Vol. 173, No. 1, 1990 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
November 30, 1990 Pages 87-91

INDUCTION OF ADIPOSE FATTY ACID BINDING PROTEIN (a-FABP) BY INSULIN-LIKE GROWTH FACTOR-1 (IGF-1) IN 3T3-L1 PREADIPOCYTES

William L. Blake and Steven D. Clarke

Unit of Performance Enhancement, Unit 7921 Bldg. 25 Lab 418,
The Upjohn Company, Kalamazoo, MI 49001

Received September 18, 1990

Murine 3T3-L1 cells were cultured in the presence of fetal bovine serum (FBS) washed with an anion exchange resin and charcoal. Using the abundance of a-FABP and fatty acid synthase (FAS) as criteria of differentiation, IGF-1 was found to be 10-fold more potent than insulin as an inducer of preadipocyte differentiation. As little as 0.5 nM IGF-1 induced expression of FAS and a-FABP mRNAs whereas a minimum of 5 nM insulin was required. The data indicate IGF-1 specifically induces the expression of a-FABP in 3T3-L1 preadipocytes whereas the effect of insulin is likely via insulin's binding to the IGF-1 receptor.

Insulin-like growth factor 1 (IGF-1), the putative second message hormone for somatotropin action, stimulates adipocyte glucose uptake, increases fatty acid synthesis, and stimulates glycerol-3-phosphate dehydrogenase expression in 3T3-L1 adipocytes (1,2). Clearly many of the IGF-1 metabolic effects are also actions attributable to insulin. Since insulin at high concentrations will bind to the IGF-1 receptor, it has been difficult in many studies to differentiate the effects of insulin from IGF-1. Recently, Rubin and colleagues (2) reported that in a media containing serum depleted of growth factors, the differentiation of 3T3-L1 preadipocytes appeared to be an IGF-1 dependent process. This evidence renders the role of insulin in fat cell differentiation much less clear. However, a-FABP expression in differentiating 3T3-L1 cells may provide a connection between the differentiation stimulus of IGF-1 and the lipogenic stimulus of insulin. Adipose FABP appears to play a role in the intracellular signaling system for insulin in the adipocyte (3). Using this concept, we

0006-291X/90 $1.50

hypothesize that IGF-1 specifically stimulates a-FABP expression in 3T3-L1 preadipocytes and that the early increase in preadipocyte a-FABP is a key event in rendering the preadipocyte insulin responsive. The initial objective was to demonstrate that a-FABP gene expression in 3T3-L1 preadipoctyes was specifically increased by IGF-1, and that this event was not an insulin dependent process.

MATERIALS AND METHODS

Materials. Resin AG1-X8 and Zeta-Probe nylon membranes were from Bio-Rad (Richmond, CA). Recombinant human IGF-1 was purchased from IMCERA Bioproducts (Terre Haute, IN). Bovine insulin, cell culture supplies, and fetal bovine serum (FBS) were obtained from GIBCO (Grand Island, NY). Random primer DNA labeling system was obtained from Bethesda Rsearch Laboratories (Gaithersburg, MD). Plasmid pBR 322 containing the 660 bp cDNA for rat liver fatty acid synthase (FAS) was provided by Dr. M. Magnuson, Vanderbilt University (4). Plasmid pBR 322 containg the 672 bp cDNA corresponding to 3T3-L1 a-FABP was provided by Dr. D. Bernlohr, University of Minnesota (5). Fetal bovine serum (FBS) was washed with AG1-X8 resin and charcoal by the procedure of (2).

Culture conditions. 3T3-L1 fibroblasts were grown (37 C, 5% CO^2/95% air) to confluence in Dulbecco's modified Eagle's medium containing 20 mM Hepes, 2 mM glutamine, 1 um biotin, 100 U/ml penicillin and 100 ug/ml streptomycin plus 10% resin/charcoal-washed FBS (2). At confluence the 3T3-L1 fibroblasts were treated with 0.5 mM dexamethasone, 0.5 mM 1-methyl-3-isobutylxanthine for 48 h followed by the addition of insulin or recombinant IGF-1 (concentrations cited in Figures).

RNA isolation, Northern and slot-blot analyses. Total RNA was isolated from cells according to Chirgwin et al. (6). Cells were solubilized with guanidinium thiocyanate and total RNA pelletd through cesium chloride cushion. The RNA pellet was dissolved in 10 mM Tris-HCl, 5 mM EDTA, 0.5% SDS, pH 7.4 The solution was extracted once with cholorform: butanol (3:1), and the RNA subsequently precipitated with absolute ethanol. The RNA precipitate was dissolved in water and its quality determined by Northern analysis (4). The amount of FAS and a-FABP was quantified by slot blot analysis (4). Total RNA (0.25 and 0.5 ug/slot) was denatured by heating in 10X SSC and 6.15 M formaldehyde. The RNA was cross-linked to the nylon filter by UV exposure. The membrane was prehybridized and hybridized with radiolabeled FAS or a-FABP cDNA (4), and the autoradiographic signal was quantified by laser densitometry (4).

RESULTS

FAS mRNA was not induced in 3T3-L1 preadipocytes cultured with resin/charcoal-washed FBS, but upon addition of insulin FAS mRNA increased several fold (Fig. 1). Thus, the extracted

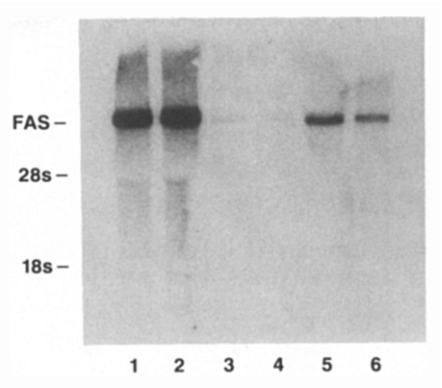
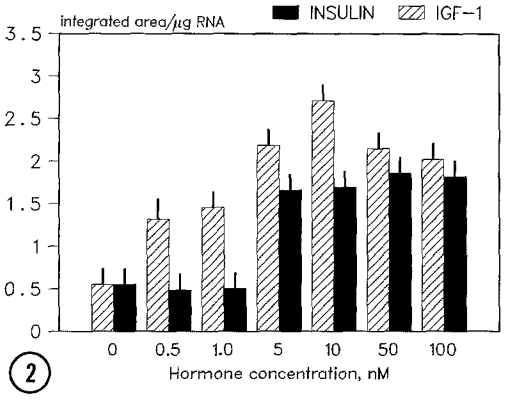


Figure 1. Abundance of fatty acid synthase in 3T3-L1 cells cultured in the presence of treated or untreated fetal bovine serum.
Total RNA (5 μg/lane) was isolated from 3T3-L1 cells cultured in FBS or FBS-extracted with anion exchange resin and charcoal. Lanes 1 and 2 represent RNA from cells cultured in treated FBS plus 1 μM insulin. Lanes 3 and 4 represent RNA from cells cultured in treated FBS only. Lanes 5 and 6 contain RNA from cells cultured in untreated FBS.

serum was not toxic to the preadipocytes, and the extraction process effectively removed factors from serum that were required for 3T3-L1 differentiation. Using the expression of a-FABP and FAS as criteria, IGF-1 was found to be a more potent inducer of 3T3-L1 differentiation than insulin (Figs. 2 and 3). As little as 0.5 nM IGF-1 induced a-FABP mRNA to near maximal levels and increased FAS mRNA 3-4 fold (Figs. 2 and 3). IGF-1 was at least 10-fold more potent than insulin as an inducer of a-FABP and FAS gene expression (Figs. 2 and 3). At all concentrations examined, IGF-1 increased the level of a-FABP to a greater extent than did insulin (Fig. 3), whereas above 10 nM IGF-1 and insulin were equipotent stimulators of FAS mRNA accumulation (Fig.2).

DISCUSSION

Adipose FABP is thought to play a role in the insulin recruitment of glucose transporters and may be part of the intracellular insulin signaling system (3,7). Consistent with this hypothesis was an observation that a-FABP gene expression precedes the accumulation of lipoprotein lipase and glucose transporter-IV mRNAs (unpublished data). In the present report, the expression of a-FABP (presumeably an early event of preadipocyte differentiation) was found to be under the control of IGF-1 and not insulin dependent. One argument that



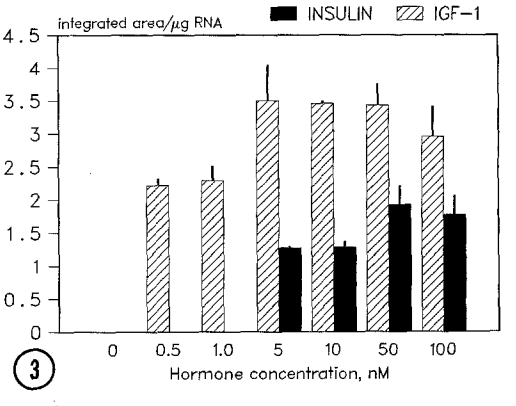


Figure 2. Effect of IGF-1 and insulin on abundance of fatty acid synthase mRNA in 3T3-L1 cells.
Total RNA was isolated from 3T3-L1 cells cultured in the presence of IGF-1 or insulin for 3 d. FAS mRNA abundance was determined by slot blot hybridization. Intensity of hybridization was quantified by laser densitometry analysis of the radiograph. Abundance is expressed as mean integrated area/μg RNA ± SEM (n=3). Analysis of variance revealed a significant ($P<.05$) increase in abundance of FAS mRNA with IGF-1 treatment (0.5, 1.0 and 5 nM) of cells in comparison to insulin.

Figure 3. Effect of IGF-1 and insulin on abundance of a-FABP mRNA in 3T3-L1 cells.
Total RNA was isolated from 3T3-L1 cells cultured in the presence of IGF-1 or insulin for 3 d. Abundance of a-FABP mRNA was determined by slot blot hybridization. Intensity of hybridization was quantified by laser densitometry analysis of the radiograph. Abundance is expressed as mean integrated area/μg RNA ± SEM (n=3). Analysis of variance revealed a significant ($P<.01$) increase in abundance of a-FABP mRNA at all concentrations of IGF-1 in comparison to insulin treatment.

could be proposed for the IGF-1 effect is that IGF-1 may have bound to the insulin receptor of the preadipocyte. However, near maximal induction of a-FABP, as well as FAS, was achieved with a concentration of IGF-1 (0.5 nM) at which virtually no IGF-1 could be bound to the insulin receptor (8). The minimal amount of insulin required to elevate a-FABP and FAS mRNA levels was 5 nM, and nearly 100-fold more insulin was required to achieve a level of a-FABP mRNA comparable to that observed with 0.5 nM IGF-1 (Figs. 2 and 3). The fold difference in sensitivity of preadipocytes between IGF-1 and insulin was consistent with the 70-fold less affinity the IGF-1 receptor possesses for insulin (2,8). Thus, with regard to early stages of differentiation of preadipocytes, a more likely argument is that insulin induction of 3T3-L1 differentiation is the consequence of crossover binding to the IGF-1 receptor (2). The increase in FAS and a-FABP mRNAs during

differentiation is accomplished by increased rates of gene transcription (5). Perhaps the FAS and a-FABP genes share a common cis-acting IGF-1 response element in their 5'-regulatory region, and/or share an IGF-1 regulated trans-acting factor. In this regard, several genes which are expressed during preadipocyte differentiation, including a-FABP, glycerol-phosphate dehydrogenase, glutamine synthase, and adipsin (9), all share two common fat specific cis element (FSE1 and 2) sequences. These cis elements may bind nuclear regulatory proteins (e.g. c-fos) which are under IGF-1 control and govern the expression of differentiation dependent proteins such as a-FABP (9). Obviously such a mechanism is speculative, and is likely to be only one component of the differentiation mechanism.

In summary, our data indicate that IGF-1 specifically stimulates the expression of a-FABP in 3T3-L1 preadipocytes. Since IGF-1 expression appears to be regulated by somatotropin (10), a logical scenario for fat cell differentiation may be that somatotropin induces IGF-1 and the autocrine action of IGF-1 stimulates the expression of a-FABP which permits the differentiating fat cell to respond to insulin and complete the differentiation process. The specificity and sensitivity of the 3T3-L1 preadipocyte to the IGF-1 stimulus indicates this cell system will provide a useful model in which to examine this hypothesis.

REFERENCES

1. Zapf, J., Froesch, E.R., and Humbel, R.E. (1981) Curr. Top. Cell. Regul. 19,257-309.
2. Smith, P.J., Wise, L.S., Berkowitz, R., Wan, C., and Rubin, C.S. (1988) J. Biol. Chem. 263,9402-9408.
3. Hresko, R., Bernier, M., Hoffman, R.D., Flores-Riveros, J.R., Liao, K., Laird, D.M., and Lane, M.D. (1988) Proc. Natl. Acad. Sci. USA 85, 8835-8839.
4. Clarke, S.D., Armstrong, M.K., and Jump, S.B. (1990) J. Nutr. 120,218-224.
5. Bernlohr, S.A., Bolanowski, M.A., Kelley, T.J., and Lane, M.D. (1985) J. Biol. Chem. 260, 5563-5567.
6. Chirgwin, J.M., Przybla, A.E., MacDonald, R.J., and Rutter, W.J. (1979) Biochemistry 18, 5294-5299.
7. Bernier, M., Laird, D.M., and Lane, M.D. (1988) J. Biol. Chem. 13626-13634.
8. Nissley, S.P., Haskell, J.F., Sasaki, N., DeVroede, M.A., and Rechler, M.M. (1985) J. Cell Sci. (Suppl.) 3, 39-51.
9. Distel, R.J., Ro, H.S., Rosen, B.S., Groves, D.L., Spiegelman, B.M. (1987) Cell 49, 835-844.
10. Doglio, A., Dani, C., Fredrikson, G., Grimaldi, P.I. and Ailhaud,G. (1987) EMBO 6, 4011-4016.