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Biochemical and Biophysical Research Communications 332 (2005) 174–180
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Posttranslational processing of SREBP-1 in rat hepatocytes is regulated by insulin and cAMP

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Received 20 April 2005
Available online 28 April 2005

Abstract

Insulin and cAMP have opposing effects on de novo fatty acid synthesis in liver and in cultured hepatocytes mediated by sterol-regulatory element binding protein (SREBP). To determine whether these agents regulate the cleavage of full-length SREBP to generate the transcriptionally active N-terminal fragment (nSREBP) in primary rat hepatocytes, an adenoviral vector (Ad-SREBP-1a) was constructed to constitutively express full-length SREBP-1a. Insulin increased, and dibutyryl (db)-cAMP inhibited, generation of nSREBP-1a from its full-length precursor. Insulin stimulated processing of SREBP-1a within 1 h, and the effect was sustained for at least 24 h. The initial stimulation of SREBP processing by insulin preceded measurable reduction in Insig-2 mRNA levels. Rat hepatocytes were also infected with an adenovirus expressing the nuclear form of SREBP-1c (Ad-nSREBP-1c). Insulin increased the half-life of constitutively expressed nSREBP-1c, and this effect of insulin was also inhibited by db-cAMP.
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Keywords: SREBP-1; Insulin; cAMP; Hepatocyte; Insig; Ubiquitin

Sterol-regulatory element binding proteins (SREBPs) are transcription factors that regulate genes controlling intracellular lipid metabolism (reviewed in [1,2]). Two separate genes coding for SREBPs have been identified, *SREBP-1* and *-2*. Of these, SREBP-2 primarily regulates genes associated with cholesterol synthesis or uptake, whereas SREBP-1 regulates genes that control lipogenesis as well. The gene for SREBP-1 has alternative promoters leading to expression of SREBP-1a or SREBP-1c, which differ only in their first exon [3]. SREBP-1c is the predominant product derived from this gene in liver and adipose, while SREBP-1a is the predominant SREBP-1 isoform in other cell types and in actively growing cell lines [4].

Nascent SREBPs contain two transmembrane domains and are integrally inserted into the endoplasmic reticulum with the N- and C-terminal domains exposed to the cytoplasm. The mechanism by which processing of full-length SREBP to generate the transcriptionally active N-terminal fragment (nSREBP) is regulated by cholesterol has been delineated (reviewed in [5,6]). The C-terminal domain of SREBP is associated with SREBP-cleavage activating protein (SCAP), another integral endoplasmic reticulum component with eight transmembrane domains [7,8] that form a sterol-sensing segment homologous to that in HMG-CoA reductase. In cholesterol-replete cells, this segment of SCAP is bound to insulin-induced gene product (Insig)-1 or Insig-2 [9]. In the absence of cholesterol, SCAP undergoes a conformational change and dissociates from Insig [10]. SREBP and associated SCAP are then incorporated into COPII

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0006-291X/$ - see front matter. Published by Elsevier Inc.
doi:10.1016/j.bbrc.2005.04.112

coated vesicles and are transported to the Golgi [11]. In the Golgi, two successive cleavages, catalyzed by Site-1 protease and by Site-2 protease, liberate the N-terminal portion of SREBP (about 65 kDa), which migrates to the nucleus as a transcription factor.

Regulation of SREBP processing by factors other than cholesterol has been less clearly defined. However, it has been suggested that insulin could stimulate SREBP processing in liver cells by reducing levels of Insig-2 [12]. The two isoforms of Insig-2-specific mRNA, the liver-specific Insig-2a and the constitutively expressed Insig-2b, are transcribed via alternative promoters [12]. Both mRNAs generate an identical translation product. Insulin has been reported to reduce levels of Insig-2a mRNA in cultured rat hepatocytes [12]. Reducing the cellular content of Insig-2 could free larger amounts of SREBP–SCAP complex for export to the Golgi and therefore stimulate posttranslational processing of full-length SREBP. Consistent with this, depletion of Insig-1 in CHO cells through ER stress accelerates proteolysis of SREBP-2 [13], while over-expression of Insig-1 or Insig-2 inhibits processing of SREBP-1a, SREBP-1c, and SREBP-2 [14–16].

In the present study, we report that insulin stimulates processing of ectopically expressed full-length SREBP-1a in rat hepatocytes and that this effect is inhibited by db-cAMP. This effect of insulin precedes measurable reduction in Insig-2 mRNA, although suppression of Insig-2 expression might contribute to the sustained stimulatory effect of insulin. In addition, we report that insulin reduces the rate of turnover of the nuclear form of SREBP-1c and that this effect of insulin is also inhibited by db-cAMP.

Materials and methods

Preparation of recombinant adenovirus

Ad-SREBP-1a. The insert from pBluescript-SREBP-1a (ATCC, Manassas, VA) containing the full coding sequence of human SREBP-1a was subcloned into pShuttle-CMV vector (Stratagene; La Jolla, CA). The resulting plasmid was linearized with *Pme*I and transformed into *Escherichia coli* BJ5183-AD-1. Recombinant Ad plasmid was digested with *Pac*I and used to transfect AD-293 cells for the preparation of primary adenovirus stock (Ad-SREBP-1a). Ad-SREBP-1a was propagated in HEK-293 cells and purified by CsCl density gradient centrifugation. Control Ad-lacZ was isolated by the same procedure. Recombinant SREBP and control viruses were stored in PBS containing 10% glycerol at −80 °C until use. Hepatocytes were infected at 10–30 MOI for 12–16 h.

Ad-nSREBP-1c. The insert from pSport-ADD1 (1–403), encoding the nuclear form of rat SREBP-1c (the kind gift of Dr. Bruce Spiegelman, Dana Farber Cancer Institute, Boston, MA), was cloned into pShuttle-CMV vector and used to construct Ad-nSREBP-1c as described above.

Primary hepatocyte culture

Hepatocytes were obtained from livers of male Sprague–Dawley rats (approximately 300 g; Harlan Laboratories, Indianapolis, IN) by collagenase perfusion as previously described [17]. Cells were suspended in RPMI medium (Invitrogen, Carlsbad, CA) containing 5% fetal bovine serum (Sigma, St. Louis, MO), 10 mM glucose, 1 μM dexamethasone, and 100 nM insulin. Each 60 mm culture dish, coated with rat tail collagen (Collaborative Biochemical Products, Bedford, MA), was seeded with 3×10^6 cells; after 4 h, non-adherent cells were removed, and adherent cells were infected for 12–18 h in DMEM without serum or hormones.

Analysis of nuclear and precursor SREBP

Following infection with Ad-SREBP-1a, hepatocytes were incubated for a further 24 h in DMEM containing 5.5 mM glucose, 100 nM dexamethasone, with or without insulin (100 nM), db-cAMP (100 μM) or insulin (100 nM) + db-cAMP (100 μM). For the last 6 h the cells were treated with 25 μg/ml acetylleucyl-leucyl-norleucinal (ALLN) to prevent degradation of liberated nSREBP-1a [18]. Cells were harvested, and membrane and nuclear extracts were prepared by a modification of the procedure described by Dignam et al. [19]. In brief, hepatocytes from five 60 mm plates were washed twice with PBS and collected. Pelleted cells were then suspended by passing through a 25-gauge needle 10 times in 1 ml lysis buffer (20 mM Hepes, pH 7.9, 10 mM NaCl, 1.5 mM $MgCl_2$, 0.2 mM EDTA, 1 mM DTT, 1 mM PMSF, 0.5% Nonidet P40, and protease inhibitor cocktail) and allowed to stand for 10 min on ice. Nuclei were pelleted by a 10-min centrifugation (500*g*) at 4 °C and washed once in the same buffer. The pelleted nuclei were resuspended in a hypertonic buffer (20 mM Hepes, pH 7.9, 0.42 M NaCl, 1.5 mM $MgCl_2$, 2.5% glycerol, 1 mM EDTA, 1 mM EGTA, 1 mM DTT, 25 μg/ml ALLN, and protease inhibitor cocktail). Nuclei were incubated for 30 min at 4 °C and a clear nuclear extract was obtained by centrifugation at 25,000*g* for 30 min at 4 °C. The supernatant from the initial low-speed centrifugation (500*g*) was re-centrifuged at 100,000*g* for 60 min to obtain a pellet of the crude membrane fraction. The pellet was resuspended in 10 mM Tris–HCl, pH 6.8, 100 mM NaCl, 1% SDS, 1 mM EDTA, 1 mM EGTA, 1 mM DTT, and protease inhibitor cocktail.

To analyze the turnover of nSREBP-1c following infection with Ad-nSREBP-1c, hepatocytes were incubated with or without insulin (100 nM), db-cAMP (100 μM), or insulin (100 nM) plus db-cAMP (100 μM) for 24 h. Medium was then replaced with fresh DMEM containing cycloheximide (10 μM) and the corresponding additions of insulin and db-cAMP. Cells were harvested at different time points (0, 2, 4, and 6 h) and cell lysates were prepared as described above.

SDS–PAGE and immunoblot analysis of SREBP proteins

The protein concentrations of nuclear extract and membrane suspensions were measured with a BCA kit (Pierce Biotechnology, Rockford, IL). Aliquots of nuclear extract or membrane suspension containing equal amounts of protein were subjected to 7.5% SDS–PAGE and transferred to nitrocellulose membranes (Amersham Biosciences, Amersham, UK). Membranes were blocked for 1 h with 5% non-fat dried milk in TBS-T (pH 8.0). The membranes were incubated overnight with 1 μg/ml mouse monoclonal anti-SREBP-1 (E-4; Santa Cruz Biotechnology, Santa Cruz, CA) followed by incubating for 1 h with HRP-linked anti-mouse IgG (Amersham Biosciences, Amersham, UK). Blots were developed using the ECL Western Blotting Detection kit (Amersham Biosciences, Amersham, UK) and images were captured with a Phosphorimager (Bio-Rad Laboratories, Hercules, CA). The intensity of bands was quantified with the National Institutes of Health IMAGE software. To detect ubiquitinated nSREBP-1c, blots were probed with monoclonal anti-mouse ubiquitin (Sigma–Aldrich, St. Louis, MO).

Measurement of steady state levels of mRNAs by Northern hybridization

Total RNA was extracted with RNA Stat-60 (Tel-Test, Friendswood, TX) and quantified by measuring absorbance at 260 nm. Twenty micrograms of total RNA was loaded per lane of a formaldehyde/0.8% agarose gel, electrophoresed in 1× MOPS buffer, blotted onto Nytran SuperCharge membranes (Schleicher & Schuell, Keane, NH), and UV-cross-linked. Ribosomal RNA bands were visualized by staining with ethidium bromide prior to transfer. Blots were prehybridized for 3 h at 42 °C in 50% formamide, 5× SSPE, 5× Denhardt's solution (5Prime-3Prime, Boulder, CO), 7.5% dextran sulfate, 1.5% sodium dodecyl sulfate (SDS), and 100 μg/ml sheared salmon sperm DNA (Ambion, Austin, TX). Overnight hybridization with the cDNA probes, ^{32}P-labeled by the random primer method using a commercial kit (Invitrogen, Carlsbad, CA), was done at 42 °C. Unbound probe was removed by washing twice with 2× SSC + 0.1% SDS at room temperature and then twice with 0.1× SSC + 0.1% SDS at 65 °C for 30 min each. Membranes were exposed to Bio-Max MS film (Eastman Kodak, Rochester, NY); a digital image of the developed film was created and RNA bands were quantified by densitometry (Alpha Innotech, San Leandro, CA). Probes for Insig-1 and Insig-2 mRNA were derived by excising the inserts from pCMV-Insig 2 and pCMV-Insig 1 (expressing full-length mouse Insig-2 and human Insig-1, respectively (ATCC, Manassas, VA)).

Results and discussion

Insulin stimulates and db-cAMP inhibits processing of full-length SREBP-1a in rat hepatocytes

In intact liver and in cultured hepatocytes, insulin increases the abundance of both the full-length SREBP-1c available for processing and of nSREBP-1c derived from it [20]. Increased abundance of the precursor would itself be expected to result in the generation of larger amounts of processed product. To determine whether insulin also directly stimulates processing of SREBP in rat hepatocytes, and to avoid the ambiguity resulting from insulin effects on the size of the precursor pool, we constructed an adenoviral vector to constitutively express human full-length SREBP-1a (Ad-SREBP-1a). Hepatocytes were infected overnight with Ad-SREBP-1a and then incubated for 24 h in the presence of insulin (100 nM) or in control medium. The nuclear form of SREBP is subjected to ubiquitination and subsequent proteasomal degradation [18]. To permit accumulation of nSREBP-1a, the proteasomal inhibitor ALLN (25 μg/ml) was added during the last 6 h of incubation [18]. The amount of nSREBP-1a that accumulated during this period in insulin treated cells was increased by 2.51 ± 0.70-fold over that produced in cells in control medium ($p = 0.034$; data from eight hepatocyte preparations; Fig. 1).

Induction of *SREBP-1c* by insulin is opposed by db-cAMP [21]. To determine whether a stimulatory effect of insulin on SREBP processing would also be opposed by db-cAMP, hepatocytes infected with Ad-SREBP-1a in the same series of experiments were incubated for 24 h with db-cAMP (100 μM) or insulin (100 nM) plus

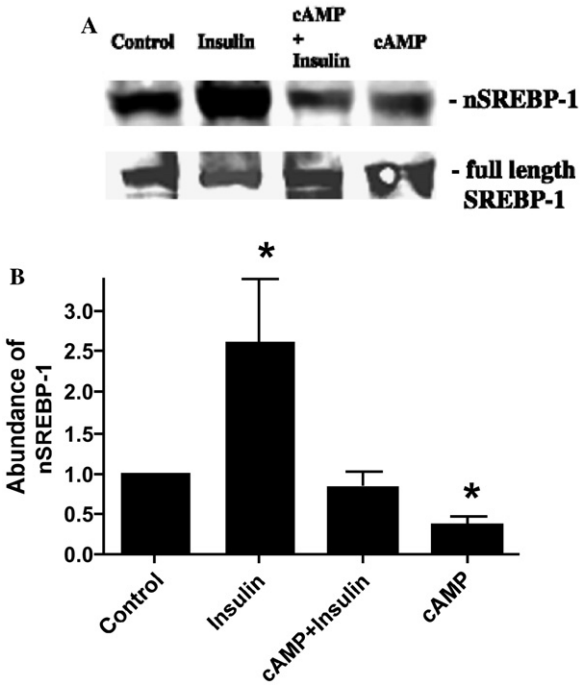

Fig. 1. Insulin stimulates processing of ectopically expressed SREBP-1a in rat hepatocytes. Hepatocytes were infected with Ad-SREBP-1a to constitutively express human full-length SREBP-1a. Cells were then incubated in medium containing insulin (100 nM), db-cAMP (100 μM), or insulin (100 nM) + db-cAMP (100 μM), or without additions. ALLN (25 μg/ml) was added after 18 h and cells were harvested after 24 h of incubation. Cells were lysed, and nuclear extracts and total membrane suspensions were prepared as described in Materials and methods. Aliquots containing equal amounts of protein were resolved by SDS–PAGE and transferred to nitrocellulose membranes for Western analysis. (A) A representative immunoblot showing increased levels on nSREBP-1a in nuclei of hepatocytes treated with insulin. Addition of db-cAMP reduced the generation of nSREBP-1a in the presence and absence of insulin. Levels of full-length SREBP-1a in membrane suspensions prepared from the same cells were not affected by insulin or db-cAMP. (B) Insulin increased the abundance of nSREBP-1a 2.51 ± 0.70-fold relative to levels in control cells (mean ± SEM; $N = 8$). Db-cAMP reduced levels of nSREBP-1a to 0.38 ± 0.09 that in control cells (mean ± SEM; $N = 3$) and reduced levels in insulin treated cells to 0.84 ± 0.19 that in untreated hepatocytes (mean ± SEM; $N = 5$). *Different than control, $p < 0.05$.

db-cAMP (100 μM). The accumulation of nSREBP-1a in the absence of insulin was reduced by 62 ± 9% by db-cAMP ($p = 0.011$; data from three hepatocyte preparations; Fig. 1) and db-cAMP completely blocked the increase in nSREBP-1a accumulation produced by insulin (abundance of nSREBP-1a: 0.84 ± 0.20 times control; data from five hepatocyte preparations; Fig. 1). Insulin and db-cAMP did not affect levels of full-length SREBP-1a present in membrane suspensions (ratio of abundance in insulin treated cells to abundance in control cells: 0.97 ± 0.06; data from eight hepatocyte preparations). No bands corresponding to precursor SREBP-1a or product nSREBP-1a were visible in samples from uninfected cells or cells infected with Ad-lacZ (data not shown).

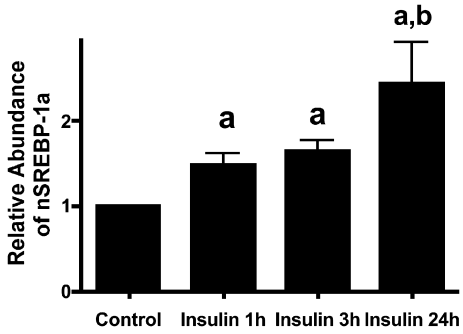

Fig. 2. Stimulation of processing of ectopically expressed SREBP-1a by insulin is apparent within 1 h and is sustained for at least 24 h. Rat hepatocytes were infected with Ad-SREBP-1a to constitutively express human full-length SREBP-1a. Cells were then incubated in DMEM for an additional 24 h. Insulin (100 nM) was added initially or after 21 or 23 h of incubation or no addition was made (control cells). ALLN (25 μg/ml) was added after 23 h and cells were harvested after 24 h of incubation. Cells were lysed, and nuclear extracts and total membrane suspensions were prepared as described in Materials and methods. Aliquots containing equal amounts of protein were resolved by SDS–PAGE and transferred to nitrocellulose membranes for Western analysis. The level of nSREBP-1a was increased in insulin treated cells to 1.49 ± 0.13 times control ($N = 4$) after 1 h, to 1.64 ± 0.13 times control ($N = 4$) after 3 h, and to 2.45 ± 0.47 times control ($N = 4$) after 24 h. [a]Different than control, $p < 0.05$; [b]Different from value at 1 h, $p < 0.05$.

To determine how rapidly insulin effects on processing of SREBP-1a can be detected, hepatocytes were infected with Ad-SREBP-1a overnight and then incubated for 24 h in DMEM with no supplement, or with insulin (100 nM) added for the last 1 h, the last 3 h, or for 24 h. In all groups, ALLN (25 μg/ml) was present during the last hour of incubation. As shown in Fig. 2, in the presence of insulin for 1 h prior to termination of the incubation, the nuclear content of nSREBP-1a was increased to 1.49 ± 0.13 times control ($p = 0.017$; data from four hepatocyte preparations). After 3 h of insulin treatment, the level of nSREBP-1a was increased to 1.64 ± 0.13 times control ($p = 0.008$; data from four hepatocyte preparations), and after 24 h to 2.45 ± 0.47 times control ($p = 0.048$; data from four hepatocyte preparations).

Since turnover of nSREBP-1a was blocked in these experiments, the increased levels of nSREBP-1a in the presence of insulin and decreased levels of nSREBP-1a in the presence of db-cAMP presumably reflect changes in the rate of processing of the full-length precursor. An increase in the cleavage of full-length SREBP-1a was discernable within 1 h of addition of insulin and was sustained for at least 24 h.

Insulin reduces levels of Insig-2 mRNA in rat hepatocytes

Insulin has been reported to reduce the levels of Insig-2 mRNA in rat hepatocytes [12]. Reduction in Insig-2a levels may contribute to the stimulatory effect of insulin on processing of SREBP-1a. We measured the levels of Insig-1 and Insig-2 mRNA by Northern analysis (Fig. 3). After 24 h of exposure, insulin reduced the level of Insig-2 mRNA to 0.46 ± 0.08 that in control cells ($p = 0.006$; data from four hepatocyte preparations; Fig. 3B). Levels of Insig-1 mRNA were unaltered by insulin at this time point (ratio of Insig-1 mRNA in insulin treated cells to that in untreated cells: 0.92 ± 0.29; data from four hepatocyte preparations).

Although accumulation of nSREBP-1a is increased after 1 h of exposure to insulin (Fig. 2), the inhibitory effect of insulin on the levels of Insig-2 mRNA was not

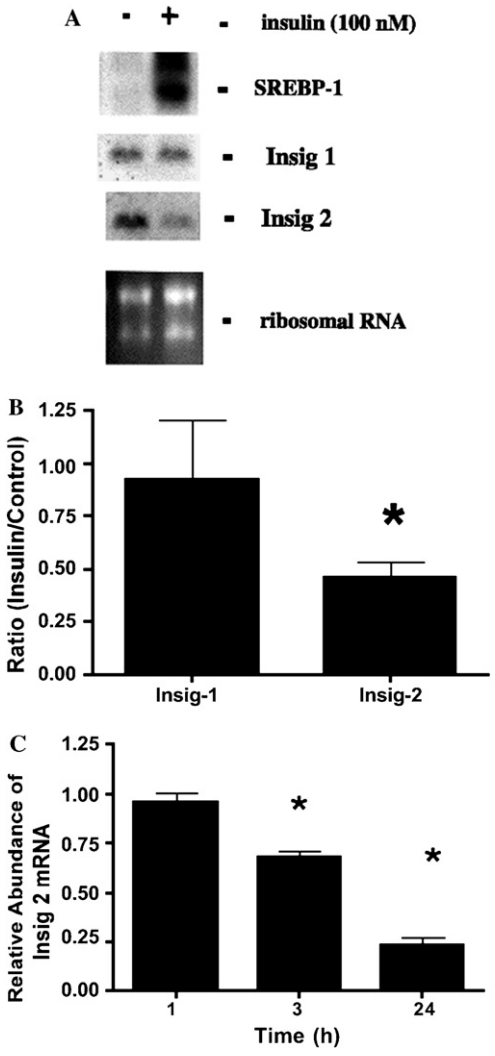

Fig. 3. Insulin reduces the levels of Insig-2 mRNA. (A) A representative Northern blot of RNA extracted from rat hepatocytes incubated for 24 h with or without insulin (100 nM) is shown. As expected, insulin increased the levels of SREBP-1 mRNA. Levels of Insig-2 mRNA were decreased by insulin, while the abundance of Insig-1 mRNA was not significantly altered ($N = 4$). (B) After 24 h of exposure, insulin reduced the level of Insig-2 mRNA to 0.46 ± 0.08 that in control cells ($N = 4$). (C) The inhibitory effect of insulin on levels of Insig-2 mRNA was not apparent after 1 h, but had become significant after 3 h and was sustained for at least 24 h ($N = 2$). *Different from control without insulin ($p < 0.05$).

apparent after 1 h (level in insulin treated cells 0.96 ± 0.05 that in control cells; Fig. 3C), but had become significant after 3 h (level in insulin treated cells 0.68 ± 0.04 that in control cells, $p = 0.029$). After 24 h, levels of Insig-2 mRNA were reduced to 0.23 ± 0.05 times control ($p = 0.038$). Reduced levels of Insig-2 are thus unlikely to be responsible for the initial enhancement of processing of SREBP-1a brought about by insulin but may contribute to the sustained insulin stimulation.

Insulin inhibits turnover of nSREBP-1c in rat hepatocytes, and this effect is blocked by db-cAMP

The previous set of experiments indicates that insulin stimulates the generation of nSREBP-1a from full-length SREBP-1a precursor. To determine whether insulin also affects the metabolic stability of nuclear SREBP in rat hepatocytes, we constructed an adenoviral vector to constitutively express rat nSREBP-1c (Ad-nSREBP-

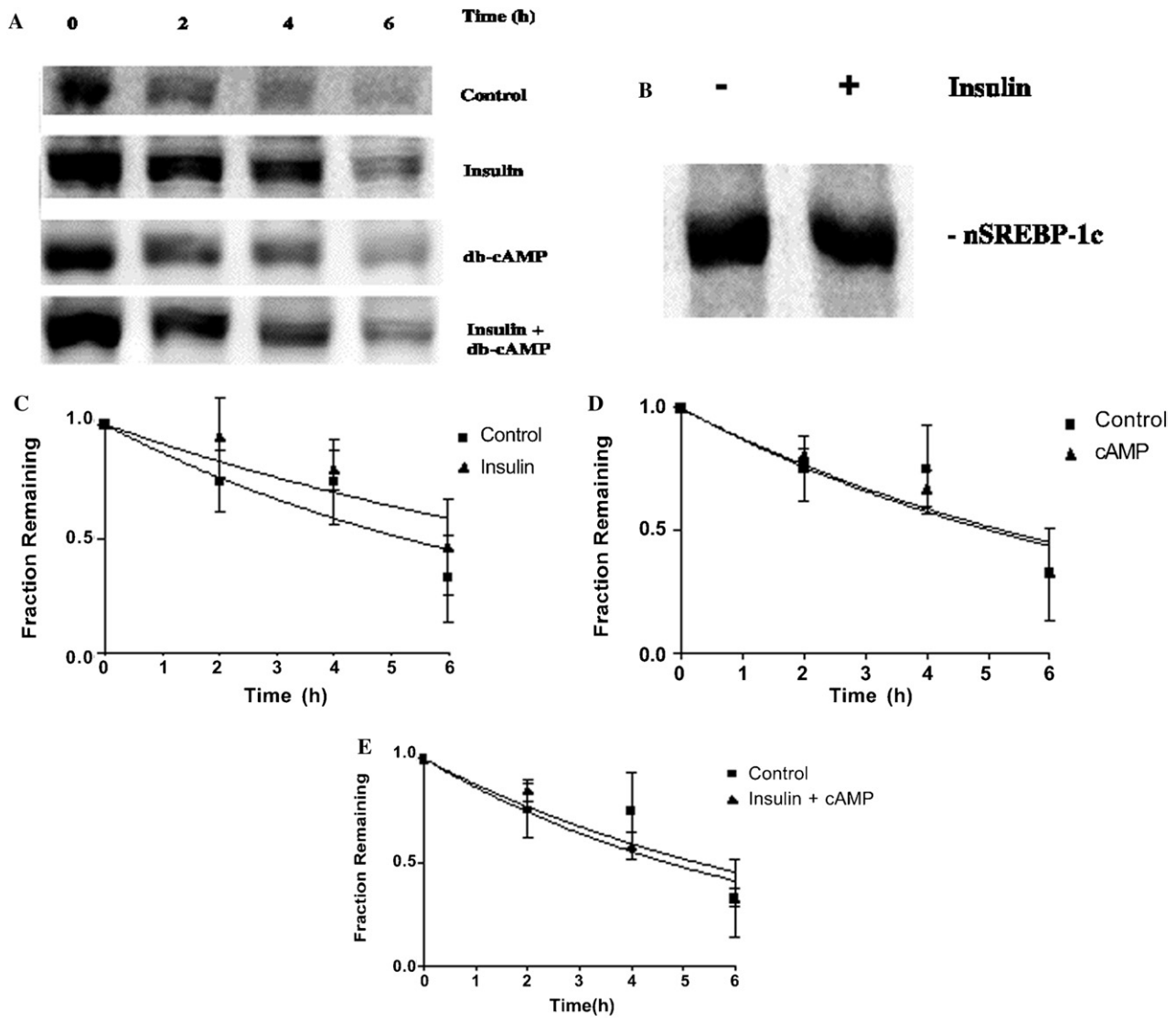

Fig. 4. Insulin decreases the rate of turnover on nSREBP-1c in rat hepatocytes, and this effect is inhibited by db-cAMP. Hepatocytes were infected with Ad-nSREBP-1c to constitutively express rat nSREBP-1c. Cells were then incubated in medium containing insulin (100 nM), db-cAMP (100 μM), insulin (100 nM) plus db-cAMP (100 μM), or without additions. Cycloheximide (10 μM) was added and cells were harvested immediately or after 2, 4, or 6 h of further incubation. Cells were lysed, and nuclear extracts were prepared as described in Materials and methods. Aliquots containing equal amounts of protein were resolved by SDS–PAGE and transferred to nitrocellulose membranes for Western analysis. (A) A representative immunoblot showing depletion of nSREBP-1c in nuclei of control hepatocytes and hepatocytes treated with insulin, db-cAMP, or insulin plus db-cAMP. No significant difference was observed in the initial level of nSREBP-1c among the four treatment groups. (B) Insulin does not reduce the level of ubiquitinated nSREBP-1c in rat hepatocyte nuclei. Nuclear proteins from hepatocytes incubated for 24 h with or without insulin (100 nM) were resolved by SDS–PAGE and transferred to nitrocellulose membranes. Membranes were probed with polyclonal antibody to ubiquitin (Sigma; St. Louis, MO). A representative immunoblot is shown ($N = 3$). (C) Insulin increased the half-life of nSREBP-1c from 4.58 ± 1.18 h to 8.02 ± 1.16 h ($P = 0.0359$; $N = 5$). Half-life of nSREBP-1c was not different than control in hepatocytes treated with db-cAMP (4.70 ± 0.43 h; $N = 2$; D) or insulin plus db-cAMP (5.05 ± 0.29 h; $N = 2$; E). *Different than control, $p < 0.05$.

1c). Hepatocytes were infected overnight with Ad-nSREBP-1c and then incubated for 24 h in the presence of insulin (100 nM), db-cAMP (100 μM), insulin (100 nM) plus db-cAMP (100 μM), or in control medium. Fresh media containing the same additions plus cycloheximide (10 μM) were then added to prevent further synthesis of protein, and samples were collected after 0, 2, 4, and 6 h of further incubation. The half-life of nSREBP-1c was increased from 4.58 ± 1.18 h in control medium to 8.02 ± 1.16 h in the presence of insulin ($p = 0.036$; data from five hepatocyte preparations; Figs. 4A and C). These values are comparable to the reported 3 h half-life of nSREBP-1a in COS cells [18]. The stabilization of nSREBP-1c produced by insulin was prevented by db-cAMP (Figs. 4A and E); db-cAMP by itself had no effect on the turnover of nSREBP-1c (Figs. 4A and D).

To determine if insulin stabilizes nSREBP-1c in rat hepatocytes by decreasing the rate at which nSREBP-1c is ubiquitinated, Western blots of nuclear extract from control and insulin treated hepatocytes constitutively expressing nSREBP-1c were reprobed with antibody directed against ubiquitin. No difference was observed in the level of ubiquitinated nSREBP-1c in the presence or absence of insulin (ratio of abundance of ubiquinated nSREBP-1c in insulin treated cells to that in control cells: 0.87 ± 0.19; data from three hepatocyte preparations; Fig. 4B). It appears that the stabilization of nSREBP-1c is due to an effect on a step(s) subsequent to or independent of ubiquitin modification of the transcription factor.

Conclusion

It was recently reported that insulin acutely stimulates the generation of nSREBP-1c from its full-length precursor in rat hepatocytes in which maturation of full-length SREBP-1c was partially blocked by the LXR agonist TO901317 [22]. Our finding that insulin stimulates processing of constitutively expressed SREBP-1a provides further support for a stimulatory effect of insulin on the maturation of SREBP isoforms in rat hepatocytes and now shows that this effect is sustained for at least 24 h. In this study, we found that the stimulatory effect of insulin precedes any measurable decline in levels of Insig-2 mRNA and is therefore at least partially independent of this effect. In addition, we found that db-cAMP inhibits maturation of SREBP-1a and blocks the stimulatory effect of insulin on this process. We also found that insulin slows the turnover of the nuclear form of SREBP-1c in rat hepatocytes. Although db-cAMP does not itself stimulate the rate of nSREBP-1c turnover, it does block the inhibitory effect of insulin.

Insulin is a potent inducer of the enzymes catalyzing de novo synthesis of fatty acids in liver and adipose. The stimulatory effect of insulin on the expression of these enzymes in hepatocytes is mediated by SREBP-1c (reviewed in [2]). Insulin stimulates the transcription of *SREBP-1c* in intact liver [20,23] and in rat hepatocytes in culture [21]. The effect of insulin to increase transcription of lipogenic enzymes is opposed by exogenous cAMP or by agents such as glucagon that elevate intracellular cAMP concentrations [23,24]. Dibutyryl-cAMP inhibits transcriptional stimulation of *SREBP-1* c by insulin [21].

Therefore, insulin increases levels of nSREBP-1c in hepatocytes by at least three different mechanisms: (1) transcriptional up-regulation of *SREBP-1c*; (2) increased rate of liberation of the active form of SREBP from its full-length precursor; and (3) decreased rate of turnover of the active form of SREBP following liberation. All of these effects are opposed by cAMP, the physiological antagonist of the pro-lipogenic effects of insulin in the liver.

Acknowledgments

We thank Poonam Kumar for expert technical assistance. This work was supported in part by grants from the Office of Research and Development, Department of Veterans Affairs (DVA), from the American Heart Association, Southeast Affiliate, the University of Tennessee Vascular Biology Center of Excellence, and by a grant from the NIH DK-059368 (E.A.P.). Dr. Yellaturu is a recipient of a Postdoctoral Fellowship Award from the American Heart Association, Southeast Affiliate. Dr. Rajendra Raghow is a senior research scientist of the DVA.

References

[1] H. Shimano, Sterol-regulatory element binding protein family as global regulators of lipid synthetic genes in energy metabolism, Vitam. Horm. 65 (2002) 167–194.
[2] D. Eberlé, B. Hegarty, P. Bossard, P. Ferré, F. Foufelle, SREBP transcription factors: master regulators of lipid homeostasis, Biochimie 86 (2004) 839–848.
[3] X. Hua, J. Wu, J.L. Goldstein, MS. Brown, H.H. Hobbs, Structure of the human gene encoding sterol regulatory element binding protein-1 (SREBF1) and localization of SREBF1 and SREBF2 to chromosomes 17p11.2 and 22q13, Genomics 25 (1995) 667–673.
[4] I. Shimomura, H. Shimano, J.D. Horton, J.L. Goldstein, M.S. Brown, Differential expression of exons 1a and 1c in mRNAs for sterol regulatory element binding protein-1 in human and mouse organs and cultured cells, J. Clin. Invest. 99 (1997) 838–845.
[5] R.G. Anderson, Joe Goldstein and Mike Brown: from cholesterol homeostasis to new paradigms in membrane biology, Trends Cell Biol. 13 (2003) 534–539.
[6] R.B. Rawson, The SREBP pathway—insights from Insigs and insects, Nat. Rev. Mol. Cell Biol. 4 (2003) 631–640.
[7] M.S. Brown, J.L. Goldstein, A proteolytic pathway that controls the cholesterol content of membranes, cells, and blood, Proc. Natl. Acad. Sci USA 96 (1999) 11041–11048.

[8] P.A. Edwards, D. Tabor, H.R. Kast, A. Venkateswaran, Regulation of gene expression by SREBP and SCAP, Biochim. Biophys. Acta 1529 (2000) 103–113.
[9] D. Yabe, M.S. Brown, J.L. Goldstein, Insig-2, a second endoplasmic reticulum protein that binds SCAP and blocks export of sterol regulatory element-binding proteins, Proc. Natl. Acad. Sci. USA 99 (2002) 12753–12758.
[10] C.M. Adams, J.L. Goldstein, M.S. Brown, Cholesterol-induced conformational, change in SCAP enhanced by Insig proteins and mimicked by cationic amphiphiles, Proc. Natl. Acad. Sci. USA 100 (2003) 10647–10652.
[11] P.J. Espenshade, W.-P. Li, D. Yabe, Sterols block binding of COPII proteins to SCAP, thereby controlling SCAP sorting in ER, Proc. Natl. Acad. Sci. USA 99 (2002) 11694–11699.
[12] D. Yabe, R. Komuro, G. Liang, J.L. Goldstein, M.S. Brown, Liver-specific mRNA for insig-2 down-regulated by insulin: implications for fatty acid biosynthesis, Proc. Natl. Acad. Sci. USA 100 (2003) 3155–3160.
[13] J.N. Lee, J. Ye, Proteolytic activation of sterol regulatory element-binding protein induced by cellular stress through depletion of insig-1, J. Biol. Chem. 279 (2004) 45257–45265.
[14] T. Yang, P.J. Espenschade, M.E. Wright, D. Yabe, Y. Gong, R. Aebersold, J.L. Goldstein, M.S. Brown, Crucial step in cholesterol homeostasis: sterols promote binding of SCAP to INSIG-1, a membrane protein that facilitates retention of SREBPs in the ER, Cell 110 (2002) 489–500.
[15] L.J. Engelking, H. Kuriyama, R.E. Hammer, J.D. Horton, M.S. Brown, J.L. Goldstein, G. Liang, Overexpression of insig-1 in the livers of transgenic mice inhibits SREBP processing and reduces insulin-stimulated lipogenesis J, Clin. Invest. 113 (2004) 1168–1175.
[16] K. Takaishi, L. Duplomb, M.-Y. Wang, J. Li, R.H. Unger, Hepatic insig1 or insig2 overexpression reduces lipogenesis in obese Zucker diabetic fatty rats and in fasted/refed normal rats, Proc. Natl. Acad. Sci. USA 101 (2004) 7106–7111.
[17] F.E. Thorngate, R. Raghow, H.G. Wilcox, C.S. Werner, M.B. Elam, Insulin promotes the biosynthesis and secretion of apolipoprotein B-48 by dramatically altering apolipoprotein B mRNA editing, Proc. Natl. Acad. Sci. USA 91 (1994) 5392–5396.
[18] Y. Hirano, Y. Minoru, M. Shimizu, R. Sato, Direct demonstration of rapid degradation of nuclear sterol regulatory element-binding proteins by the ubiquitin–proteasome pathway, J. Biol. Chem. 276 (2001) 36341–36437.
[19] J.D. Dignam, R.M. Lebovitz, R.G. Roeder, Accurate transcription initiation by RNA polymerase II in a soluble extract from isolated mammalian nuclei, Nucleic Acids Res. 11 (1983) 1475–1489.
[20] D. Azout-Marniche, D. Bécard, C. Guichard, M. Foretz, P. Ferré, F. Foufelle, Insulin effects on sterol regulatory element binding protein 1c (SREBP-1c) transcriptional activity in rat hepatocytes, Biochem. J. 350 (2000) 389–393.
[21] X. Deng, L.M. Cagen, H.G. Wilcox, E.A. Park, R. Raghow, M.B. Elam, Regulation of the rat SREBP-1c promoter in primary rat hepatocytes, Biochem. Biophys. Res. Commun. 290 (2002) 256–262.
[22] B.D. Hegarty, A. Bobard, I. Hainault, P. Ferre, P. Bossard, F. Foufelle, Distinct roles of insulin and liver X receptor in the induction and cleavage of sterol regulatory element-binding protein-1c, Proc. Natl. Acad. Sci. USA 102 (2005) 791–796.
[23] M. Foretz, C. Guichard, P. Ferré, F. Foufelle, Sterol regulatory element binding protein 1c is a major mediator of insulin action on the hepatic expression of glucokinase and lipogenesis-related genes, Proc. Natl. Acad. Sci. USA 96 (1999) 12737–12742.
[24] V.S. Rangan, B. Oskouian, S. Smith, Identification of an inverted CCAAT box motif in the fatty acid synthase gene as an essential element for the modification of transcriptional regulation by cAMP, J. Biol. Chem. 271 (1996) 2307–2312.