Comparative studies on the regulation of insulin-like growth factor-binding protein-1 (IGFBP-1) and sex hormone-binding globulin (SHBG) production by insulin and insulin-like growth factors in human hepatoma cells

T. Kalme*, H. Koistinen, M. Loukovaara, R. Koistinen, P. Leinonen

Department of Obstetrics and Gynecology, Helsinki University Central Hospital, Haartmaninkatu 2, FIN-00029 Huch, Finland

Abstract

Production of insulin-like growth factor-binding protein-1 (IGFBP-1) by the liver is efficiently inhibited by insulin both in vivo and in vitro. Consequently, serum IGFBP-1 concentration reflects insulin bioactivity in portal vein. Sex hormone-binding globulin (SHBG) is another insulin-regulated liver-derived protein that has appeared promising in detecting individuals with portal hyperinsulinemia. We compared the regulation of IGFBP-1 and SHBG production by insulin and insulin-like growth factors (IGF-I and IGF-II) in human hepatoma cell cultures. Insulin equipotently inhibited IGFBP-1 and SHBG production, with maximal decrease in culture medium concentrations being about 35% for both proteins during 48 h of culture in serum-free medium. IGF-I and IGF-II also inhibited the IGFBP-1 and SHBG levels. We conclude that IGFBP-1 and SHBG are equally sensitive to ambient insulin concentrations in human hepatoma cell cultures, and the production of both proteins is also attenuated by the IGFs.
© 2003 Elsevier Ltd. All rights reserved.

Keywords: Insulin-like growth factor-binding protein-1; Sex hormone-binding globulin; Insulin; Insulin-like growth factors

1. Introduction

Insulin-like growth factor-binding protein-1 (IGFBP-1), primarily produced by the liver, is one of the six IGF-binding proteins that regulate IGF actions in various tissues [1,2]. Insulin and, in a lesser degree, also IGF-I and IGF-II downregulates IGFBP-1 production in the liver in vivo [3,4].

Since portal hyperinsulinemia is a key feature of metabolic syndrome [5], serum IGFBP-1 concentration might serve as a marker for hyperinsulinemia and insulin resistance by indicating insulin bioactivity in portal vein. An easily measurable marker would be needed, as the current gold standard for assaying insulin sensitivity, the insulin clamp technique [6] is invasive and technically demanding procedure not suited for large populations. Clinical studies indicate that IGFBP-1 correlates with cardiovascular risk factors [7]. Clinical studies have also shown an association between hyperinsulinemic insulin resistance and sex hormone-binding globulin (SHBG) [8], another insulin-regulated protein produced by the liver.

The human hepatoma cell line HepG2 has been used to study the regulation of IGFBP-1 and SHBG production in vitro [9–12]. Earlier studies have shown the inhibitory effect of insulin on the production of both proteins [4,9,12,13]. In previous studies, the effects of IGF-I on IGFBP-1 production have been variable ranging from inhibitory to no effect at all [4,9,14]. IGF-II appears to have an inhibitory effect on IGFBP-1 as well [4], but its effect on SHBG production in liver or hepatoma cells is not known.

The relative potencies of insulin and insulin-like growth factors in the regulation of the production of IGFBP-1 and SHBG by the liver is still unresolved, therefore we carried out comparative studies on the regulation of these proteins in cell cultures.

2. Materials and methods

The study was approved by the Institutional Review Board, Department of Obstetrics and Gynecology, Helsinki University Central Hospital.

* Corresponding author. Tel.: +358-9-8611; fax: +358-9-8615944.
 E-mail address: tarja.kalme@hus.fi (T. Kalme).

2.1. Cell cultures

Human hepatoma cells (HepG2 cells, American Type Culture Collection, Rockville, MD) were grown at 37 °C in 92% air and 8% CO_2, in Dulbecco's Modified Eagle's Medium without phenol red, supplemented with 10% heat-inactivated fetal calf serum, 2 mM L-glutamine, 1 mM non-essential amino acids, 100 U/ml penicillin, and 100 µg/ml streptomycin (penicillin–streptomycin solution) (all from Gibco, Paisley, UK). The HepG2 cells were grown to subconfluency for 3 days. The subconfluent cell layers were washed twice with phosphate-buffered saline (PBS; Orion diagnostica, Espoo, Finland), after which the cells were cultivated for 1 day under serum-free conditions. The medium was then replaced by fresh serum-free medium, and the cells were cultivated in the presence or absence of the hormones for another 2 days (for IGFBP-1 and SHBG protein measurement) or 6 h (for RNA experiments). For protein assays, each experiment was performed three times with six observations for each dose of the hormones. Human insulin was obtained from Boehringer Mannheim (Mannheim, Germany), and human IGF-I and IGF-II from Sigma (St. Louis, MO). At the end of the incubation, the media were removed and stored at −20 °C until assayed. Cell viability was determined by trypan blue uptake. The cells for RNA extraction were used immediately.

2.2. IGFBP-1 and SHBG assays

The IGFBP-1 concentration of the media was determined using specific immunofluorometric assay (IFMA) performed essentially as described earlier [15] using two monoclonal antibodies (F34-15C9 and F36-9G3). The sensitivity of the assay was 0.1 µg/l, intra-assay variation 3–11% and the linear measuring range 0.1–100 µg/l. The SHBG concentrations from medium samples were measured by an immunofluorometric assay (Delfia SHBG assay, Wallac Oy, Turku, Finland) based on the direct sandwich technique in which polyclonal rabbit anti-SHBG antibodies and monoclonal mouse anti-SHBG antibodies were used. The intra- and inter-assay coefficients of variation were 7.8 and 9.1%, respectively.

2.3. Isolation of total RNA and Northern blot analysis

Total hepatocyte RNA was extracted by dissolving the cells in 4 M guanidium isothiocyanate, followed by acid–guanidium thiocyanate–phenol–chloroform extraction [16]. RNA was quantified by measuring ultraviolet absorbance at 260 nm.

Total RNA (20 µg) was subjected to electrophoresis and transferred to nylon membrane (Magna Nylon Transfer Membrane, Finnzymes, Espoo, Finland). As a IGFBP-1 probe, the entire 1.5 kb long human IGFBP-1 cDNA probe [17] was used. SHBG cDNA (402 bp long) was cloned from HepG2 cells by RT-PCR and TA cloning kit (Invitrogen, San Diego, CA). 5′-TTG AGG TTC GAA CCT GGG A-3′ was used as a sense primer and 5′-AGA TCT CGG CCT GTT TGT C-3′ as an antisense primer. Labeling of *Eco*RI fragment was performed by Rediprime DNA-labeling system (Amersham, Buckinghamshire, UK). The filter was hybridized overnight at 68 °C using Express Hyb hybridization solution (Clontech, Palo Alto, CA) and ^{32}P-labeled IGFBP-1 or SHBG cDNA. For autoradiography, the filter was exposed to Hyperfilm-MP film (Amersham). Ribosomal RNA was used to quantify the amount and control the quality of loaded RNA. The amount of SHBG and ribosomal RNA were estimated from autoradiographs and gels, respectively, using an UV transilluminator with or without UV-to-white converter, respectively, and GeneTools program (SynGene, Cambridge, UK).

2.4. Statistical analyses

The results were expressed as a percentage of the control (without hormones) value in each case, given as the mean ± S.E.M. for six culture wells of each dose and hormone for protein analyses. The differences in IGFBP-1 and SHBG levels between cultures with and without the hormones were evaluated by Mann–Whitney *U*-test. $P < 0.05$ was considered statistically significant.

3. Results

Insulin attenuated both IGFBP-1 and SHBG production in a dose-dependent fashion by about 35% from the control value at 30 nM concentration at 48 h (Fig. 1). At 6 h, no changes were observed in IGFBP-1 or SHBG production (data not shown). At 30 nM concentration, IGF-I inhibited IGFBP-1 and SHBG production by 20 and 24%, respectively (Fig. 1). IGF-II inhibited IGFBP-1 production by 32%, but for SHBG the decrease caused by IGF-II was only 11% (Fig. 1). Over 90% of the cells were viable at the end of the experiments, and no difference in the viability between control and experiment wells was found (data not shown).

IGFBP-1 RNA levels decreased 40% by IGF-I and 52% by insulin and IGF-II (Table 1). The changes in SHBG RNA levels by all hormones were smaller than in IGFBP-1 (10–25%), and only IGF-I caused change was statistically significant (Table 1).

Table 1
IGFBP-1 and SHBG RNA values calculated in proportion with ribosomal RNA

	n	IGFBP-1 RNA	P-value	SHBG RNA	P-value
IGF-I (30 nM)	4	60 ± 7.3	0.009	75 ± 3.3	0.025
IGF-II (60 nM)	3	48 ± 3.4	0.013	90 ± 3.8	0.20
Insulin (120 nM)	2	48 ± 15	0.034	75 ± 29	0.81

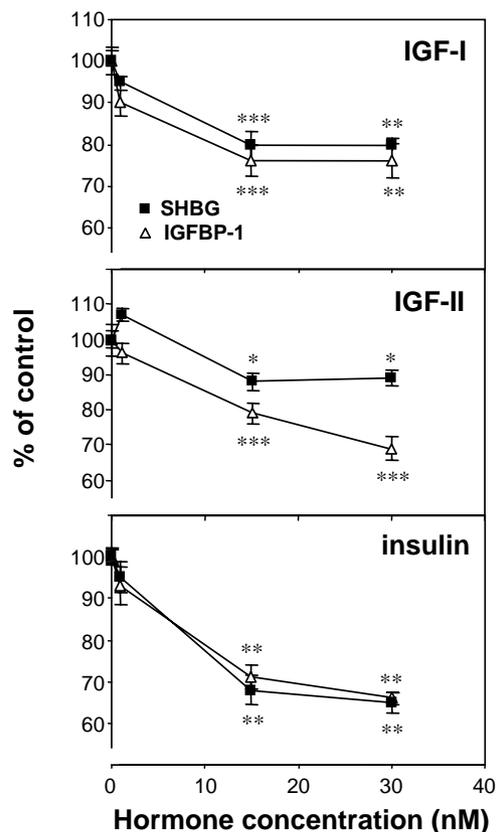The values are given as percent of control ± S.E.M. For control, $n = 9$.

Fig. 1. Effect of IGF-I, IGF-II and insulin treatments on HepG2 cell IGFBP-1 and SHBG production. Data are expressed as percent of control ± S.E.M. (*$P < 0.05$, **$P < 0.01$ and ***$P < 0.001$ as compared to control).

4. Discussion

Insulin was found to inhibit the production of IGFBP-1 and SHBG from HepG2 hepatoma cells at 48 h in the same extent. In vivo, insulin infusion studies have shown that insulin regulates IGFBP-1 levels [3] and a non-linear inverse correlation between portal vein insulin concentration and serum IGFBP-1 level has been reported [18]. The sensitivity of this regulation corresponds to the capacity of insulin to inhibit lipolysis [19], and is four- to five-fold greater than the ability of insulin to regulate glucose production [20]. In insulin clamp studies, IGFBP-1 values have decreased 18-fold as compared to SHBG [21]. In this study using hepatoma cells no such difference was observed, and the decrease of production is equal for both proteins. No changes were observed in IGFBP-1 and SHBG concentrations by insulin at 6 h. As insulin was the most potent hormone used in this study, it is likely that IGF-I or IGF-II would give similar results. There is also no evidence in the literature that changes in IGFBP-1 and SHBG would be likely to occur in the cell media this early.

Also IGF-I was found to significantly decrease SHBG and IGFBP-1 production, but to a lesser extent than insulin did.

Our result is in keeping with a previous study [4], where a reduction of IGFBP-1 production by IGF-I was observed. However, this has not been observed in all studies [14]. The changes in SHBG were in accordance with previous studies using HepG2 cells [12,13]. Like insulin, IGF-I regulated IGFBP-1 and SHBG in the same extent. Clinical findings have shown that patients treated with either IGF-I or growth hormone, which increases IGF-I levels, show inverse correlation between plasma insulin and SHBG levels, whereas no correlation existed between IGF-I and SHBG levels [22]. Similarly, in postmenopausal breast cancer patients, multivariate analysis revealed that plasma SHBG correlated negatively with insulin, but not with IGF-I [23]. Studies on serum IGFBP-1 and IGF-I levels also showed inverse correlations between IGFBP-1 and IGF-I [23–25].

There are not many clinical or experimental studies addressing the effect of IGF-II on IGFBP-1 or SHBG synthesis. Similarly to insulin and IGF-I, we found that IGF-II decreased the production of both IGFBP-1 and SHBG. The reduction was smaller for SHBG than for IGFBP-1.

The reduced IGFBP-1 concentrations are likely to be caused by reduced synthesis because we observed that IGF-I and IGF-II, and insulin downregulate IGFBP-1 RNA levels. This is in concordance with previous studies [4,26]. SHBG RNA levels were significantly different only between IGF-I-treated and control cells. Reduction of SHBG mRNA levels caused by insulin has been shown in a previous study, in which the incubation time was much longer, 48 h as opposed to 6 h in this study [27]. The more pronounced decrease in IGFBP-1 than in SHBG RNA levels after 6 h incubation suggests that the changes in SHBG production are slower than for IGFBP-1.

In summary, insulin, IGF-I and IGF-II decreased both IGFBP-1 and SHBG protein levels with similar sensitivities and maximal responses. These results suggest that both IGFBP-1 and SHBG concentrations could act as highly sensitive indicators of insulin bioactivity in portal vein, but comparative clinical studies would be needed to clarify this.

References

[1] G. Povoa, M. Isaksson, H. Jörnvall, K. Hall, The somatomedin-binding protein isolated from human hepatoma cell line is identical to the amniotic fluid somatomedin-binding protein, Biochem. Biophys. Res. Commun. 128 (1985) 1071–1078.

[2] P.D.K. Lee, C.A. Conover, D.R. Powell, Regulation and function of insulin-like growth factor-binding protein-1, Proc. Soc. Exp. Biol. Med. 204 (1993) 4–29.

[3] A.-M. Suikkari, V.A. Koivisto, E.M. Rutanen, H. Yki-Järvinen, S.L. Karonen, M. Seppälä, Insulin regulates the serum levels of low molecular weight insulin-like growth factor binding protein, J. Clin. Endocrinol. Metab. 66 (1988) 266–272.

[4] P.D.K. Lee, A. Suwanickul, L.A. DePaolis, M.B. Snuggs, S.L. Morris, D.R. Powell, Insulin-like growth factor (IGF) suppression of IGFBP-1 production: evidence for mediation by the type I IGF receptor, Regul. Pept. 48 (1993) 199–206.

[5] P. Björntorp, Abdominal obesity and the development of non-insulin-dependent diabetes mellitus, Diabetes Metab. Rev. 4 (1988) 615–622.

[6] R.A. DeFronzo, J.D. Tobin, R. Andres, Glucose clamp technique: a method of quantifying insulin secretion and resistance, Am. J. Physiol. 237 (1979) E214–E223.

[7] M. Harrela, R. Koistinen, J. Tuomilehto, A. Nissinen, M. Seppälä, Low serum insulin-like growth factor-binding globulin-1 is associated with an unfavourable cardiovascular risk profile in elderly men, Ann. Med. 32 (2000) 424–428.

[8] J.E. Nestler, Sex hormone binding globulin: a marker for hyperinsulinemia and/or insulin resistance? J. Clin. Endocrinol. Metab. 76 (1993) 273–274.

[9] C.A. Conover, P.D.K. Lee, Insulin regulation of IGF binding protein production in cultured HepG2 cells, J. Clin. Endocrinol. Metab. 70 (1990) 1062–1067.

[10] M.S. Khan, B.B. Knowles, D.P. Aden, W. Rosner, Secretion of testosterone–estradiol-binding globulin by a human hepatoma-derived cell line, J. Clin. Endocrinol. Metab. 53 (1981) 448–449.

[11] B.B. Knowles, C.C. Howe, D.P. Aden, Human hepatocellular carcinoma cell lines secrete the major plasma proteins and hepatitis B surface antigen, Science 209 (1980) 497–499.

[12] J.C. Crave, H. Lejeune, C. Brebant, C. Baret, M. Pugeat, Differential effects of insulin and insulin-like growth factor I on the production of plasma steroid-binding globulins by human hepatoplastoma-derived (HepG2) cells, J. Clin. Endocrinol. Metab. 80 (1995) 1283–1289.

[13] S.R. Plymate, R.E. Joes, L.A. Matej, K.E. Friedl, Regulation of sex hormone binding globulin (SHBG) production in HepG2 cells by insulin, Steroids 52 (1988) 339–340.

[14] A. Singh, D. Hamilton-Fairley, R. Koistinen, M. Seppälä, V.H.T. James, S. Franks, M.J. Reed, Effect of insulin-like growth factor-type I (IGF-I) and insulin on the secretion of sex hormone binding globulin and IGF-I binding protein (IBP-I) by human hepatoma cells, J. Endocrinol. 124 (1990) R1–R3.

[15] H. Koistinen, R. Koistinen, L. Selenius, O. Ylikorkala, M. Seppälä, Effect of marathon run on serum IGF-I and IGF-binding protein 1 and 3 levels, J. Appl. Physiol. 80 (1996) 760–764.

[16] P. Chomczynski, N. Sacchi, Single-step method of RNA isolation by acid–guanidium thiocyanate–phenol–chloroform extraction, Anal. Biochem. 162 (1987) 156–159.

[17] M. Julkunen, R. Koistinen, K. Aalto-Setälä, M. Seppälä, O.A. Jänne, K. Kontula, Primary structure of human insulin-like growth-factor binding protein/placental protein and tissue-specific expression of its mRNA, FEBS Lett. 236 (1988) 295–303.

[18] C.A. Conover, P.D.K. Lee, J.A. Kanaley, J.T. Clarkson, M.D. Jensen, Insulin regulation of insulin-like growth factor binding protein-1 in obese and non-obese humans, J. Clin. Endocrinol. Metab. 74 (1992) 1355–1360.

[19] M.D. Jensen, M. Caruso, V. Heiling, J.M. Miles, Insulin regulation of lipolysis in nondiabetic and IDDM subjects, Diabetes 38 (1989) 1595–1601.

[20] R.A. Rizza, L.J. Mandarino, J.E. Gerich, Dose–response characteristics of insulin on production and utilisation of glucose in man, Am. J. Physiol. 240 (1981) E630–E639.

[21] P. Ebeling, U.-H. Stenman, M. Seppälä, V.A. Koivisto, Acute hyperinsulinemia, androgen homeostasis and insulin sensitivity in healthy man, J. Endocrinol. 146 (1995) 63–69.

[22] M. Gafny, A. Silbergeld, B. Klinger, M. Wasserman, Z. Laron, Comparative effects of GH, IGF-I and insulin on serum sex hormone binding globulin, Clin. Endocrinol. 41 (1994) 169–175.

[23] P.E. Lonning, S.I. Helle, D.C. Johanssen, H. Adlercreutz, E.A. Lien, M. Tally, D. Ekse, T. Fotsis, G.B. Anker, K. Hall, Relations between sex hormones, sex hormone binding globulin, insulin-like growth factor-I and insulin-like growth factor binding protein-1 in postmenopausal breast cancer patients, Clin. Endocrinol. 42 (1995) 23–30.

[24] M. Wang, D.M. Wilson, P.D.K. Lee, Serum levels of the 25 kDa insulin-like growth factor binding protein in children with insulin-dependent diabetes mellitus, in: S.L.S. Drop, R.L. Hintz (Eds.), Insulin-Like Growth Factor Binding Proteins, Excerpta Medica, Amsterdam, 1989, pp. 39–45.

[25] K. Hall, G. Lundin, G. Povoa, Serum levels of the low molecular weight form of insulin-like growth factor binding protein in healthy subjects and patients with growth hormone deficiency, acromegaly and anorexia nervosa, Acta Endocrinol. 118 (1988) 321–326.

[26] D.R. Powell, A. Suwanichkul, M.L. Cubbage, L.A. DePaolis, M.B. Snuggs, P.D.K. Lee, Insulin inhibits transcription of the human gene for insulin-like growth factor-binding protein-1, J. Biol. Chem. 266 (1991) 18868–18876.

[27] M. Loukovaara, M. Carson, H. Adlercreutz, Regulation of production and secretion of sex hormone-binding globulin in HepG2 cell cultures by hormones and growth factors, J. Clin. Endocrinol. Metab. 80 (1995) 160–164.