Vol. 134, No. 1, 1986 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
January 14, 1986 Pages 427-435

Insulin-like Growth Factors (IGF I and IGF II) Mimic the Effect of Insulin on Plasma Protein Synthesis and Glycogen Deposition in Cultured Hepatocytes

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Received December 6, 1985

Monolayer cultures of chick embryo hepatocytes were used to compare the effects of insulin-like growth factors, IGF I and IGF II, with insulin on two hepatic functions: plasma protein production and glycogen deposition. Just as with exposure of the cells to insulin, addition of either IGF I or IGF II to the otherwise hormone-free medium elicited a dramatic change in the production of secretory proteins as well as the development of glycogen deposits equivalent to in vivo (fed) levels. No major differences between insulin, IGF I or IGF II were observed in terms of the degree of stimulation or potency of the hormones.

The insulin-like growth factors, IGF I and IGF II, are polypeptide hormones that regulate the metabolism and growth of many tissues (1-3). They share a high degree of sequence homology with insulin (4-6), but have their own cell surface receptors (7,8). Differences in the relative potencies of these hormones with respect to a spectrum of common biological activities have been noted in a variety of target tissues (for review, see ref. 3): in general, insulin is more potent in fat and muscle cells, while IGF is more potent in fibroblasts and chondrocytes.

Two recent reports have addressed the ex vivo effects of these agents in model systems for the liver (9,10), the organ responsible for synthesis of the IGFs and their carrier proteins (11,12). Verspohl et al. (9) measured the incorporation of [^{3}H]glucose into glycogen in cultures of HEP-G2 cells, a human hepatocarcinoma cell line, and found that the IGFs, like insulin, stimulated glucose uptake but differed from insulin in potency. In the study by Widmer et al. (10), which employed primary liver cell cultures, the IGFs were found to be as potent as insulin in stimulating growth indices but perhaps as much as 5- to 10-fold weaker in stimulating [^{14}C]glucose incorporation into glycogen.

0006-291X/86 $1.50

In the present study, we have employed primary monolayer cultures of chicken embryo hepatocytes to compare the effects produced by the IGFs and insulin on another differentiated hepatic function: plasma protein production. A comparative evaluation of the glycogenic effects of insulin and the IGFs was also made based upon direct assay of glycogen deposits, as opposed to measurement of labeled glucose incorporation, the parameter most often described by others (9,10).

MATERIALS AND METHODS

IGFs purified from human serum were generously provided by Dr. René Humbel (University of Zürich, Switzerland). Bovine pancreas insulin (crystalline, Sigma; 24.3 units/mg) was used routinely. Stock solutions of peptide hormones were prepared and stored in 0.01 N HCl.

Primary monolayer cultures of liver cells from 16-day old chick embryos were established as described (13,14). In short, suspensions of hepatocytes were prepared by perfusion, mechanical disruption, and treatment of the embryonic liver with purified dissociating enzymes. Hepatocytes were washed, plated, and, unless otherwise specified, maintained in modified Ham's F12 medium which contains 25 mM glucose, but no hormones or serum supplement, so that the cells were cultured from the onset in a completely chemically defined medium, free of added macromolecules. No lactate or pyruvate and no other sugar but glucose is present in modified Ham's F12 medium.

Hepatocyte suspensions were plated in untreated plastic culture dishes (Falcon). Culture medium was replaced with an equal volume of fresh medium every 24 h unless otherwise indicated. Hormones were introduced only after removal of unattached (damaged) cells at the first medium change, thereby avoiding effects on plating efficiency. These non-dividing cultures suffer no loss of viability for several days either in the presence or absence of hormonal medium supplement (13,15,16).

Crossed immunoelectrophoresis was employed for simultaneously visualizing many secreted proteins (15). Electroimmunoassays with monospecific antisera were used to measure secreted plasma proteins individually in 3 µl samples (15,17). For determination of glycogen content, monolayers were treated as described (13). Determination of DNA content was made from cells scraped from the culture dishes, as detailed previously (15).

For electron microscopic analysis, monolayers were fixed briefly in a modified Karnovsky fixative (18) [2% glutaraldehyde-2% paraformaldehyde mixture in 0.1 M sodium cacodylate buffer, pH 7.3]. Cells were then gently scraped from culture dishes, transferred in the fixative to 0.5 ml microfuge tubes and centrifuged for 2 min. Pellets were removed and placed into vials of fresh fixative for a minimum of 2 h at room temperature. They were then washed in buffer for 1 h and post-fixed for 2 h in buffered 1% osmium tetroxide. Following dehydration in a graded series of ethanol solutions, and clearing in propylene oxide the samples were embedded in EPON 812 (19). Thin sections were cut on a Sorvall MT-2B ultramicrotome, contrasted with uranyl and lead stains (20,21) and examined with a Philips 301 electron microscope.

RESULTS

Primary monolayer cultures of chick embryo hepatocytes synthesized and secreted a broad spectrum of plasma proteins for several days when maintained, from the onset of culture, in a chemically defined medium devoid of hormonal and macromolecular supplement (Fig.1, panel A, and ref. 15). A 24-h exposure of the cells to either IGF I or IGF II, as the sole hormone additive in the culture medium, produced dramatic changes in the pattern of secreted proteins (panels B

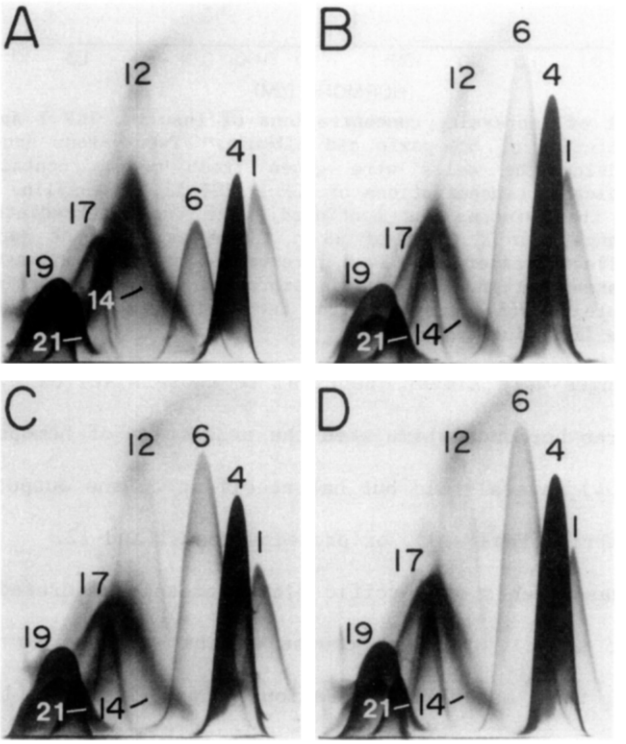

Figure 1. Crossed immunoelectrophoresis of plasma proteins secreted in the presence and absence of IGF I, IGF II, and insulin. The hepatocytes were plated in standard methionine-containing medium. After 23 h, they were given fresh medium with and without hormone plus 10 μCi/ml of [^{35}S]methionine. Spent culture media were collected after 24 h of exposure to the hormones, concentrated 60-fold by ultrafiltration (Amicon, PM30 filter), and analyzed by crossed immunoelectrophoresis. Media samples (8 μl), were applied in the lower left corner of each panel. Electrophoresis in the first dimension was performed from left to right and in the second dimension from bottom to top. The second dimension gels contained polyvalent antisera similar to that described (15) recognizing most plasma proteins. Autoradiograms of the gels are shown. The amount of each protein is reflected by the area and intensity of its respective peak. Peaks were identified immunologically and are numbered according to ref.15: peak 4, albumin; peak 6, hemopexin (α_1-globulin "M"); peak 14, lipoprotein; peak 17, α-antitrypsin; peak 19, transferrin; peak 21, fibrinogen. (A) no hormone; (B) IGF I, 10 nM; (C) insulin, 35 nM; (D) IGF II, 10 nM.

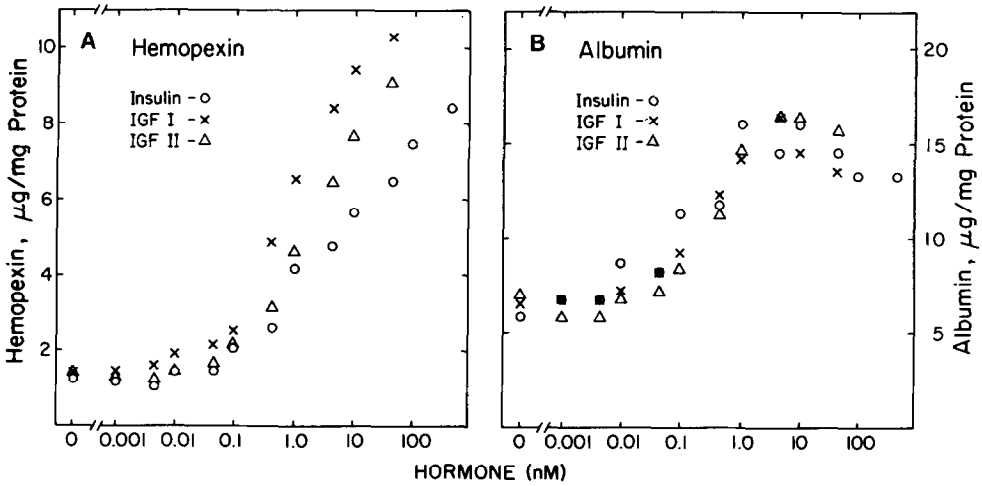

Figure 2. Effect of increasing concentrations of insulin, IGF I and IGF II on secretion of hemopexin and albumin. Twenty-four hours after plating the cells were given fresh medium containing the indicated concentrations of IGF I, IGF II, or insulin. Exposure to the hormones was continued, with one intermediate medium change, for a total of 48 h. Samples of spent media were collected after each 24 h interval of exposure and assayed for plasma protein. Values are expressed in μg of protein/mg cell protein. (A) Hemopexin from second interval; (B) albumin from the first interval.

and D). These changes were almost identical to those elicited by insulin alone (panel C). All three hormones stimulated the production of hemopexin[1] (Peak 6) and albumin (Peak 4) several-fold but had no effect on the output of fibrinogen (Peak 21), transferrin (Peak 19), or proteins Nos. 1 and 12.

Based on measurements of specific plasma proteins secreted over a period of three days (not shown), the time course of the response to IGFs I and II closely paralleled that established previously for insulin (15), including further acceleration of both albumin and hemopexin production upon prolonged exposure to the hormone. Cellular DNA content was not affected by the presence of insulin (35nM) (15,16) or the IGFs (10 nM), and remained constant (18.3 $\pm$ 0.4 μg DNA/dish) throughout this period.

Sensitivity of hemopexin production to varying concentrations of IGFs I and II was evaluated following a 48-h exposure and compared with that for insulin (Fig. 2A). For all three agents, stimulated hemopexin production was first

[1]The protein (peak 6) referred to in earlier studies as α_1-globulin "M", the function of which was previously unknown, has recently been shown to be the heme-binding protein, hemopexin, a developmental protein which is a major acute phase reactant in the chicken (Grieninger, G., Liang, T.J., Beuving, G., Goldfarb, V., Metcalfe, S.A., and Muller-Eberhard, U., submitted).

detected at a hormone concentration of approximately 0.1 nM and reached its maximum, representing an increase of about 10-fold over basal levels,at hormone concentrations in the range of 10 to 350 nM.[2] Albumin production exhibited a similar sensitivity, except that 3.5 nM hormone elicited maximal early response (evaluated after 24 h of exposure) (Fig. 2B) while roughly 10-fold higher levels were required to elicit the later, more pronounced response (after 48 h; data not shown).

Ultrastructural analysis of the cultured hepatocytes revealed that addition of either IGF I (Fig. 3B) or IGF II (not shown) as sole hormone to the medium stimulated production of large, cytoplasmic masses of densely staining α and β glycogen granules identical to those elicited by insulin, which were indistinguishable from those seen in vivo (13); only small, scattered patches of glycogen granules were seen in cells cultured under hormone-free conditions (Fig. 3A).

All three hormones were about equally effective in stimulating glycogenesis (Fig. 4). The lowest concentration of hormone that stimulated glycogen deposition was approximately 0.35 nM, while hormone in the range of 2 to 10 nM elicited a half-maximal response.[2] In another experiment (Table I), glycogen deposits were monitored in 75 rather than 25 mM glucose. It has been shown that higher levels of glucose increase the rate of glycogen synthesis without affecting the sensitivity of the cells to insulin (13). In the case of each hormone, maximally stimulated glycogen levels, assayed on the third day of exposure, were equivalent to those found in the liver of the fed chicken (ca. 200 μg glycogen/mg protein, ref. 13).

Simultaneous addition of insulin and the IGFs to the cells' environment did not elicit any greater response of plasma protein production or glycogen deposition than was achieved by exposure to the agents singly (data not shown).

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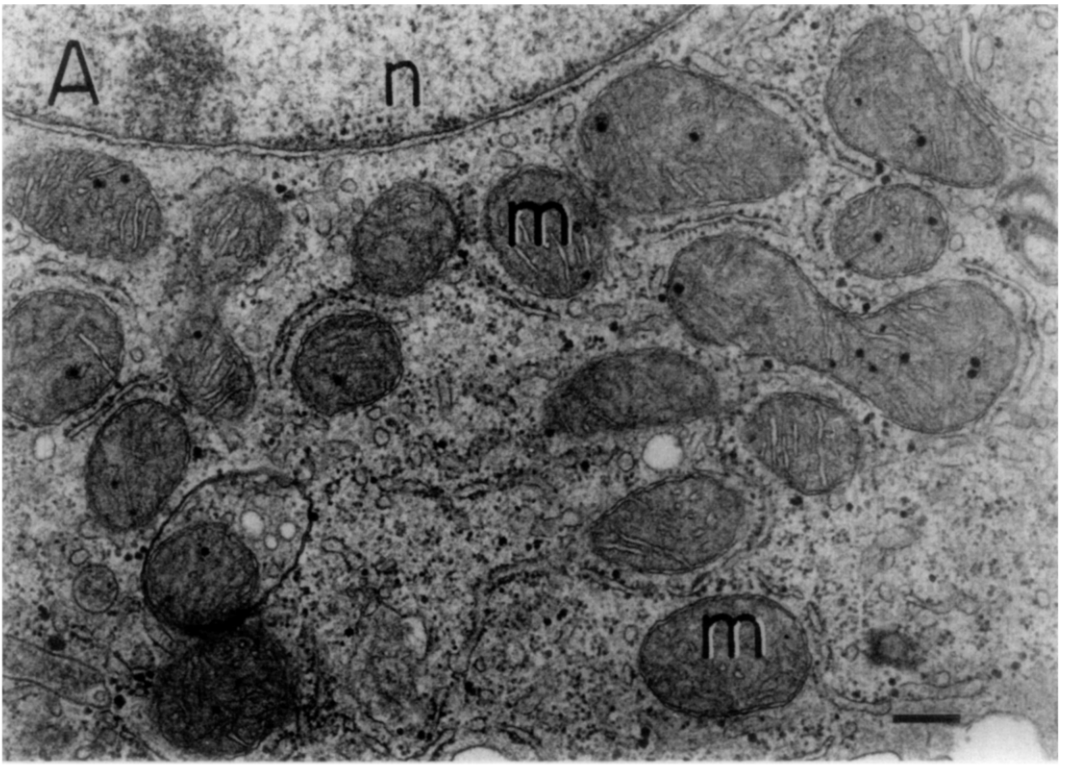
[2]Because of scarcity of material, IGF concentrations higher than 35 nM were tested in other, smaller scale experiments; they were found to have no further stimulatory effect on either plasma protein production or glycogen deposition.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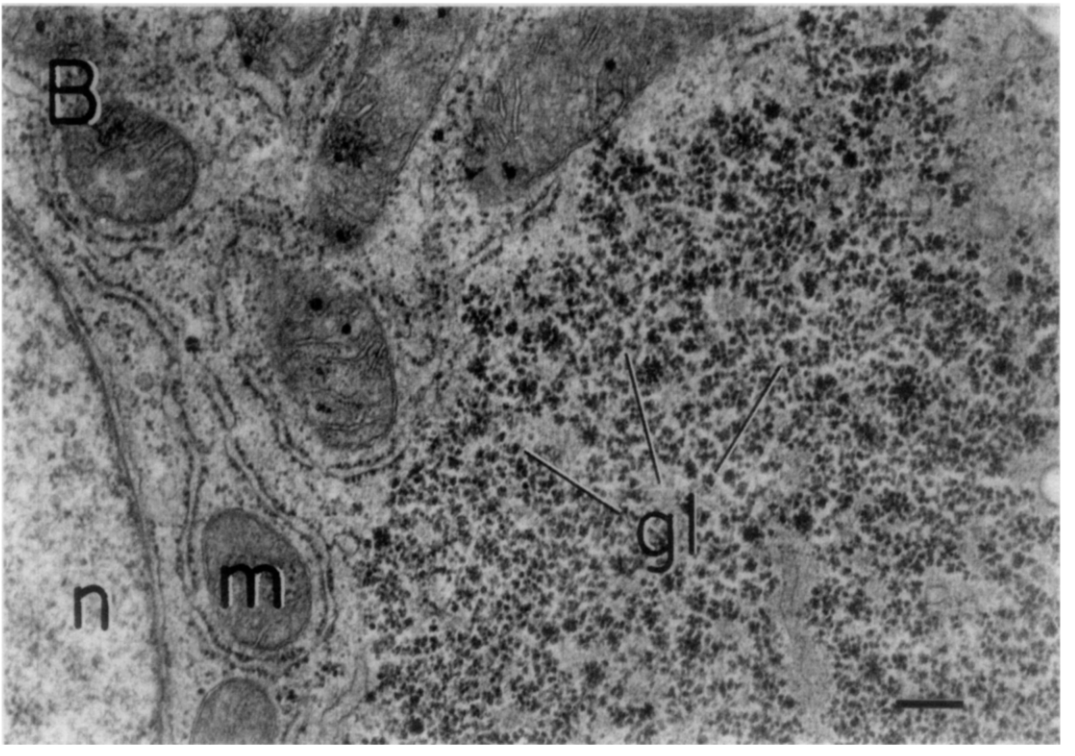
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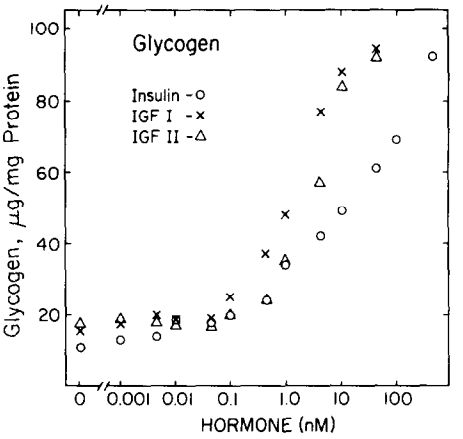

Figure 4. Effect of increasing hormone concentration on glycogen deposition. Twenty-four hours after plating, the cells were exposed to fresh medium (25 mM glucose) containing various concentrations of either IGF I, IGF II, or insulin. Cells were harvested for determination of glycogen content after a total of 48 h of hormone exposure. Values are expressed as μg glycogen/mg cell protein.

DISCUSSION

Evidence is presented that IGF I and IGF II can each independently mimic the direct actions of insulin on specific hepatocellular functions: IGFs I and

Table I

Effect of Prolonged Hormone Exposure on Glycogen Levels

Additions:	μg glycogen/mg protein
None	40 ± 1.4
IGF I	198 ± 6.0
IGF II	212 ± 1.8
Insulin	183 ± 2.4

Three hours after plating in standard hormone-free medium (25 mM glucose), cells were washed once and incubated in test media containing 75 mM glucose, alone or with either IGF I (10 nM), IGF II (10 nM), or insulin (35 nM). At this higher glucose concentration, the concentration of NaCl was adjusted accordingly to keep the osmotic pressure of the medium constant. However, Na^+ remained the predominant cation of the external milieu at least 10-fold in excess of K^+. Cells were given fresh test media every 24 h. After 70 h of hormone exposure, cells were harvested and glycogen levels determined. The data represent the average of duplicate dishes ± range.

Figure 3. Electron microscopic evaluation of glycogen deposition in cultured hepatocytes. Three hours after plating, cells were given fresh medium with or without hormone. They were maintained under these conditions for 70 h, then prepared for evaluation by electron microsopy. gl = glycogen; n = nucleus; m = mitochondria. (A) no hormone; (B) IGF I, 10 nM. Note: The morphology of hepatocytes exposed to either IGF II or insulin was identical to that of cells incubated with IGF I. The bar represents 250 nm in both panels.

II exhibited nearly the same pattern of plasma protein stimulation as insulin and, like insulin, each individually restored glycogen deposits to the maximal in vivo levels seen in the fed chicken.

There were no profound differences between insulin and the IGFs in this system with regard to potency in stimulating either hepatocellular plasma protein production or glycogen deposition. Sensitivity of these parameters to the IGFs was similar to that found for other functions in liver-derived systems (9,10). While dose-response curves for hemopexin production and glycogen deposition might suggest a somewhat lower potency for insulin relative to the IGFs, this trend is not supported by the curves for albumin production. In no case did the potency of insulin exceed that of the IGFs, as reported for effects on hepatocellular [^{14}C]glucose incorporation into glycogen (10). Minor differences aside, it is clear that the widely differing potencies of insulin and the IGFs noted in other cell types do not extend to the hepatocyte.

IGF I, IGF II and insulin were equally effective individually, as when combined, in stimulating hemopexin, albumin, and glycogen synthesis. This suggests that a common mechanism of action is involved.

ACKNOWLEDGEMENTS

We thank Mary Ann Chiasson for skilled technical assistance as well as Kathe M. Hertzberg and Gisela K. Haselbacher for helpful discussions in the preparation of this manuscript. This project was supported by grants HL-09011 and AM-27097 from the National Institutes of Health. J.L.P. was funded by Institutional Grant NRSA5T32 HL-07331 from the National Institutes of Health. The work was carried out during the tenure of an American Heart Association Established Investigatorship to G.G.

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