Dual Regulation of Glycogen Metabolism by Insulin and Insulin-like Growth Factors in Human Hepatoma Cells (HEP-G2)

Analysis with an Anti-receptor Monoclonal Antibody

Eugen J. Verspohl, Richard A. Roth, Riccardo Vigneri, and Ira D. Goldfine

Cell Biology Laboratory and Department of Medicine, Mount Zion Hospital and Medical Center, San Francisco, California 94120; Departments of Medicine and Physiology, University of California, San Francisco 94143

Abstract. Insulin and the insulinlike growth factors (IGF-I and IGF-II) are members of a family of hormones that regulate the metabolism and growth of many tissues. Cultured HEP-G2 cells (a minimal deviation human hepatoma) have insulin receptors and respond to insulin by increasing their glycogen metabolism. In the present study with HEP-G2 cells, we used ^{125}I-labeled insulin, IGF-I, and IGF-II to identify distinct receptors for each hormone by competition-inhibition studies. Unlabeled insulin was able to inhibit ^{125}I-IGF-I binding but not ^{125}I-IGF-II binding. A mouse monoclonal antibody to the human insulin receptor that inhibits insulin binding and blocks insulin action inhibited 75% of ^{125}I-insulin binding, but inhibited neither ^{125}I-IGF-I nor ^{125}I-IGF-II binding. When glycogen metabolism was studied, insulin stimulated [^{3}H]glucose incorporation into glycogen in a biphasic manner; one phase that was 20–30% of the maximal response occurred over 1–100 pM, and the other phase occurred over 100 pM–100 nM. The anti-receptor monoclonal antibody inhibited the first phase of insulin stimulation but not the second. Both IGF-I and IGF-II stimulated [^{3}H]glucose incorporation over the range of 10 pM–10 nM; IGF-I was three to fivefold more potent. The monoclonal antibody, however, was without effect on IGF regulation of glycogen metabolism. Therefore, these studies indicate that insulin as well as the IGFs at physiological concentrations regulate glycogen metabolism in HEP-G2 cells. Moreover, this regulation of glycogen metabolism is mediated by both the insulin receptor and the IGF receptors.

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Introduction

Insulin and insulinlike growth factors belong to a family of polypeptide hormones that exhibit a similar spectrum of biological activities and have a high degree of sequence homology (1–5). However, insulin and the insulinlike growth factors are distinct immunologically, and their relative potencies in eliciting various cellular effects differ (6, 7). Typically, insulin has more potent short-term metabolic effects, whereas the insulinlike growth factors (IGFs)[1] have potent long-term growth effects (3, 6, 8). The insulinlike growth factors include IGF-I (also called somatomedin C) and IGF-II (which is similar to multiplication stimulation activity). These factors are two separate hormones and IGF-I is under the control of growth hormone (6, 9, 10).

In addition to having sequence homology, these hormones can interact to some extent with each other's receptors. Both the insulin and IGF-I receptors are oligomeric tetramers with two alpha and two beta subunits linked by sulphydryl bonds (11–14). In contrast, the IGF-II receptor has a relative molecular weight of ~240,000 and has no discernible subunit structure (11, 13). While the subunits of the insulin and IGF-I receptors are similar, they are reported to have slightly different molecular weights (15) and to differ antigenically (16–19). Insulin and IGF-I can react with each other's receptors, but with relative differences in affinity ranging from 100- to 1,000-fold (4, 13). Also, IGF-II can react with both the IGF-I receptor and the

1. *Abbreviations used in this paper:* GH, growth hormone; PRL, prolactin; IGF, insulinlike growth factor.

insulin receptor (4, 13). However, IGF-I, but not insulin, can react readily with the IGF-II receptor.

Two approaches have been used to distinguish whether an effect of insulin on target cells is occurring either via its own receptor or via the IGF-I receptor. One approach is to use purified hormones and analyze the sensitivity of the dose-response curves. In most instances, insulin acts on its own receptor at picomolar or nanomolar concentrations, whereas it acts on the IGF-I receptor at micromolar concentrations (18, 20). The other approach is to use antibodies specific to the insulin receptor that either mimic or inhibit the action of insulin but not IGF-I (18, 19). In one report, an Fab fragment of a polyclonal antibody was used to distinguish between insulin and IGF effects on human fibroblasts (18).

Recently, we reported the production of a species-specific mouse monoclonal anti-receptor antibody that is directed towards the human insulin receptor (19, 21). This antibody reacts with the insulin binding site and competitively blocks insulin binding; moreover, this antibody antagonizes the biological actions of insulin (21). Since this antibody reacts only weakly with IGF-I receptors, and does not react with the IGF-II receptor (19), it can be used to probe whether an effect of insulin occurs via its own receptor or via an IGF receptor.

A major function of insulin is to regulate the hepatic metabolism of glycogen, but the relative roles of insulin and insulinlike growth factor on this function are unknown. Recently, it has been reported that cultured HEP-G2 hepatoma cells, obtained from a minimal deviation human hepatoma (22), have insulin receptors (23, 24), and in these cells insulin activates glycogen synthesis (23). Accordingly, we have used the monoclonal antibody to the insulin receptor to investigate the regulation of glycogen metabolism by insulin and the insulinlike growth factors in HEP-G2 cells. We report herein that there is evidence for dual regulation of glycogen metabolism in these cells by both insulin, via the insulin receptor, and IGF, via the IGF receptor.

Materials and Methods

Materials. The following were purchased: bovine serum albumin (fraction V) from Reheis Chemical Co. (Chicago, IL); rabbit liver glycogen type III, from Sigma Chemical Co. (St. Louis, MO); crystalline porcine insulin (27.3 U/mg) from Elanco Products Co. (Indianapolis, IN); D-[1-^{3}H]glucose (15 Ci/mmol) from New England Nuclear (Boston, MA); Dulbecco's Modified Eagle's Medium (DME) with minimal Eagle's medium amino acid supplement, penicillin, streptomycin, amphotericin B, and fetal calf serum from the Cell Culture Facility, University of California, San Francisco; tissue culture flasks from Falcon Plastics (Los Angeles, CA); 35-mm plastic tissue culture dishes from Corning Glass Works (Corning, NY); 24-sample multiwell plates from Costar (Cambridge, MA); Liquiscint from National Diagnostics (Somerville, NJ); and protein assay reagent from BioRad Laboratories (Richmond, CA).

The following were gifts: porcine proinsulin from Dr. R. Chance, Eli Lilly Research Laboratories (Indianapolis, IN); a semipurified preparation of mixed IGF (12% pure and containing equal amounts of both IGF-I and IGF-II),[2] and pure IGF-I and IGF-II from R. Humbel (Zürich, Switzerland); synthetic cholecystokinin octapeptide from the Squibb Institute for Medical Research (Princeton, NJ); and beef growth hormone (GH) and prolactin (PRL) from the Hormone Distribution Program, National Institutes of Health (Bethesda, MD).

Mouse monoclonal antibody to the human insulin receptor was prepared as previously described (21). All other chemicals and reagents were of analytical grade.

Hepatoma cell cultures. HEP-G2 cells were obtained from Dr. B. Knowles, Wistar Institute, Philadelphia (22). For binding studies, cells (2×10^5/ml) were plated on 35-mm plastic tissue culture dishes in DME containing 10% (vol/vol) fetal calf serum, glutamine (2 mM), penicillin (100 U/ml), streptomycin (50 μg/ml), and amphotericin B (2.5 μg/ml) in a humidified incubator at 37°C under an atmosphere of 5% CO_2/95% air. Under these conditions, HEP-G2 cells had a doubling time of ~27 h. 3 d after plating, when these cells were still in log phase, the incubation medium was replaced by DME with Earle's salts supplemented with 20 mM Hepes, pH 7.4, to stabilize the pH during various additions. ^{125}I-Labeled hormone binding was studied immediately thereafter. For measuring the biological effect of insulin, cells (1×10^5/ml) were placed into multiwell plates with the same media with 1 mg/ml of glucose and were treated similarly.

^{125}I-labeled hormone binding. ^{125}I-Insulin, ^{125}I-IGF-I, and ^{125}I-IGF-II were prepared by a modified chloramine-T method (25) to specific activities of 140, 80, and 80 μCi/μg, respectively. To measure total hormone binding, HEP-G2 cells (180–210 μg protein per dish) were incubated at 37°C in a 2-ml vol with ^{125}I-insulin (50 or 80 pM), ^{125}I-IGF-I (80 pM), or ^{125}I-IGF-II (80 pM) and various concentrations of unlabeled ligand as indicated. At specified times, the incubation medium was removed, and the cell layer was washed twice at 4°C with 154 mM sodium chloride with 10 mM Tris (hydroxymethyl)aminomethane, pH 7.8 (Tris-saline). The cells were then scraped off each dish and collected with 0.9 ml of the aforementioned Tris-saline into 12 × 75-mm glass test tubes, followed by the addition of 0.1 ml 1 N NaOH. The radioactivity associated with the cells was measured in a γ-scintillation counter. An aliquot of the incubation medium was also counted to determine the total radioactivity in the medium. After counting, the protein content of the solubilized cells was measured (26). Nonspecific binding was determined by incubating cells with the same concentrations of labeled hormone plus 10 μM unlabeled insulin (for ^{125}I-insulin), either 100 μM unlabeled insulin or 100 nM unlabeled mixed IGF (for ^{125}I-IGF-I), and 100 nM unlabeled mixed IGF (for ^{125}I-IGF-II), respectively.

^{125}I-Hormone degradation. ^{125}I-Insulin and ^{125}I-IGF-I and ^{125}I-IGF-II degradation in the incubation media was determined by precipitability in 10% trichloroacetic acid (TCA). In addition, ^{125}I-insulin degradation was also measured by the ability of medium ^{125}I-insulin to bind to human IM-9 lymphocytes (27).

Incorporation of [^{3}H]glucose into glycogen. Assay of insulin-stimulated incorporation of [^{3}H]glucose into glycogen was a modification of that described by Hofmann et al. (28). Monolayer HEP-G2 cell cultures were incubated with 4 μCi of D[1-^{3}H]glucose) for 2 h. After incubation, cells were rinsed twice with Tris-saline at 4°C. 1 ml of 30% KOH with 2 mg/ml carrier glycogen was then added followed by a 30-min incubation at 37°C. The solubilized mixture plus another 1

2. The subsequent data for mixed IGF concentrations were corrected for the degree of purity and the molarity of the material was calculated using a molecular weight of 7,500 for the IGFs.

ml KOH-rinse were transferred to glass tubes, and the mixture was boiled for 30 min or until clear. After cooling to 4°C, glycogen was precipitated from 1 ml of the suspension by adding 2.3 ml of 95% ethanol. The precipitate was pelleted at 2,000 *g*, washed with 70% ethanol, dissolved in water, and counted for radioactivity in Liquiscint in a liquid scintillation counter. Blanks were obtained by immediately processing incubated cells 6 s after addition of labeled glucose. Protein content was measured by the method of Lowry et al. (29).

Statistics. The *t* test for paired observations was used for the evaluation of statistical differences.

Results

^{125}I-Insulin binding studies. Total binding of 80 pM ^{125}I-insulin to HEP-G2 cells at 37°C was one-half maximal within 10 min of incubation, reached a maximal value of 3.2% per mg protein at 60 min of incubation, and declined thereafter (Fig. 1, lower). Nonspecific binding (in the presence of 10 μM unlabeled insulin) was only 10–15% of total binding.

Degraded ^{125}I-insulin slowly appeared in the incubation medium (Fig. 1, upper). After 120 min of incubation, 7% of the ^{125}I-insulin was degraded as measured by the TCA method, and 23% was degraded by the IM-9 receptor binding method.

The specificity of insulin binding to HEP-G2 cells was assessed by competitive binding experiments using unlabeled native insulin, proinsulin, and unrelated hormones (Fig. 2). Native insulin one-half maximally inhibited ^{125}I-insulin binding at 0.26±0.09 nM (mean±SEM, n = 5). Proinsulin was 1% as potent. In contrast, cholecystokinin and growth hormone had negligible effects. Of interest was the observation that prolactin at 1 μM inhibited binding by 20–30%.

Effect of insulin on [^{3}H]glucose incorporation into glycogen. After a 30-min lag, [^{3}H]glucose incorporation into glycogen was linear from 30 to 180 min when 1 μM insulin was added to HEP-G2 cells (data not shown). At 120 min of incubation, the time at which the subsequent studies were performed, basal [^{3}H]glucose incorporation was 20.7±9.2 fmol/mg protein (which was normalized to 100%). A maximal stimulating insulin concentration (1 μM) increased uptake over basal levels by 99.8±4.8% (mean±SEM, n = 4) (Fig. 3). When the insulin dose-response curves were evaluated, the effect of the hormone was biphasic (Fig. 3). The first phase of stimulation occurred over the range of 1–100 pM (one-half maximal concentration 4–9 pM), and the second phase occurred over the range of 100 pM–100 nM (one-half maximal concentration 0.7 to 1 nM). The effect of insulin at low concentrations (first phase) accounted for 20–30% of the maximal insulin effect. Proinsulin induced a parallel stimulation of glucose incorporation but was only 1% as potent as insulin. Growth hormone and cholecystokinin were without effect. Prolactin in concert with its small effect on insulin binding had a small stimulating effect on glycogen metabolism.

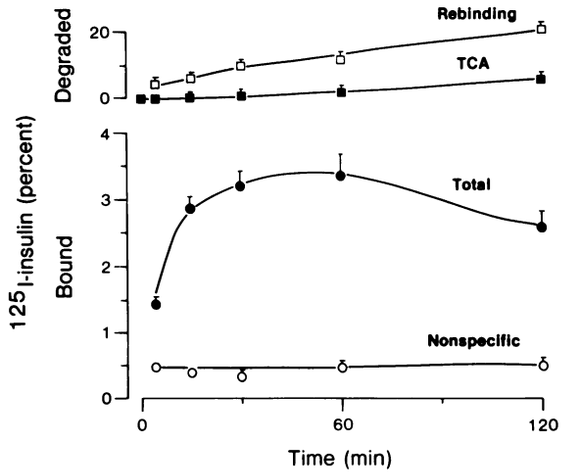

Figure 1. Time course of total ^{125}I-insulin binding to and degradation by HEP-G2 cells. ^{125}I-insulin (80 pM) was incubated with HEP-G2 cells in the absence (total binding) and presence of 10 μM unlabeled insulin (nonspecific binding). Degraded ^{125}I-insulin in the incubation media (*upper*) was measured in samples used for total binding by using both the TCA precipitation method and the receptor binding method with IM-9 lymphocytes. Each value is the mean±SEM of four individual experiments.

Influence of monoclonal anti-insulin receptor antibody. Anti-receptor antibody competitively inhibited the binding of ^{125}I-insulin to HEP-G2 cells. A detectable effect was seen at 100 pM and a maximal effect was seen at 100 nM (Fig. 4 *A*). At this concentration, however, only 75% of the specific insulin

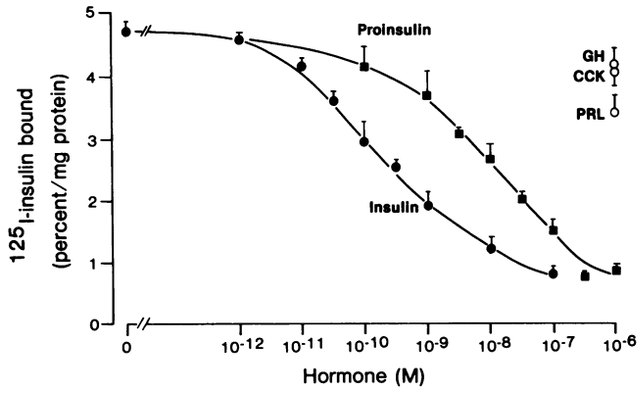

Figure 2. Effect of unlabeled insulin and other hormones on total ^{125}I-insulin binding to HEP-G2 cells. ^{125}I-insulin (50 pM) was incubated for 45 min with HEP-G2 cells plus increasing concentrations of unlabeled insulin and proinsulin, and 1 μM GH, cholecystokinin octapeptide (CCK_8) and PRL. Values are the mean±SEM of five individual experiments.

3. In this paper the accumulation of ^{125}I-hormone by HEP-G2 cells is referred to as binding. This process includes both the initial interaction of hormone with the cell surface as well as the internalization of the hormone and subsequent processing.

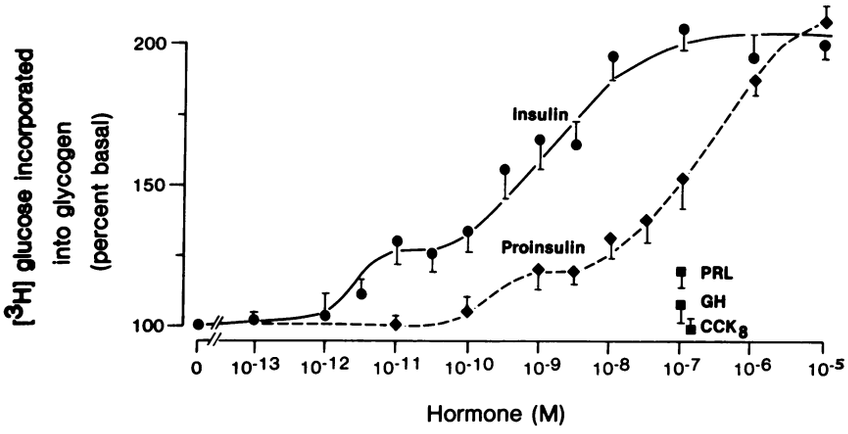

Figure 3. Effect of insulin and other hormones on [^{3}H]glucose incorporation into glycogen of HEP-G2 cells. Cells were incubated in the presence of various concentrations of hormone and 4 μCi/well of [^{3}H]glucose for 120 min. Data are normalized to percent basal and are the mean±SEM of four individual experiments.

binding was inhibited. Normal mouse IgG had little or no effect.

To determine whether the residual ^{125}I-insulin binding that was not inhibited by anti-receptor antibody was via either the insulin receptor or another receptor, HEP-G2 cells were first preincubated with 100 nM anti-receptor antibody. Next the cells were incubated with ^{125}I-insulin, and either unlabeled insulin or IGF (Fig. 4 *B*). IGF was found to be more potent than insulin itself in inhibiting ^{125}I-insulin binding in the presence of anti-receptor antibody.

When 100 nM anti-receptor antibody was added with insulin, the antibody completely inhibited the first phase of insulin stimulation of [^{3}H]glucose incorporation into glycogen (Fig. 5). In contrast, the antibody was without effect on the second phase of stimulated glucose incorporation. Normal mouse IgG was without effect on either phase.

To study the effects of the antibody further, increasing concentrations of antibody were added to two concentrations of insulin: 100 pM (sufficient to maximally stimulate the first phase of[^{3}H]glucose incorporation) and 100 nM (sufficient to stimulate the second phase) (Fig. 6). High concentrations of the antibody (100 nM) inhibited the stimulation induced by 100 pM insulin but were ineffective on the effect of 100 nM insulin. Normal mouse IgG was without effect on either concentration of insulin.

IGF binding studies. The prior studies of insulin binding and action suggested that insulin may have been interacting in part with a receptor other than the insulin receptor. Accordingly, the binding of 80 pM ^{125}I-IGF-I and ^{125}I-IGF-II to HEP-G2 cells was then studied. Total binding of ^{125}I-IGF-I was one-half maximal within 10 min of incubation, reached a maximal value of 11% per mg protein at 60 min of incubation, and then a slight decrease in binding was observed (Fig. 7). ^{125}I-IGF-II bound with a similar time course but to a lesser degree (maximal value of 7% per mg protein) than ^{125}I-IGF-I. Nonspecific binding (in the presence of 100 nM partially purified IGF for labeled IGF-II, and 100 μM insulin for labeled IGF-I) was <17% (IGF-I) and 26% (IGF-II) of total hormone binding, respectively. Degraded ^{125}I-IGF-I and ^{125}I-IGF-II slowly appeared in the incubation medium (Fig. 7). After 120 min of incubation, only 5 and 4% of the IGF-I and IGF-II, respectively, was degraded by the TCA method.

The abilities of IGF, insulin, anti-receptor antibody, and normal mouse IgG to compete with ^{125}I-IGF-I binding were studied (Fig. 8). Mixed IGF one-half maximally inhibited binding at 3.1±0.6 nM. Insulin also inhibited binding, but was 7% as active as IGF. Proinsulin was considerably less potent than insulin (data not shown). Anti-receptor antibody had weak effects and inhibited only at the highest dose tested (100 nM); however, at this concentration, normal IgG also inhibited ^{125}I-IGF-I binding.

IGF also inhibited ^{125}I-IGF-II binding (Fig. 8). In contrast to studies with ^{125}I-IGF-I, neither insulin, anti-receptor antibody, nor normal IgG had an inhibitory effect on ^{125}I-IGF-II binding.

Effects of IGF on [^{3}H]glucose incorporation into glycogen. Mixed IGF stimulated [^{3}H]glucose incorporation into glycogen (Fig. 9); a one-half maximal effect was seen at 0.3 nM. The effect of this IGF preparation, however, was not inhibited by preincubation with 100 nM anti-receptor antibody. Highly purified IGF-I and IGF-II were then studied and both hormones alone also stimulated [^{3}H]glucose incorporation into glycogen (Fig. 9, inset); IGF-I was slightly more potent than IGF-II. When 10 nM of mixed IGF was added with 10 μM insulin, the resultant stimulation of [^{3}H]glucose incorporation was no different than when IGF was added alone (data not shown).

Discussion

Cell lines in tissue culture have been a valuable tool to study hormone action in liver-derived tissue. Rat hepatoma cells, previously used to investigate the mechanism of action of the glucocorticoids (30–32), have also proved important in the study of insulin action. These cells have characteristics that are stable through many passages, and have been used to study enzyme regulation (28, 33–35), insulin receptor phosphorylation and hormone-sensitive tyrosine kinase activity (36), and second

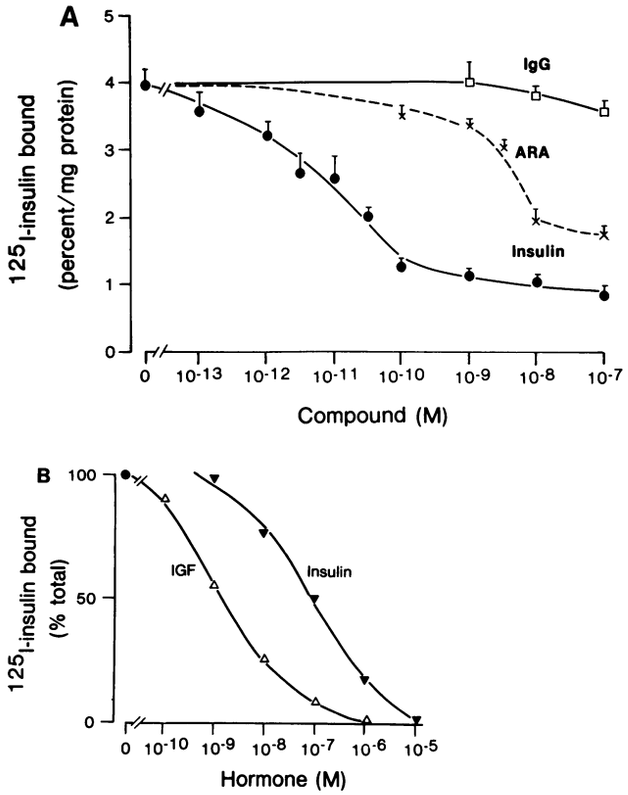

Figure 4. (*A*) Effect of unlabeled insulin, monoclonal antibody, and IgG on total ^{125}I-insulin binding to HEP-G2 cells. HEP-G2 cells were preincubated with various concentrations of insulin, monoclonal antibody to the human insulin receptor (ARA) and normal mouse immunoglobulin (IgG) for 10 min, followed by the addition of 50 pM ^{125}I-insulin for 45 min. Values are mean±SEM of three individual experiments. (*B*) Effect of unlabeled insulin and IGF on residual ^{125}I-insulin binding to HEP-G2 cells preincubated with a monoclonal antibody to the human insulin receptor. HEP-G2 cells were preincubated with 100 nM monoclonal antibody (ARA) for 10 min, followed by the addition of 50 pM ^{125}I-insulin and increasing concentrations of both unlabeled insulin and mixed IGF. After 45 min, binding was measured. Values are normalized to the maximal ^{125}I-insulin bound (1.89±0.12 percent of total [mean±SD]) in the presence of anti-receptor antibody but in the absence of unlabeled hormones. Each value is the mean of two individual experiments.

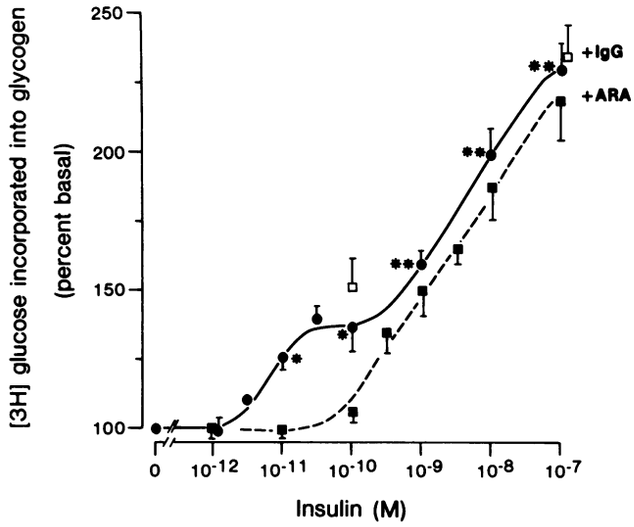

Figure 5. Effect of a monoclonal antibody to the human insulin receptor on insulin-induced incorporation of [^{3}H]glucose into glycogen of HEP-G2 cells. Cells were preincubated for 20 min with either 100 nM monoclonal antibody (ARA) (■), 100 nM normal mouse IgG (□), or without addition (●), followed by the addition of various concentrations of insulin and of 4 μCi/well [^{3}H]glucose for 120 min. Data are normalized to percent basal and given as the mean±SEM of three individual experiments. *$P < 0.01$ and **NS indicate the statistical differences in the presence and absence of ARA.

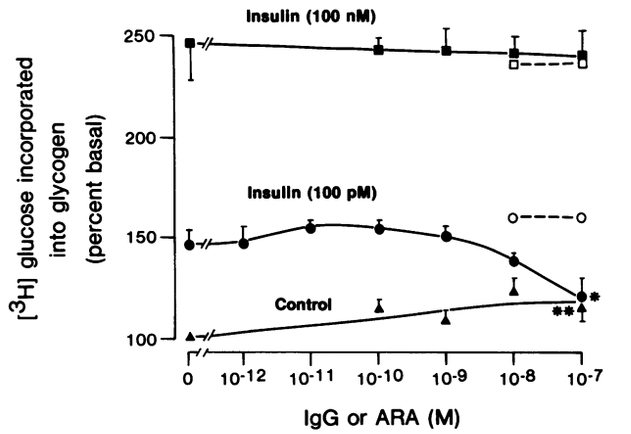

Figure 6. Concentration-dependent effect of monoclonal antibody on incorporation of [^{3}H]glucose into glycogen of HEP-G2 cells. HEP-G2 cells were preincubated for 10 min with various concentrations of monoclonal antibody (ARA) (closed symbols) or normal mouse IgG (open symbols) followed by the addition of 100 nM insulin, 100 pM insulin, or no insulin (control) plus 4 μCi/well [^{3}H]glucose for 120 min. Data are normalized to percent basal and given as the mean±SEM of three individual experiments. Studies of IgG on control cells were no different than those with ARA and, therefore, are not shown. *$P < 0.01$ and **NS indicate the statistical differences in the presence and absence of ARA.

messenger generation (37). Moreover, these cells are very sensitive to insulin and concentrations as low as 1.0 pM have been shown to regulate enzyme activity (28, 33–35). In contrast to these studies with cultured rat hepatoma cells, relatively little is known about insulin receptors and insulin action in cultured human hepatoma cells.

In the present study, we investigated the regulation of glycogen metabolism in human hepatoma cells (HEP-G2) by both insulin and the insulinlike growth factors, IGF-I and IGF-II. In target cells, insulin and the insulinlike growth factors

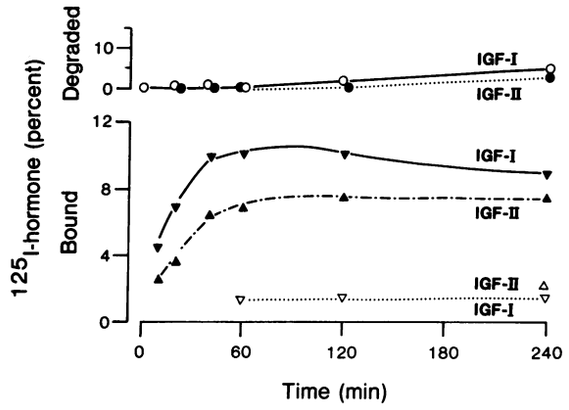

Figure 7. Time course of total ^{125}I-IGF-I and II binding to and degradation by HEP-G2 cells. ^{125}I-IGF-I or II (80 pM) were incubated with HEP-G2 cells in the absence (closed symbols) or presence (open symbols) of 100 μM unlabeled insulin (nonspecific binding for IGF-I) or of 100 nM unlabeled mixed IGF (nonspecific binding for IGF-II). Degraded ^{125}I-hormone in the medium was measured using the TCA precipitation method. Each value is the mean of two individual experiments.

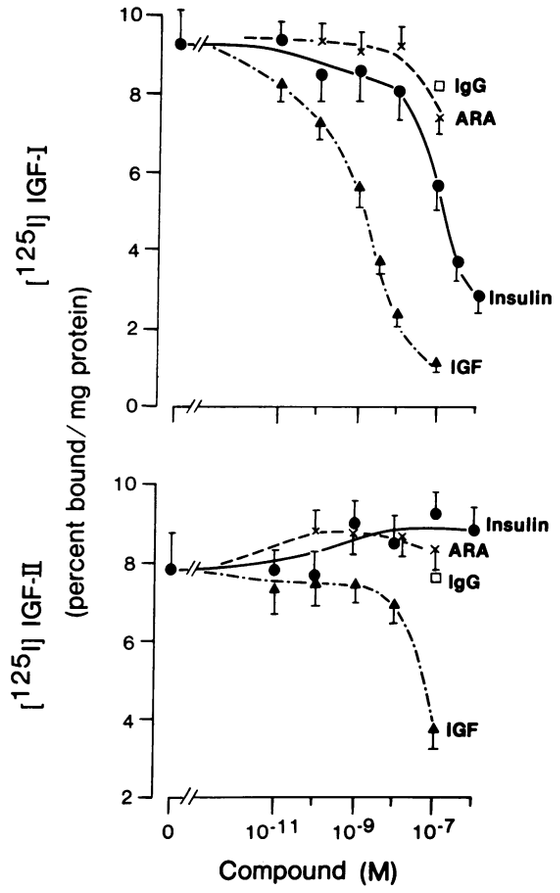

Figure 8. Effect of IGF, insulin, monoclonal antibody, and mouse immunoglobulin on total ^{125}I-IGF-I or II binding to HEP-G2 cells. Cells were preincubated with a monoclonal antibody to human insulin receptor (ARA) and normal mouse immunoglobulin (IgG) for 10 min followed by the addition of insulin and 80 pM of either ^{125}I-IGF-I or II for 90 min. Values are the mean±SEM of five individual experiments.

regulate both rapid metabolic effects and long-term growth promoting effects (3, 6, 38). For many of the metabolic effects, insulin is a more potent agent; whereas for many of the long-term growth effects, the insulinlike growth factors are more potent agents (3, 6, 8).[4] In HEP-G2 cells, we found that the effect of insulin on glucose incorporation into glycogen was biphasic. Insulin, at low concentrations (1–100 pM), appeared to regulate glycogen metabolism through the insulin receptor, and at higher concentrations (100 pM–100 nM) appeared to regulate metabolism through another receptor, presumably a receptor for IGF. The stimulation, by high concentrations of insulin, of biological effects via IGF receptors has previously been reported in human fibroblasts (18), rat sertoli cells (46), and chick myocytes (8).

This biphasic insulin dose-response curve for stimulation of [^{3}H]glucose incorporation into glycogen was then analyzed with a species-specific monoclonal antibody directed at the binding portion of the human insulin receptor. As previously shown in human fibroblasts and adipocytes, this antibody is an antagonist of insulin action (21). This antagonism also occurred in HEP-G2 cells, but only at insulin concentrations <100 pM. At higher insulin concentrations, the antibody was without effect. Therefore, this observation provided further evidence that, at lower concentrations, insulin was acting via the insulin receptor, whereas at higher concentrations insulin was acting via another receptor.

Competitive binding studies of ^{125}I-insulin in HEP-G2 cells with both native insulin and mouse anti-receptor antibody was in concert with these observations. Native insulin competitively inhibited the binding of ^{125}I-insulin to its receptor.[5] In contrast, the antibody was only able to inhibit 75% of ^{125}I-insulin binding. These data also suggested that insulin was acting on two populations of receptors, only one of which was the insulin receptor. Moreover, the observation that IGF was more

4. Insulin in certain instances can increase cell growth (38–40), and IGF in certain instances can enhance metabolic functions (41–45).

5. A nonlinear two-dimensional least squares analysis (four-parameter fit) (47) of the data in Fig. 2 indicated the presence of two orders of binding sites; a high affinity binding site with a dissociation constant K_d of 0.26±0.09 nM and a lower affinity binding site with a K_d of 46±13 nM. However, in view of both insulin degradation (Fig. 1) and insulin processing (unpublished data) by HEP-G2 cells, these values for binding affinities must only be considered as approximations (48).

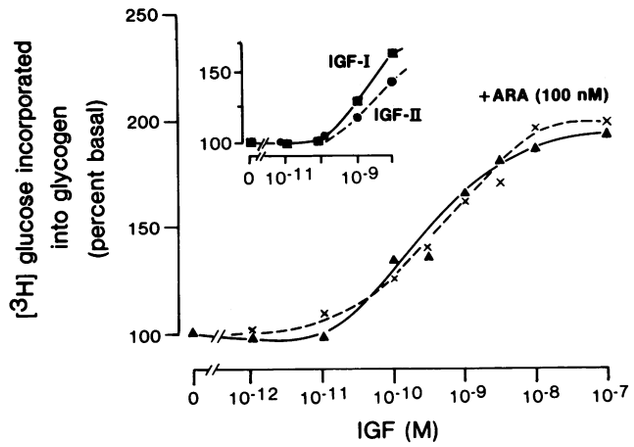

Figure 9. Effect of monoclonal antibody on IGF-induced incorporation of [^{3}H]glucose into glycogen of HEP-G2 cells. Cells were preincubated for 10 min either with (× - - - ×) or without (▲ —— ▲) 100 nM monoclonal antibody to the human insulin receptor (ARA) followed by the addition of increasing concentrations of mixed IGF plus 4 μCi/well of [^{3}H]glucose for 120 min. The inset shows the effect of pure IGF-I and II in the absence of ARA. Data are normalized to percent basal and given as the mean of two experiments.

potent than insulin in competing for the residual ^{125}I-insulin binding (not blocked by anti-receptor antibody) indicated that an IGF receptor was the second binding site for insulin in HEP-G2 cells.

Since insulin can react with the IGF-I receptor in other tissues, we studied the binding and biological effects of the insulinlike growth factors, IGF-I and IGF-II, on HEP-G2 cells. Both hormones specifically bound to these cells. The binding of ^{125}I-IGF-I, but not ^{125}I-IGF-II, was inhibited by high concentrations of insulin. This observation indicated, therefore, that in HEP-G2 cells insulin could interact with the IGF-I receptor. In HEP-G2 cells, the anti-receptor antibody was without effect on influencing the binding of either IGF-I or IGF-II. When studies of[^{3}H]glucose incorporation into glycogen were studied, a mixture of IGF-I and IGF-II, as well as the pure hormones themselves, stimulated this function. Moreover, they did so at concentrations of 1 nM or less. However, unlike the effect of insulin, the effects of the IGFs on glycogen metabolism were not inhibited by anti-receptor antibody. These data indicated, therefore, that the IGFs were acting directly through their own receptors to regulate glycogen metabolism.

The biological significance of the metabolic regulation of liver glycogen metabolism by the insulinlike growth factors remains to be elucidated. In several other diverse cell types the IGFs at nanomolar concentrations or lower mediate the stimulation of glucose and amino acid transport, glycolysis, and glycogen synthesis (41–45). These studies in liver and other tissues suggest, therefore, that in addition to their long-term effects on cell growth, IGFs may have important short-term effects on metabolic functions.

Acknowledgments

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References

1. Rinderknecht, E., and R. E. Humbel. 1978. The amino acid sequence of human insulin-like growth factor I and its structural homology with proinsulin. *J. Biol. Chem.* 253:2769–2776.

2. Rinderknecht, E., and R. E. Humbel. 1978. Primary structure of human insulin-like growth factor II. *FEBS (Fed. Eur. Biochem. Soc.) Lett.* 89:283–286.

3. Zapf, J., E. Schoenle, and E. R. Froesch. 1978. Insulin-like growth factors I and II: some biological actions and receptor binding characteristics of two purified constituents of non-suppressible insulin-like activity of hormone serum. *Eur. J. Biochem.* 87:285–296.

4. Rechler, M. M., J. Zapf, S. P. Nissley, E. R. Froesch, A. C. Moses, J. M. Podskalny, E. E. Schilling, and R. E. Humbel. 1980. Interactions of insulin-like growth factor I and II and multiplication-stimulating activity with receptors and serum carrier proteins. *Endocrinology.* 107:1451–1457.

5. Blundell, T. L., S. Bedarkar, E. Rinderknecht, and R. E. Humbel. 1978. Insulin-like growth factor: a model for tertiary structure accounting for immunoreactivity and receptor binding. *Proc. Natl. Acad. Sci. USA.* 75:180–184.

6. Zapf, J., E. Rinderknecht, R. E. Humbel, and E. R. Froesch. 1978. Nonsuppressible insulin-like activity (NSILA) from human serum. Recent accomplishments and their physiologic implications. *Metab. Clin. Exp.* 27:1803–1827.

7. Van Wyk, J. J., and L. E. Underwood. 1978. The somatomedins and their actions. *In* Biochemical Actions of Hormones. G. Litwack, editor. Academic Press, Inc., New York. 5:101–148.

8. Schmid, C. H., T. H. Steiner, and E. R. Froesch. 1983. Preferential enhancement of myoblast differentiation by insulin-like growth factors (IGF I and IGF II) in primary cultures of chicken embryonic cells. *FEBS (Fed. Eur. Biochem. Soc.) Lett.* 161:117–121.

9. Chochinov, R. H., and W. H. Daughaday. 1976. Current concepts of somatomedin and other biologically related growth factors. *Diabetes.* 25:995–1007.

10. Zapf, J., H. Walter, and E. R. Froesch. 1981. Radioimmunological determination of insulinlike growth factors I and II in normal subjects and in patients with growth disorders and extrapancreatic tumor hypoglycemia. *J. Clin. Invest.* 68:1321–1330.

11. Kasuga, M., E. Van Obberghen, S. P. Nissley, and M. M. Rechler. 1981. Demonstration of two subtypes of insulin-like growth factor receptors by affinity cross-linking. *J. Biol. Chem.* 256:5305–5308.

12. Massagué, J., and M. P. Czech. 1982. Role of disulfides in the subunit structure of the insulin receptor. *J. Biol. Chem.* 257:6729–6738.

13. Massagué, J., and M. P. Czech. 1982. The subunit structures of two distinct receptors for insulin-like growth factors I and II and their relationship to the insulin receptor. *J. Biol. Chem.* 257:5038–5045.

14. Czech, M. P., and J. Massagué. 1982. Subunit structure and dynamics of the insulin receptor. *Fed. Proc.* 41:2719–2723.

15. Stuart, C. A., R. Pietrzyk, A. K. Q. Siu, and R. W. Furlanetto. 1984. Size discrepancy between somatomedin-C and insulin receptors. *J. Clin. Endocrinol. Metab.* 58:1–5.

16. Jonas, H. A., R. C. Baxter, and L. C. Harrison. 1982. Structural differences between insulin and somatomedin-C/insulin-like growth factor-1 receptors revealed by autoantibodies to the insulin receptor. *Biochem. Biophys. Res. Comm.* 109:463–470.

17. Kull, F. C., S. Jacobs, Y. F. Su, M. E. Svoboda, J. J. Van Wyk, and P. Cuatrecasas. 1983. Monoclonal antibodies to receptors for insulin and somatomedin C. *J. Biol. Chem.* 258:6561–6566.

18. King, G. L., C. R. Kahn, M. M. Rechler, and S. P. Nissley. 1980. Direct demonstration of separate receptors for growth and metabolic activities of insulin and multiplication-stimulating activity (an insulin-like growth factor) using antibodies to the insulin receptor. *J. Clin. Invest.* 66:130–140.

19. Roth, R. A., B. Maddux, K. Y. Wong, D. M. Styne, G. Van Vliet, R. E. Humbel, and I. D. Goldfine. 1983. Interactions of a monoclonal antibody to the insulin receptor with receptors for insulin-like growth factors. *Endocrinology.* 112:1865–1867.

20. Rechler, M. M., J. M. Podskalny, and S. P. Nissley. 1977. Characterization of the binding of multiplication stimulating activity to a receptor for growth polypeptides in chick embryo fibroblasts. *J. Biol. Chem.* 252:3898–3910.

21. Roth, R. A., D. J. Cassell, K. Y. Wong, B. A. Maddux, and I. D. Goldfine. 1982. Monoclonal antibodies to the human insulin receptor block insulin binding and inhibit insulin action. *Proc. Natl. Acad. Sci. USA.* 79:7312–7316.

22. Knowles, B. B., C. C. Howe, and D. P. Aden. 1980. Human hepatocellular carcinoma cell lines secrete the major plasma proteins and hepatitis B surface antigen. *Science (Wash. DC).* 209:497–499.

23. Podskalny, J. M., and P. Gorden. 1983. Insulin receptors in a human liver cell line. *Endocrinology.* 112:284. (Abstr.)

24. Ciechanover, A., A. L. Schwartz, and H. F. Lodish. 1983. The asialoglycoprotein receptor internalizes and recycles independently of the transferrin and insulin receptors. *Cell.* 32:267–275.

25. Goldfine, I. D., and G. J. Smith. 1976. Binding of insulin to isolated nuclei. *Proc. Natl. Acad. Sci. USA.* 77:1427–1432.

26. Bradford, M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* 72:248–254.

27. Freychet, P., C. R. Kahn, and J. Roth. 1972. Function interactions with liver plasma membranes: independence of binding of the hormone and its degradation. *J. Biol. Chem.* 247:3953–3961.

28. Hofmann, C., J. W. Marsh, B. Miller, and D. F. Steiner. 1980. Cultured hepatoma cells as a model system for studying insulin processing and biological responsiveness. *Diabetes.* 29:865–874.

29. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* 193:265–275.

30. Rousseau, G. G., J. D. Baxter, S. J. Higgins, and G. M. Tomkins. 1973. Steroid-induced nuclear binding of glucocorticoid receptors in intact hepatoma cells. *J. Mol. Biol.* 79:539–554.

31. Thompson, E. B., D. Avid, and M. E. Lippman. 1977. Variants of HTC cells with low tyrosine aminotransferinase inducibility and apparently normal glucocorticoid receptors. *Endocrinology.* 100:406–419.

32. Thompson, E. B., D. K. Granner, T. Gelehrter, J. Erikson, and G. L. Hager. 1979. Unlinked control of multiple glucocorticoid-induced processes in HTC cells. *Mol. Cell. Endocr.* 15:135–150.

33. Iwamoto, Y., K. Y. Wong, and I. D. Goldfine. 1981. Insulin action in cultured HTC and H35 rat hepatoma cells: receptor binding and hormone sensitivity. *Endocrinology.* 108:44–51.

34. Wicks, W. D., C. A. Barnett, and J. B. McKibbin. 1974. Interaction between hormones and cyclic AMP in regulating specific hepatic enzyme synthesis. *Fed. Proc.* 33:1105–1111.

35. Crettaz, M., and C. R. Kahn. 1983. Analysis of insulin action using differentiated and dedifferentiated hepatoma cells. *Endocrinology.* 113:1201–1209.

36. Kasuga, M., I. A. Karlsson, and C. R. Kahn. 1982. Insulin stimulates the phosphorylation of the 95,000-dalton subunit of its own receptor. *Science (Wash. DC).* 215:185–187.

37. Parker, J. C., F. L. Kiechle, and L. Jarett. Partial purification from hepatoma cells of an intracellular substance which mediates the effect of insulin on pyruvate dehydrogenase and low Km cyclic AMP phosphodiesterase. *Arch. Biochem. Biophys.* 215:339–344.

38. Goldfine, I. D. 1981. Effects of insulin on intracellular functions. *In* Biochemical Actions of Hormones. G. Litwack, editor. Academic Press, Inc., New York. 8:273–305.

39. Massagué, J., L. A. Blinderman, and M. P. Czech. 1982. The high affinity insulin receptor mediates growth stimulation in rat hepatoma cells. *J. Biol. Chem.* 257:13958–13963.

40. Koontz, J. W., and M. Iwahashi. 1980. Insulin as a potent, specific growth factor in a rat hepatoma cell line. *Science (Wash. DC).* 211:947–949.

41. Poggi, C., Y. Le Marchand-Brustel, J. Zapf, E. R. Froesch, and P. Freychet. 1979. Effects and binding of insulin-like growth factor I in the isolated soleus muscle of lean and obese mice: comparison with insulin. *Endocrinology.* 105:723–730.

42. Berhanu, P., and J. M. Olefsky. 1981. Effects of insulin and insulin-like agents on the glucose transport system of cultured human fibroblasts. *Diabetes.* 30:523–529.

43. Williams, J. A., A. Bailey, R. Humbel, and I. D. Goldfine. 1984. Insulinlike growth factors bind to specific receptors in isolated pancreatic acini. *Am. J. Physiol.* 246:G96–G99.

44. Knight, A. B., M. M. Rechler, J. A. Romanus, E. E. Van Obberghen-Schilling, and S. P. Nissley. 1981. Stimulation of glucose incorporation and amino acid transport by insulin and an insulin-like growth factor in fibroblasts with defective insulin receptors cultured from a patient with leprechaunism. *Proc. Natl. Acad. Sci. USA.* 78:2554–2558.

45. Schmid, C. H., T. G. Steiner, and E. R. Froesch. 1983. Insulin-like growth factors stimulate synthesis of nucleic acids and glycogen in cultured calvaria cells. *Calcif. Tissue Int.* 35:578–585.

46. Borland, K., M. Mita, C. L. Oppenheimer, L. A. Blinderman, J. Massagué, P. F. Hall, and M. P. Czech. 1984. The actions of insulin-like growth factors I and II on cultured sertoli cells. *Endocrinology.* 114:240–246.

47. Dixon, W. J., editor. 1974. BMD Biomedical Computer Programs. University of California, Berkeley. 387–396.

48. Beck, J. S., and H. J. Goren. 1983. Simulation of association curves and Scatchard plots of binding reactions where ligand and receptors are degraded or internalized. *J. Recept. Res.* 3:561–577.