor 1 (ADD1), a transcription factor that binds to the insulin-responsive E box, we demonstrated that ADD1 was required for Ang II regulation of the FAS gene in 3T3-L1 adipocytes. Furthermore, ADD1 expression was also up-regulated by Ang II. With the use of transfections as well as glucose transport assays, we further demonstrated that Ang II stimulation of the FAS gene was dependent on glucose. In conclusion, this is the first report that Ang II regulates adipocyte FAS gene transcription via insulin response sequences in a glucose-dependent manner and that this regulation is mediated at least in part via the ADD1 transcription factor.

Key words: gene transcription, glucose, DNA regulatory sequences, lipogenesis, reporter gene.

INTRODUCTION

Angiotensin II (Ang II) is a hypertensive hormone classically known to regulate blood pressure [1]. However, studies from our laboratories and others have shown that this hormone also regulates adipocyte differentiation, metabolism and gene expression [2–4]. Components of the renin angiotensin system have been identified in several tissues, including adipose tissue [2,5–7]. We and others have provided several lines of evidence for the role of the adipocyte-derived Ang II in lipid metabolism [2,7]. First, the Ang II precursor, angiotensinogen, is expressed and nutritionally and hormonally regulated in adipose tissue [8–11]. Secondly, expression of the angiotensinogen gene is dependent on differentiation, and treatment of cultured adipocytes with Ang II increases the expression of differentiation markers [3]. Furthermore, we have shown that treatment of cultured adipocytes with Ang II increases triglyceride storage and the activities of lipogenic enzymes including fatty acid synthase (FAS) [2].

FAS is a key lipogenic gene that catalyses the synthesis of the long-chain fatty acid palmitate from acetyl-CoA and malonyl-CoA in the presence of NADPH [12]. FAS concentrations in adipose tissue are highly sensitive to nutritional, hormonal and developmental states [12,13]. Fasting and diabetes in rats lead to diminished synthesis of FAS, whereas obesity, a high carbohydrate diet or insulin treatment increases FAS synthesis [14]. The gene encoding FAS is primarily regulated at the transcriptional level [15–19] and we have previously identified an insulin-responsive element (IRE) in the FAS gene that mediates the transcriptional up-regulation of this gene by insulin in

adipocytes [16]. Because our previous studies indicate that Ang II is a lipogenic hormone that exerts an insulin-like effect on adipocyte gene expression, we hypothesized that Ang II and insulin regulate FAS gene expression via similar mechanisms.

We report here for the first time that Ang II increases FAS gene transcription in adipocytes via an angiotensin-responsive element that is identical with the insulin-responsive region (E box) in the proximal FAS promoter. Furthermore, we show that Ang II effect is dependent on glucose and we identify adipocyte determination and differentiation factor 1/sterol-regulatory-element-binding protein 1c (ADD1/SREBP1c) as a transcription factor involved in the regulation of the FAS gene by Ang II.

MATERIALS AND METHODS

3T3-L1 cell culture

3T3-L1 cells were grown and differentiated as described previously [2,19]. In brief, cells were grown to confluence in standard DMEM (Dulbecco's modified Eagle's medium) supplemented with 10% (v/v) fetal bovine serum. At confluency, cells were induced to differentiate by the addition of dexamethasone (250 nM) and isobutylmethylxanthine (0.5 mM) to standard medium for 72 h. Cells were maintained for a further 3 days in standard medium, then changed to serum-free medium [containing 1% (w/v) BSA] for 12 h followed by treatment with Ang II and/or insulin as indicated in the figure legends. All treatments were performed in serum-free medium to test individual and direct hormone effects. However, the responsiveness of the FAS gene to both Ang II and insulin can be demonstrated in the

Abbreviations used: Ang II, angiotensin II; ADD1, adipocyte determination and differentiation factor 1; CAT, chloramphenicol acetyltransferase; E box, insulin-responsive region; FAS, fatty acid synthase; IRE, insulin-responsive element; SREBP1c, sterol-regulatory-element-binding protein 1c.

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presence of both 1 % and 10 % (v/v) serum (results not shown). Unless otherwise stated, cells were cultured in the presence of a high concentration of glucose (25 mM).

RNA isolation and Northern analysis

RNA was isolated by the CsCl density gradient method [19]. Individual culture dishes (100 mm) of cells were used for each RNA sample within treatments; three or four samples were included in each treatment group and each experiment was repeated two or three times. Total RNA (approx. 30 µg) for each sample was subjected to electrophoresis in a 0.7 % agarose gel, transferred to nylon membrane and analysed by Northern blotting, as described previously [19]. Membranes were hybridized with ³²P-labelled cDNA probes for rat FAS (kindly provided by Dr A. G. Goodridge) or ADD1 (kindly provided by Dr B. M. Spiegelman) and 18 S (purchased from Promega). Unbound probe was removed by washing membranes in 2 × saline/sodium phosphate/EDTA (SSPE) for 45 min at 25 °C and then in 0.1 × SSPE/0.1 % SDS for 60 min at 65 °C. After being washed, membranes were exposed to X-ray film (Dupont). Autoradiograms were analysed by densitometric scanning; data are expressed as a ratio of FAS to 18 S.

Nuclear run-on assay

Nuclei were isolated from control or Ang II-treated 3T3-L1 cells as described previously [19]. In brief, cells were homogenized in a buffer containing Nonidet P40, and nuclei were isolated by differential centrifugation. Nuclear run-on assays and hybridizations were conducted as previously described [19]. Labelled RNA was hybridized with plasmids containing cDNA species encoding FAS and β-actin, and with vector alone (pBS) immobilized on nylon membranes. Membranes were then exposed to film and the autoradiograms were quantified by scanning laser densitometry. FAS transcription rates were normalized to those of β-actin.

FAS-reporter gene constructs

A fragment of the FAS 5'-flanking region spanning -2100 to +67 was generated by PCR with rat genomic DNA and subcloned into pGL-basic vector (Promega). Various deletions of this fragment were subsequently generated by *ExoIII*/mung bean nuclease deletion (Stratagene, La Jolla, CA, U.S.A.) or by PCR with previously published FAS promoter sequences [20]. For fusion constructs containing two direct repeats of sequences spanning the E box, -70 to -54 wild-type (CAGCCCATGTGGCGTGG) and mutant -69 to -54 (AGCCGTGACGGCGTGG) oligonucleotides of the specified sequences were synthesized, annealed then subcloned into pGL2-SV promoter luciferase plasmid. These FAS-promoter-luciferase fusion constructs were used to transfect 3T3-L1 cells. To determine the role of this IRE in the context of the native FAS promoter, we also used the native FAS promoter fragment -200 to +67 linked to the chloramphenicol acetyltransferase (CAT) reporter gene, containing either the wild-type or mutated E box [21].

Transfections

3T3-L1 cells were transfected with FAS-luciferase gene fusion constructs and pSVneo plasmid that confers neomycin resistances using the calcium phosphate-DNA co-precipitation method (Gibco BRL, Bethesda, MD, U.S.A.) as described previously [16]. Stably transfected cells were selected by maintaining transfected cells in 500 µg/ml neomycin for 2 weeks. Surviving

fibroblasts were cultured as described above for 3T3-L1 cells. The differentiated stably transfected adipocytes were maintained overnight in serum-free medium before being treated with Ang II and/or insulin as indicated in figure legends. pGL2-control plasmid, which contains the simian virus 40 promoter linked to the luciferase gene, was used as a control to determine the specificity of the effect of Ang II on FAS promoter and did not show responsiveness to insulin or Ang II (results not shown).

Reporter gene assays

For luciferase assays, the cells were lysed in 100 mM potassium phosphate (pH 7.8)/0.2 % (w/v) Triton X-100/1 mM dithiothreitol. The cytosolic extracts were used to measure luciferase activities with a luminometer (Berthold, Nashua, NH, U.S.A.) and a kit for luciferase assay (Tropix). Luciferase activity was normalized to protein measured with the Bradford assay [22]. For CAT assays the cells were lysed in a buffer containing 250 mM Tris/HCl, pH 8, and 5 mM dithiothreitol; a quantitative non-chromatographic assay was used to measure CAT activity in the cell extracts, as described previously [21].

Adenovirus-mediated overexpression of a dominant-negative form of ADD1 in 3T3-L1 adipocytes

An adenoviral vector overexpressing a dominant-negative version of rat ADD1 under the control of the cytomegalovirus immediate-early promoter was previously described [23]. The adenovirus vector Adnull, whose expression cassette contains the major late promoter with no exogenous gene, was used as a control. The adenoviral vectors were propagated in HEK-293 cells, purified by CsCl density centrifugation and stored. Cells were grown as described above, and 35 mm dishes containing fully differentiated 3T3-L1 adipocytes were incubated overnight in serum-free medium containing BSA. Cells were transduced with 3×10^7 plaque-forming units in DMEM alone for 4 h at 37 °C; 1 nM Ang II was then added. Transduced cells were assayed for FAS enzyme activity 48 h after viral infection.

Uptake of 2-deoxyglucose

3T3-L1 cells were differentiated as described above and incubated overnight in serum-free medium containing 2 % (w/v) BSA. Cells were then washed twice with Krebs-Ringer phosphate, pH 7.4, and incubated for 30 min at 37 °C in the presence or absence of insulin (10 nM) or Ang II (1 nM). 2-Deoxyglucose uptake was initiated by the addition of 0.2 mM 2-[³H]-deoxyglucose (500 GBq/mmol). After 5 min, cells were washed rapidly with ice-cold buffer and lysed with NaOH; radioactivity was then counted.

Statistical analysis

Experiments were repeated two to eight times as indicated in the figure legends. Analysis of variance was used to compare overall group means. Student's *t* test was used for comparison between groups. All tests were conducted with 95 % confidence intervals.

RESULTS

Effects of Ang II on FAS activity, mRNA level and gene transcription rate

We have previously reported that Ang II increases FAS gene expression in murine and human adipocytes [2]. Figure 1 shows the co-ordinated effects of Ang II on FAS activity, mRNA level and the rate of gene transcription. Ang II increased FAS activity, mRNA level and gene transcription by approx. 2.5-fold, 4.5-fold

Figure 1 Effects of Ang II on FAS enzyme activity, mRNA level and gene transcription rate

3T3-L1 adipocytes were treated with 1 nM Ang II (Ang II). Cells were then harvested after 48 h for FAS enzyme activity (measured in nmol of NADPH oxidized/min per mg of protein) or after 24 h for mRNA analysis and gene transcription assays (measured in arbitrary densitometric units). Results are presented as percentages of control. * $P < 0.01$ compared with controls from a total of eight experiments.

Figure 2 Effects of Ang II on luciferase activity in 3T3-L1 adipocytes transfected with various FAS promoter constructs

Adipocytes were transfected with FAS-luciferase constructs containing the -200 to $+67$ region (A) or the -70 to $+67$ region (B) of the FAS promoter and treated for 48 h with Ang II (Ang II) and/or insulin (Ins). Luciferase activity was then measured (in arbitrary units) and normalized to protein levels. Results are means for six different experiments. * $P < 0.01$ compared with control; # $P < 0.01$ compared with 1 nM Ang II.

and 2.5-fold respectively. These results demonstrate that FAS gene is regulated by Ang II at the transcriptional level.

Effects of Ang II on FAS promoter activity: comparison with insulin

To investigate further the mechanisms of transcriptional regulation of FAS by Ang II, we searched for Ang II-responsive

Figure 3 Insulin-responsive E box mediates Ang II responsiveness of the FAS promoter

Upper panel: the IRE (E box) linked to a heterologous promoter mediates the effects of Ang II (Ang II) and insulin on luciferase activity. Two copies of the insulin-responsive region (-70 to -54) containing the wild-type (hatched columns) or mutated (black columns) E box were subcloned into pGL2 SV-luciferase plasmid and transfected into 3T3-L1 adipocytes. Cells were then treated with or without 1 nM Ang II and/or insulin for 48 h; luciferase activity and protein content were then measured. Results are reported in arbitrary units of luciferase/mg of protein. This experiment was performed in triplicate. ** $P < 0.01$, Ang II effect compared with control; * $P < 0.05$, insulin effect compared with control; # $P < 0.05$, mutation effect compared with control. Lower panel: mutation of the IRE (E box) in the native FAS promoter abolishes the effects of Ang II (Ang II) on the CAT reporter gene. Fusion constructs containing the wild-type (hatched columns) or mutated (black columns) E box within the FAS promoter (-200 to $+67$) linked to the CAT reporter gene were transfected into 3T3-L1 adipocytes. Cells were then treated with or without 1 nM Ang II for 48 h and harvested for assays. CAT activity was normalized to protein content. * $P < 0.01$ compared with control; this experiment was performed in triplicate.

regions in the FAS gene. Ang II significantly increases luciferase activity in 3T3-L1 cells transfected with the fusion constructs containing the regions -200 to $+67$ (Figure 2A) and -70 to $+67$ (Figure 2B) of the FAS promoter. These fragments contain the IRE previously identified in the FAS gene [16].

The magnitude of FAS promoter response to Ang II stimulation was half of that elicited by insulin but it is clear from Figure 2 that adipocytes responded similarly to Ang II and insulin. In addition we show that Ang II and insulin in combination increased luciferase activity in 3T3-L1 cells transfected with the plasmids spanning the regions -200 to $+67$ and -70 to $+67$ (Figures 2A and 2B respectively) of the FAS promoter. Importantly, the increase in luciferase activity in the combined Ang II and insulin treatments was not found to be significantly different from insulin treatments alone, suggesting that Ang II and insulin might target similar *cis*-acting elements to regulate FAS gene transcription.

Ang II-responsive sequences are the insulin-responsive sequences in the FAS gene

To determine whether the IRE might mediate the Ang II responsiveness of the FAS gene, we tested the responsiveness of

Figure 4 ADD1 is a potential transcription factor through which Ang II effects FAS gene transcription

As described in the Materials and methods section, 3T3-L1 adipocytes were infected with adenovirus either encoding or not (null) a dominant-negative form of ADD1 (ADD1-DN; multiplicity of infection of 100) and treated with 1 nM Ang II (Ang II). FAS activity was measured after 48 h and normalized to protein. * $P < 0.01$, ADD1/DN compared with null. This figure is representative of four experiments.

this element to Ang II and insulin in the context of a heterologous promoter and when linked to the native FAS promoter. Using adipocytes transfected with a construct containing only the -70 to -54 (IRE) motif linked to the simian virus 40 heterologous promoter, we tested the responsiveness of luciferase to Ang II and to insulin. We demonstrate (Figure 3, upper panel) that Ang II and insulin significantly increased luciferase activity. This indicates that the -70 to -54 region alone is sufficient to confer Ang II responsiveness on the FAS promoter and no additional sequence is needed outside this motif. In addition, this indicates that Ang II and insulin both regulate FAS gene transcription through the same responsive sequences in the FAS promoter.

To confirm this we mutated the insulin-responsive E box in the -70 to -54 region of the FAS promoter. Figure 3 (upper panel) demonstrates that the mutation of this region caused a loss of stimulation of luciferase activity in response to Ang II treatment as it did for insulin when compared with control. Additionally,

when IRE within the native -200 to $+67$ FAS promoter was mutated, stimulation of the CAT reporter gene activity by Ang II was abolished (Figure 3, lower panel).

ADD1/SREBP1c is required for Ang II-regulated FAS gene transcription

ADD1/SREBP1c has been proposed as a transcription factor mediating transcriptional effects of insulin on lipogenic genes [23,25]. Because Ang II exhibited insulin-like effects on FAS gene expression, we next investigated whether ADD1 might be the potential transcription factor through which Ang II might affect FAS gene transcription: we infected 3T3-L1 cells with a dominant-negative form of ADD1 (ADD1/DN) designed to induce the formation of transcriptionally inactive ADD1 dimers that are unable to bind DNA target sequences. Figure 4 demonstrates that Ang II-induced FAS enzyme activity was eliminated by the infection of this dominant-negative form of ADD1. This result demonstrates that ADD1, the insulin-induced transcription factor, is also required for the regulation of the FAS gene by Ang II.

Glucose-dependent effect of Ang II on FAS promoter: a proposed mechanism for regulating FAS gene transcription

Because the above results demonstrated that Ang II regulates the FAS promoter activity through the same *cis*-active sequence as insulin, and targets the same transcription factor as insulin, we next addressed the question of whether Ang II might exert its effects by mimicking a common action of insulin on glucose entry within adipocytes. Because the regulation of lipogenic genes by insulin is also dependent on glucose, we next investigated whether the regulation of FAS by Ang II still occurred when glucose was withdrawn from the culture medium and replaced by pyruvate as the energy source. 3T3-L1 adipocytes were transfected with the FAS-luciferase fusion construct used above (-70 to $+67$) and treated with or without glucose in the presence or absence of 1 nM Ang II. Figure 5(A) demonstrates that glucose alone increased FAS promoter activity by 50%. This small effect of glucose alone in adipocytes should be related to the low concentration of glucose transporters at the plasma membrane in the absence of insulin, leading to inefficient glucose entry. Interest-

Figure 5 Ang II- and glucose-dependent regulation of the FAS gene and glucose transport

(A) The effect of Ang II (Ang II) on promoter activity is dependent on glucose. Adipocytes were transfected with the SV-luciferase plasmid (described in the legend to Figure 3), which contains the insulin-responsive E box, then treated with glucose-free DMEM for 8 h before treatment for an additional 48 h with (black columns) or without (hatched columns) glucose in the presence or absence of Ang II. Luciferase activity was normalized to protein and is reported in arbitrary units. * $P < 0.05$ compared with no glucose; ** $P < 0.01$ compared with all other treatments. This experiment was repeated twice. (B) Ang II stimulates 2-deoxyglucose uptake in 3T3-L1 cells. 2-Deoxyglucose uptake was measured for 5 min in differentiated 3T3-L1 cells in the absence (basal conditions) or presence of insulin (Ins) or Ang II (Ang II) at the indicated concentrations. Values are presented as the effect of the hormones over basal unstimulated conditions. This experiment was repeated three times.

Figure 6 ADD1 expression is increased by Ang II and glucose in 3T3-L1 adipose cells

Differentiated 3T3-L1 adipocytes were treated with or without Ang II (Ang II, 1 nM) in the presence or absence of glucose (25 mM) for 24 h; ADD1 mRNA expression was analysed by Northern blotting as described in the Materials and methods section. ADD1 mRNA levels were normalized to 18 S RNA. This experiment was repeated twice.

ingly, in the absence of glucose, Ang II was almost unable to increase luciferase activity (25% stimulation), whereas it elicited a 3-fold increase in the presence of glucose. This clearly indicates that the stimulation of FAS activity by Ang II is largely dependent on glucose.

Because the stimulation of the FAS promoter by Ang II was dependent on the presence of glucose in the culture medium, we next investigated whether the effect of Ang II might be mediated through the stimulation of glucose uptake into adipocytes. Figure 5(B) shows that Ang II does indeed stimulate 2-deoxyglucose uptake in 3T3-L1 adipocytes. Even though Ang II was less potent than insulin, this effect of Ang II can provide a potential mechanism through which Ang II might exert its insulin-like effects on FAS expression in fat cells.

Regulation of ADD1 expression by Ang II and glucose

Because ADD1 mediates the nutritional and hormonal regulation of several lipogenic genes [23,25], we tested whether Ang II modulated ADD1 expression (Figure 6). Adipocytes cultured in the absence of glucose expressed very low levels of ADD1 mRNA. Consistent with the activatory effect of glucose on ADD1 expression in cultured hepatocytes [23] was the observation that glucose alone was able to induce ADD1 expression in adipocytes. This effect was further potentiated in the presence of Ang II (Figure 6). This indicates that ADD1 expression is modulated by both glucose and Ang II.

DISCUSSION

Insulin is a well-known lipogenic hormone that regulates adipocyte metabolism and gene expression. Among these genes, FAS has been well studied for its transcriptional regulation by insulin [15–18]. We have previously identified an IRE in the FAS proximal promoter [16]. This IRE is an E box that has been shown to bind ADD1/SREBP1c [23,25,26], a member of the bHLH family. In established adipose cell lines, ADD1 has been shown to promote adipocyte differentiation and the expression of genes linked to fatty acid metabolism [24]. In isolated rat adipocytes, FAS expression has been shown to be dependent on

ADD1 through the use of infection of a mutated dominant-negative form of ADD1 [23]. Moreover, in the cultured hepatocyte system, ADD1/SREBP1c is required for the regulation of lipogenic genes by glucose [23] and its expression is nutritionally regulated [23,25] in hepatocytes and fat cells, establishing it as the strongest candidate for participation in the regulation of the FAS gene by insulin.

We have shown previously that Ang II acts like insulin as an adipogenic hormone to regulate FAS and other lipogenic genes in adipocytes. Here we demonstrate that Ang II regulates FAS at the transcriptional level in 3T3 adipocytes and we searched for Ang-II-responsive sequences in the FAS gene. By using transfection assays, we mapped the Ang-II-responsive region to the IRE. Furthermore, mutation in the E box motif in this sequence abolished Ang II responsiveness, as seen in earlier work with the response to insulin when the E box was mutated [16,25]. This is the first report on the regulation of FAS gene transcription in adipocytes by Ang II and the identification of an Ang II-responsive element in the FAS gene. Very few Ang II-responsive elements have been identified in other genes. In smooth muscle, responsiveness of the α -actin gene to Ang II is partly dependent on modulation of the serum response factor binding to CC(A/T-rich)₆GG regions. The CC(A/T-rich)₆GG elements are found in the promoters of several immediate-early response genes [27]. These response elements have been shown to confer serum-induced and growth factor-induced transcription activation of these various genes [28–32]. In the present study the Ang-II-responsive element is an E box (CATGTG) that is distinct from the serum response element; there is no evidence that such elements are present in the 2 kb promoter region located immediately upstream of the transcription start site of the FAS gene.

As discussed above, the transcription factor ADD1/SREBP1c is critical in the regulation of FAS gene transcription in adipocytes. We tested the hypothesis that ADD1 might be a transcription factor mediating the regulation of FAS by Ang II. Expression of ADD1/DN in 3T3-L1 adipocytes markedly decreases FAS induction by Ang II, indicating that ADD1 is one of the transcription factors required for the up-regulation of FAS gene transcription by Ang II. This led to the conclusion that the Ang-II-responsive element, which is also the insulin-responsive sequence (E box), functions through the same transcription factor (ADD1) as insulin for the regulation of FAS gene transcription. This led us to investigate whether Ang II might produce its transcriptional effects through insulin-like effects on glucose utilization in adipocytes. Indeed, several studies have pointed out that glucose independently was able to regulate lipogenic gene expression, including FAS, and that the effect of insulin was in part dependent on glucose. Moreover, it has been demonstrated in the hepatocyte cell system that ADD1/SREBP1c was required for the transcriptional effects of glucose on the FAS gene [23]. Here we have demonstrated that stimulation of the FAS promoter activity with Ang II was largely dependent on glucose. The present results also indicate that Ang II stimulates 2-deoxyglucose uptake in adipocytes, which further supports the possibility that the effect of Ang II might be linked to glucose utilization. Together, these results provide evidence that, rather than a direct effect of Ang II on ADD1/SREBP1c, the induction of the FAS gene by Ang II is linked to increased glucose utilization, which in turn up-regulates FAS via ADD1. This led us to propose the following scheme for Ang II regulation of FAS transcription, in which the primary action of Ang II is exerted at the level of glucose entry within the adipocytes. Once transported within the fat cell, glucose can regulate the transcription of the FAS gene through ADD1/SREBP1c, which

targets the IRE. According to this scheme, insulin is not required for Ang II effects because substantial levels of ADD1/SREBP1c are present in differentiated 3T3-L1 adipocytes.

There are at least two identified types of receptor through which Ang II can signal [33]. Most studies have focused on the signalling of Ang II through the angiotensin type I receptor (AT1). The primary receptor mediating the regulation of lipogenic genes in adipocytes by Ang II identified in our laboratory and in others was the angiotensin type II (AT2) receptor [2,3]; the signalling pathway of Ang II through this receptor is largely unknown. Prostaglandin I₂ has been postulated as a signalling molecule produced by adipocytes in response to Ang II binding to AT2 receptors [2,3]. One recent study has linked Ang II and glucose transport in vascular smooth cells [34]. In that experiment, low concentrations of Ang II (0.1 nM) stimulated 2-deoxyglucose uptake. Although the receptor subtype involved in this effect was not determined, this is reminiscent of our present results showing that the effects of Ang II on FAS gene transcription might be related primarily to the insulin-like effects of Ang II on glucose metabolism. Because changes in ADD1 expression have been involved in the regulation of lipogenic genes by both insulin and glucose [23], we tested whether Ang II also modulates ADD1 expression in the presence or absence of glucose. The present study demonstrates that Ang II in the presence of glucose markedly induced ADD1 expression, suggesting that the regulation of the FAS gene in adipocytes by Ang II might be mediated by the direct modulation of ADD1 in the presence of glucose.

In conclusion, the present study demonstrates for the first time that Ang II can act on gene transcription through insulin-responsive sequences, extending results obtained by others on insulin-like mechanisms of intracellular signalling by Ang II.

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