Regulation of Insulin-Receptor mRNA Levels by Glucocorticoids

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SUMMARY

We found with IM-9 human cultured lymphocytes, that the glucocorticoid dexamethasone increased insulin-receptor mRNA levels. This increase correlated in a time- and dose-dependent manner with the increase in the biosynthesis of the insulin-receptor precursor. In addition, in AR42J cultured rat pancreatic acinar cells, dexamethasone increased insulin-receptor mRNA levels. These studies suggest, therefore, that an increase in mRNA levels is an early step in the regulation of the insulin receptor by glucocorticoids in several cell types. *Diabetes* 36:779–81, 1987

Insulin produces a wide range of effects in the intact organism and in cultured cells (1). These effects occur after insulin binds to and activates a tetrameric glycoprotein receptor located on the plasma membrane (2). This receptor consists of two α-subunits and two β-subunits linked by sulfhydryl bonds. One α- and one β-subunit are derived from a common precursor. The insulin receptor is under the control of hormones and metabolic factors (1,2). It is unknown, however, whether regulation of the receptor occurs at the level of gene expression.

It has been shown that glucocorticoids increase insulin-receptor precursor biosynthesis in several cell types (3). Because the insulin-receptor cDNA has been cloned (4,5), it is possible to directly investigate the effects of glucocorticoids on insulin-receptor gene expression. We now describe an effect of glucocorticoids on insulin-receptor mRNA levels.

MATERIALS AND METHODS

Cell culture. IM-9 lymphocytes were grown in suspension culture (6). AR42J cells were grown in subconfluent monolayer culture (7).

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Insulin-receptor precursor studies. Studies were carried out as previously described (6). Briefly, the solubilized and ^{35}S-labeled receptor precursor was reacted with either normal rabbit serum or rabbit polyclonal anti-insulin-receptor antibody (6) and subjected to electrophoresis on a 6% polyacrylamide gel followed by fluorography.

mRNA preparation and Northern transfers. Either 3×10^8 IM-9 cells or 5×10^8 AR42J cells were washed, and total RNA was prepared from IM-9 cells with proteinase K in the presence of sodium dodecyl sulfate (8) and from AR42J cells by the guanidinium–cesium chloride method (9). Poly(A)$^+$ RNA was obtained by oligo (*dt*) cellulose chromatography (10). For Northern transfers, poly(A)$^+$ RNA was denatured in formaldehyde, subjected to electrophoresis in 1% agarose, and transferred to nitrocellulose paper (11).

cDNA labeling and hybridizations. Two human insulin-receptor cDNA probes (1 and 4.2 kb; a kind gift from Dr. G. I. Bell, Howard Hughes Medical Institute, Univ. of Chicago, Chicago, IL), spanning the entire open reading frame of the receptor and extending into the poly A region (12), were labeled by nick translation (11) to specific activities of 10^9 cpm/μg. The nitrocellulose filters were prehybridized and hybridized and washed as previously described (11). A probe for β-actin (13) was nick translated and hybridized as described for the insulin receptor. Filters were exposed to Kodak XAR-5 film in the presence of two intensifying screens at −70°C for 16 h for IM-9 cells and 48 h for AR42J cells.

RESULTS AND DISCUSSION

Treatment of IM-9 cells with the potent glucocorticoid dexamethasone enhanced the synthesis of the 190,000-M_r insulin-receptor precursor protein (Fig. 1*A*), with maximal effects occurring after 4 h (Fig. 2). Dexamethasone stimulated insulin-receptor synthesis one-half maximally at 10 nM and maximally at 100 nM (Fig. 3). In addition to increasing insulin-receptor protein synthesis, as previously reported (3), dexamethasone induced a time-dependent increase (2- to 3-fold) in ^{125}I-labeled insulin binding to its receptors.

The influence of dexamethasone on insulin-receptor mRNA levels was investigated by employing cDNA probes spanning the coding region of the human insulin receptor

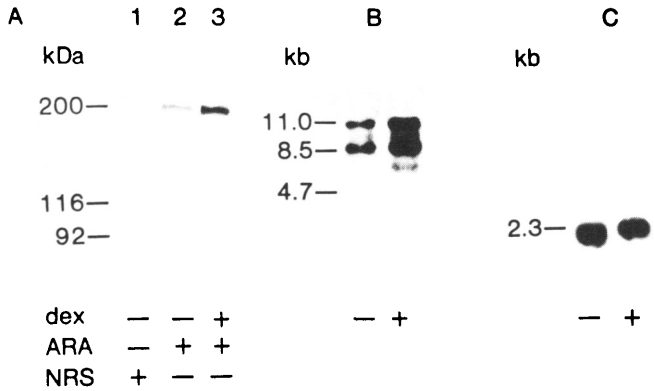

FIG. 1. Influence of dexamethasone on insulin-receptor precursor biosynthesis and mRNA levels. Either dexamethasone (dex; 100 nM) or its diluent was added to IM-9 cells for 4 h. *A*: insulin-receptor precursor studies. *Lane 1*: control receptor reacted with normal rabbit serum (NRS). *Lane 2*: control receptor reacted with anti-receptor antibody (ARA). *Lane 3*: dexamethasone-treated receptor reacted with ARA. Gels were treated with Enhance (New England Nuclear, Boston, MA) and exposed to Kodak XAR-5 film at −70°C for 7 days. *B* and *C*: mRNA studies. Each *lane* contained 5 μg poly(A)⁺ RNA hybridized to either 10^7 cpm/ml insulin-receptor cDNA (*B*) or 5 × 10^6 cpm/ml β-actin cDNA (*C*). + and − refer to the presence or absence of 100 nM dexamethasone.

(Fig. 1*B*). Multiple insulin-receptor mRNAs were observed (4,5). In IM-9 cells, dexamethasone increased all insulin receptor mRNAs by 5- to 10-fold. In contrast to this effect on insulin-receptor mRNA, there was no change in the levels of β-actin mRNA (Fig. 1*C*). Moreover, both the time course and the dose dependence of the dexamethasone effect on insulin-receptor mRNA levels were similar to the effects of dexamethasone on the synthesis of the insulin-receptor precursor (Figs. 2 and 3).

To determine whether this effect of dexamethasone on insulin-receptor mRNA levels occurred in other tissues, we studied AR42J rat pancreatic acinar cells, a cell line with characterized insulin receptors (7). As in IM-9 cells, dexamethasone induced a time-dependent two- to threefold increase in ^{125}I-insulin binding. In these cells, dexamethasone increased insulin-receptor biosynthesis (data not shown). Moreover, as in IM-9 cells, dexamethasone increased insulin-receptor mRNA levels (Fig. 4).

In addition to our in vitro studies with lymphocytes and

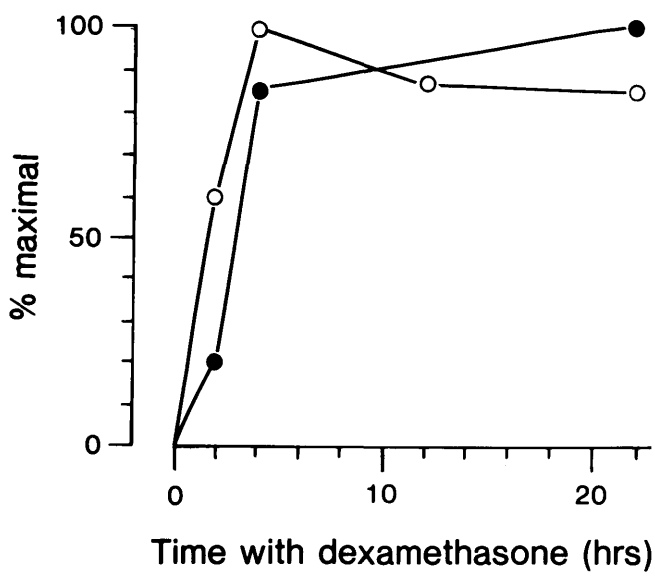

FIG. 2. Time dependence of dexamethasone-induced alterations in receptor mRNA levels (●) and precursor protein synthesis (○). IM-9 cells were incubated in presence of 100 nM dexamethasone for up to 20 h. At times indicated, cells were removed, receptors immunoprecipitated, mRNA Northern transfers performed, and fluorographs quantified. Results are percent of maximum stimulation and represent mean of 3 experiments.

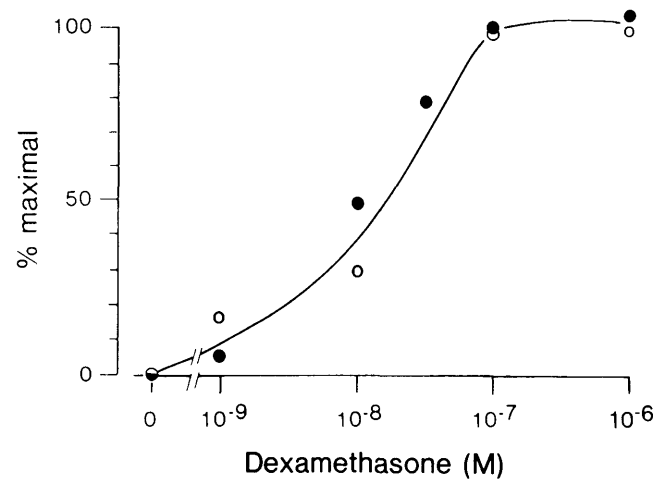

FIG. 3. Dose dependence of dexamethasone-induced alterations in insulin-receptor mRNA levels (●) and precursor protein synthesis (○). IM-9 cells were incubated for 4 h with various concentrations of dexamethasone. Insulin-receptor precursor and RNA levels were determined as in Fig. 2. Results are expressed as percentage of maximal stimulation and are mean of 3 experiments.

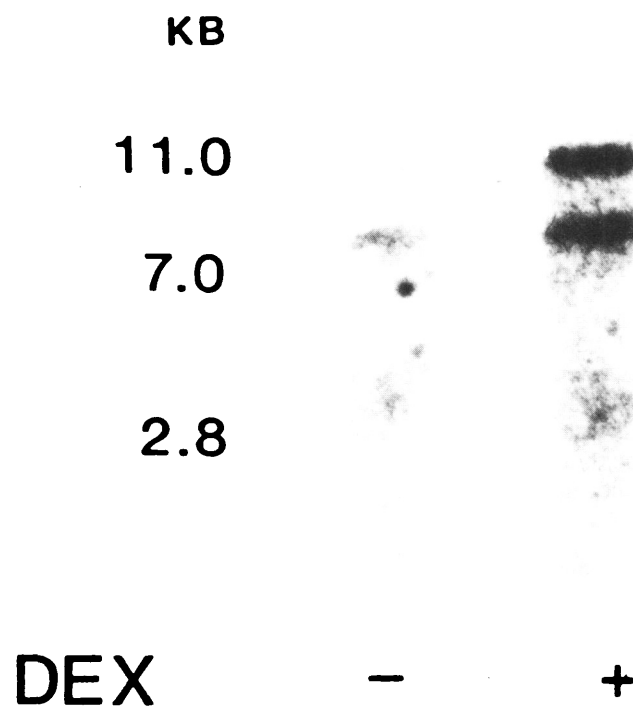

FIG. 4. Influence of dexamethasone on insulin-receptor RNA levels in AR42J cells. Northern transfers containing 20 μg poly(A)⁺ RNA from either control cells or cells treated with 100 nM dexamethasone for 12 h.

pancreatic acinar cells, others have reported that glucocorticoids increase insulin-receptor binding and biosynthesis in cultured rat liver cells (14). In vitro studies with adipose cells and adipose cell precursors have been conflicting (15,16), but an increase in receptor biosynthesis has not been reported.

Studies of glucocorticoids in vivo have yielded variable results. We previously reported that glucocorticoids in vivo decrease the binding of insulin to its receptor in liver and other tissues (17). However, it has also been reported that glucocorticoids in vivo increase insulin-receptor levels (18). The interpretation of these in vivo studies is complicated by two factors. First, glucocorticoids in vivo raise plasma insulin levels, which consequently downregulate insulin receptors (1,2). Second, most studies have employed pharmacological amounts of glucocorticoids that may induce a catabolic state, thus obscuring effects of glucocorticoids on insulin-receptor biosynthesis. Our study with two diverse cell types indicates that glucocorticoids act directly and rapidly on insulin-receptor gene expression. These studies suggest, therefore, that increased synthesis of insulin receptors is a primary effect of glucocorticoids.

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REFERENCES

1. Goldfine ID: Effects of insulin on intracellular functions. In *Biochemical Actions of Hormones*. Litwack G, Ed. New York, Academic, 1981, p. 272–305
2. Kahn CR: The molecular mechanism of insulin action. *Annu Rev Med* 36:429–51, 1985
3. Rouiller DG, McElduff A, Hedo JA, Gorden P: Induction of the insulin proreceptor by hydrocortisone in cultured lymphocytes (IM-9 line). *J Clin Invest* 76:645–49, 1985
4. Ebina Y, Ellis L, Jarnaglin K, Edery M, Graf L, Clauser E, Ou J-H, Masiarz F, Kan, YW, Goldfine ID, Roth RA, Rutter WJ: The human insulin receptor cDNA: the structural basis for hormone-activated transmembrane signalling. *Cell* 40:747–58, 1985
5. Ullrich A, Bell JR, Chen EY, Herrera R, Petruzzelli LM, Dull TJ, Gray A, Coussens L, Liao Y-C, Tsubokawa M, Masson A, Seeburg PH, Grunfeld C, Rosen OM, Ramachandran J: Human insulin receptor and its relationship to the tyrosine kinase family of oncogenes. *Nature* (Lond) 313:756–61, 1985
6. Forsayeth J, Maddux B, Goldfine ID: Biosynthesis and processing of the human insulin receptor. *Diabetes* 35:837–46, 1986
7. Mössner J, Logsdon CD, Williams JA, Goldfine ID: Insulin, via its own receptor, regulates growth and amylase synthesis in pancreatic acinar AR42J cells. *Diabetes* 34:891–97, 1985
8. Lizardi PM: Methods for the preparation of messenger RNA. *Methods Enzymol* 96:24–38, 1983
9. Chirgwin JM, Przybyla AE, McDonald RJ, Rutter WJ: Isolation of biologically active ribonucleic acid from sources enriched in ribonuclease. *Biochemistry* 24:5294–99, 1979
10. Aviv H, Leder P: Purification of biologically active globin messenger RNA by chromatography on oligothymidylic acid-cellulose. *Proc Natl Acad Sci USA* 69:1408–12, 1972
11. Maniatis, T, Fritsch EF, Sambrook J: *Molecular Cloning, A Laboratory Manual*. Cold Spring Harbor, NY, Cold Spring Harbor Lab. 1982, p. 545
12. Shaw DJ, Bell GI: RsaI polymorphism at the insulin receptor locus (INSR) on chromosome 19. *Nucleic Acids Res* 13:8661, 1985
13. Cleveland DW, Lopata MA, MacDonald RA, Cowan NJ, Rutter WJ, Kirschner MW: Number and evolutionary conservation of alpha- and beta-tubulin and cytoplasmic beta- and gamma-actin genes using specific cloned cDNA probes. *Cell* 20:95–105, 1980
14. Salhanick AI, Krupp MN, Amatruda JM: Dexamethasone stimulates insulin receptor synthesis in cultured rat hepatocytes. *J Biol Chem* 258:14130–35, 1983
15. Knutson VP, Ronnet GV, Lane MD: Control of insulin receptor level in 3T3 cells: effect of insulin-induced down-regulation and dexamethasone-induced up-regulation on rate of receptor inactivation. *Proc Natl Acad Sci USA* 79:2822–26, 1982
16. Grunfeld C, Baird K, Van Obberghen E, Kahn CR: Glucocorticoid-induced insulin resistance in vitro: evidence for both receptor and postreceptor defects. *Endocrinology* 109:1723–30, 1981
17. Kahn CR, Goldfine ID, Neville D, De Meyts P: Alterations in insulin binding induced by changes in vivo in the levels of glucocorticoids and growth hormone. *Endocrinology* 103:1054–66, 1978
18. Beck-Nielsen H, De Pirro R, Pedersen O: Prednisone increases the number of insulin receptors on monocytes from normal subjects. *J Clin Endocrinol Metab* 50:1–4, 1980