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Expression and regulation of growth hormone (GH) and prolactin (PRL) receptors in a rat insulin producing cell line (INS-1)

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Abstract

We have examined the expression of growth hormone (GH) and prolactin (PRL) receptors, and the binding parameters of human GH (hGH), on the rat insulin producing cell line INS-1. We found that, like normal insulin producing β-cells, INS-1 cells express both GH and PRL receptors, and the majority of human GH (hGH) binding sites on this cell line are of lactogenic specificity. As calculated from Scatchard plots, about 6600 hGH binding sites with a K_a of 6.2×10^8 M^{-1} are present per cell. Northern blot analysis showed two mRNA species of 4 and 1.6 kb for the GH receptor and, one major species of 10.5 kb for the PRL receptor. The PRL receptor mRNA was up-regulated by bovine GH (bGH), rat PRL (rPRL), in a time- and dose-dependent manner. On the contrary, bGH and rPRL, down-regulated the expression of GH receptor gene. The importance of this differential regulation in in vivo and in vitro studies are discussed.

Keywords: Growth hormone; Prolactin; Receptor; β-cells

1. Introduction

Growth hormone and the related family of hormones such as prolactin (PRL) and placental lactogen (PL) have been shown to exert both mitogenic and insulinotropic activities on normal β-cells in vivo (Beck and Daughaday 1967; Parson et al., 1983) and in vitro (Hellerstrom et al., 1985; Nielsen, 1985; Billestrup and Nielsen, 1991; Brelje and Sorenson, 1991; Brelje et al., 1993). Certain investigators have found the effects of GH to be more pronounced (Parson et al., 1983; Nielsen, 1985) or equal to those of PRL and PL (Billestrup and Nielsen, 1991), while others have observed higher activities for PRL and PL when used in homologous systems (islets and hormones from the same species) in vitro (Brelje and Sorenson, 1991; Brelje et al., 1993). It has been suggested that these hormones may exhibit activities in heterologous systems that do not correspond to their effects in homologous systems (Nicoll, 1972; Nicoll et al., 1986), since hGH, for example, binds equally well to receptors with somatotropic or lactogenic specificity in homologous and heterologous systems (Cunningham and Wells, 1991). Recent findings have demonstrated that the same tyrosine kinase is associated with GH and PRL receptors (Argetsinger et al., 1993), suggesting that the signal transduction following hormone binding with the corresponding receptors may be identical. In this case, the levels of activities of these hormones on their target cells could be determined by the levels of the expression of their receptors.

In the present study we have investigated the GH and PRL receptor gene expression and regulation in a pure population of β-cells. For this purpose, we chose the INS-1 cell line which exhibits a great degree of differentiation in comparison to other existing rodent cell lines (Janjic and Asfari, 1991; Asfari et al., 1992a,b; Thorens 1992; Brun et al., 1993; Scharfmann et al., 1993; Waeber et al., 1993). We have also determined the binding characteristics of hGH receptor in this cell line. The data presented here further demonstrate that the INS-1 cell line is an appropriate β-cell model to study the effects of GH and PRL and support the idea that the differences in the degree of the effects of GH and PRL observed at a given condition

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may be due to the levels of expression of the receptor of each hormone.

2. Materials and methods

2.1. Hormones

Recombinant hGH was obtained from AS-R Serono (Geneva, Switzerland), rat and ovine PRL were gifts of the National Hormone and Pituitary Program (University of Maryland School of Medicine, NIDDK, USA). bGH was obtained from USDA Reproduction Lab (Beltsville, USA).

Iodination of hGH to a specific activity of about 130 μCi/μg was performed with chloramine-T as described before (Lesniak et al., 1973).

2.2. Cell culture

Cells were maintained in complete medium (CM, RPMI 1640, 2 mM glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin, 1 mM sodium pyruvate, 10% heat inactivated fetal calf serum (HI-FCS), 10 mM HEPES, and 50 μM 2-mercaptoethanol as described before (Asfari et al., 1992). Both RINm5F (Gazdar et al., 1980) and Hepa-1 (Darlington, 1987) cell lines were maintained in RPMI 1640 medium and 10% FCS.

2.3. Binding assay

The binding assays were performed on adherent cells, cultured at 2.5×10^6 cells per 35 mm culture plates in 3 ml of CM for 5 days. The cultures were then washed once with 4 ml serum-free medium (SFM), containing 0.5% BSA and incubated in the same medium for 2 h at 37°C. The medium was then removed and the cultures were washed once with 3 ml of incubation buffer (PBS Dulbecco, pH 7.4, with 10 mM glucose and 0.5% BSA). Cells were then incubated with ^{125}I-labeled hGH (recombinant human GH obtained from AS-R Serono Geneva, Switzerland) (14×10^4 cpm, about 5 ng) alone or in the presence of varying concentrations of native hGH, bGH, or oPRL (two plates per concentration) in a total volume of 0.6 ml of incubation buffer for 1 h at 37°C. After this incubation period, the plates were placed on ice, incubation buffer was removed and cells were washed twice with 1 ml ice-cold incubation buffer and solubilized in 1.2 ml of 1 M NaOH for determination of radioactivity. Non-specific binding was determined in the presence of excess (1 μg/ml) unlabeled hGH and the specific binding was defined as the difference between the radioactivity of the cells incubated with ^{125}I-labeled hGH in the absence and presence of excess unlabeled hGH.

2.4. RNA extraction and Northern blot analysis

Cells were seeded into 100 mm tissue culture plates (at a density of 8–9 million cells/plate per 12 ml of CM) for 48–72 h. Depending on the experimental protocol, the RNA was prepared from the cells that were cultured in their respective growth medium or after treatment with hormone, as is specified in Section 3. For RNA extraction, the plates were chilled on ice and washed once with PBS and RNA was isolated in urea–lithium chloride according to Auffray and Rougeon's (1980) procedure. For RNA preparation from rat liver, the fresh tissues were washed in ice-cold PBS and cut into small pieces. About 300 mg of tissue were transferred into 15 ml of ice-cold urea–lithium chloride (Sigma), homogenized and processed as explained for cell culture.

RNAs were denatured in formamide/formaldehyde at 65°C for 10 min and fractionated on formaldehyde (2.2 M)/1% agarose gel, blotted onto a nylon membrane (Hybond Amersham, Les Ulis, France) and UV cross-linked. Blots were hybridized with ^{32}P-labeled cDNA probes overnight at 42°C in a solution containing 5× SSPE, 5× Denhardt's solution, 0.1% sodium dodecyl sulfate (SDS), and 50% formamide. After hybridization the filters were washed twice in a solution of 2× SSC, 0.1% SDS for 15 min at ambient temperature and once in the same solution at 45°C for 60 min. The filters were exposed to RX film (Fuji Medical X-Ray film) at −70°C using intensifying screens. The mRNAs on autoradiograms were quantified by scanning densitometry (Shimadzu dual wavelength TLC scanner CS-930, Kyoto, Japan).

2.5. cDNA probes

The probes were cDNA of rat GH receptor (the 563-bp *Bam*H1 fragment coding for the large part of the 5′-sequence of the extracellular domain) (Mathews et al., 1989) and PRL receptors (Boutin et al., 1988; Shirota et al., 1990) (kindly provided by Dr. Paul Kelly). For control of RNA loading, cDNA probes of 1A (a cDNA obtained from female rats uteri cDNA library with no defined genomic activities) (Hsu et al., 1988), or [γ-^{32}P]ATP labeled oligonucleotides complementary to 18S ribosomal RNA (Chan et al., 1984) were used.

3. Results

3.1. Effects of hGH, bGH and oPRL on ^{125}I-labeled hGH binding to INS-1 cells

As illustrated in Fig. 1, 50% inhibition of ^{125}I-labeled hGH binding was obtained with 20 ng/ml hGH. Both ovine prolactin (oPRL) and bovine growth hormone (bGH), which has been shown in rat liver cells, to interact only with PRL and GH receptors, respectively (Postel-Vinay, 1976; Picard and Postel-Vinay, 1984), competed with ^{125}I-labeled hGH binding to INS-1 cells although with different abilities. Fifty percent inhibition of ^{125}I-labeled hGH binding was obtained with 100 ng/ml of oPRL and 2 μg/ml of bGH. Thus, the apparent affinity for oPRL is much greater than that of bGH. When 100 ng/ml of both hormones were added together, the inhibition increased to 80% as obtained using 200 ng/ml of hGH.

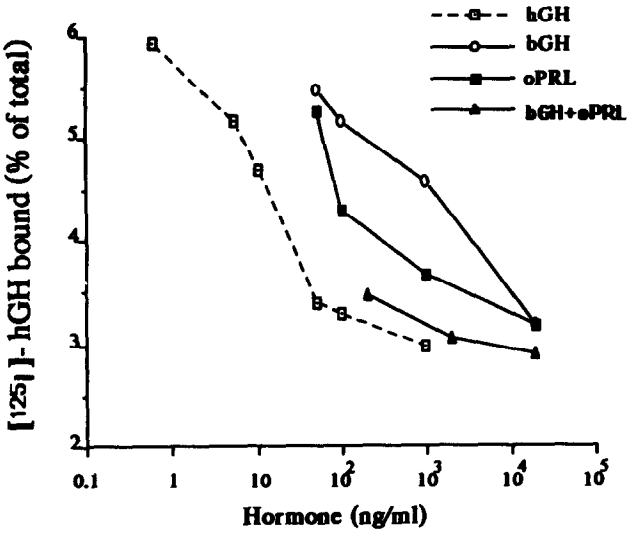

Fig. 1. Binding of ^{125}I-labeled hGH to INS-1 cells and competition by hGH, bGH, and oPRL. Cells were cultured in 35-mm culture dishes (see Section 2), and after 2 h incubation in SFM with 0.5% BSA, cultures were washed and incubated in 0.6 ml incubation buffer containing 140 000 cpm of ^{125}I-labeled hGH in the absence or presence of increasing concentrations of native hormones for 1 h. Competitive binding of native hormones with ^{125}I-labeled hGH alone (A), or in combination with other hormones (B). Each point is the mean of duplicate experiments.

3.2. Gene expression of GH receptor and PRL receptor

RNA preparations from female rat liver tissue, INS-1, RINm5F, and a rat hepatoma cell line (Hepa1), were hybridized to rat prolactin and rat GH receptor cDNA probes (Fig. 2). mRNAs encoding the PRL receptor in female rat liver were of one major species of 1.8 kb and a minor one of 3.4 kb. In INS-1 cells. PRL receptor mRNA was of one major species of 10.5 kb (Fig. 2 A). The same size PRL receptor mRNA was detected in INS-1 cells when 6 mg of poly-A RNA was hybridized to either the long or short form of PRL receptor cDNA probe (data not shown). The PRL receptor mRNA in RINm5F cells (which have a common tumor origin with INS-1 cells) or in Hepa1 cells was beyond detectability.

GH receptor mRNA in rat liver tissue was found to be of three mRNA species of 4, 1.6 and 0.8 kb (Fig. 2B). In INS-1 cells, the GH receptor mRNAs were of two species of 4 and 1.6 kb. The same forms of mRNA were found in RINm5F cells.

3.3. Regulation of PRL and GH receptor gene expression

PRL receptor mRNA was up-regulated by rPRL and bGH and FCS in a time- and dose-dependent manner (Figs. 3 and 4). PRL receptor mRNA was reduced 2.8-

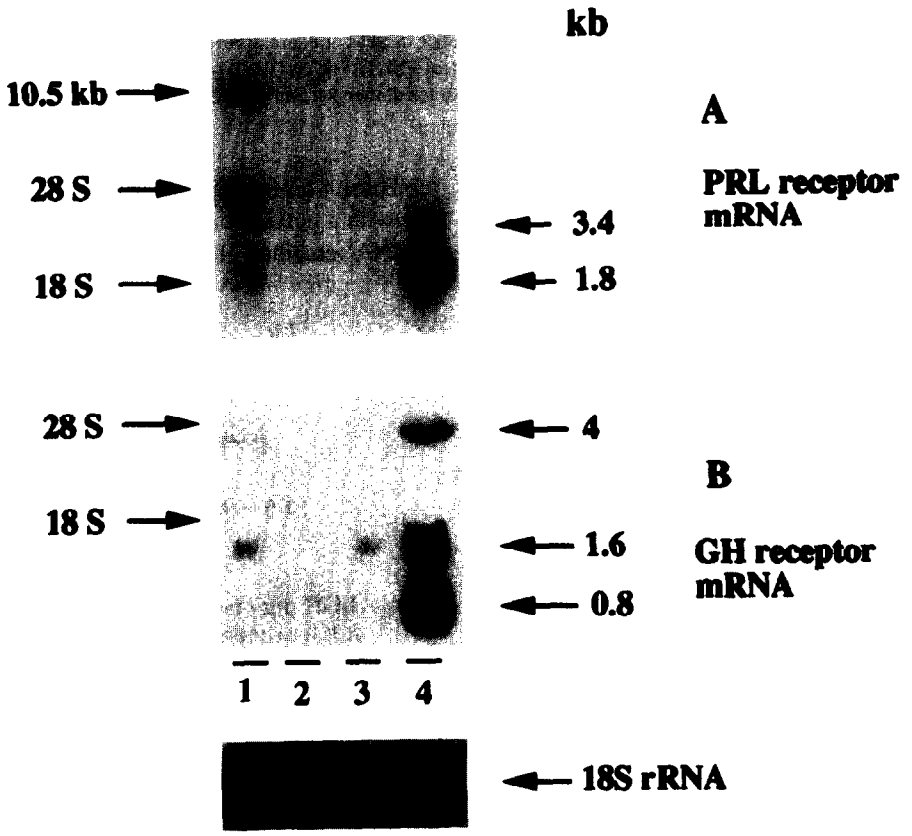

Fig. 2. GH and PRL receptor gene expression in INS-1 and rat liver tissue. Twenty micrograms of total RNA were fractionated on agarose, blotted onto nylon membranes and hybridized to rat PRL receptor (short form) (the same size mRNA for PRL receptor was obtained when the blots were hybridized with long form PRL receptor cDNA probe) (A) or GH (B) receptor cDNA probes. (A) Lane 1, INS-1 cells total RNA; 2, rat hepatoma cell line (Hepa1); 3, RINm5F cells; 4, rat liver tissue. (B) The same blot (B) dehybridized and rehybridized with rGH cDNA probe.

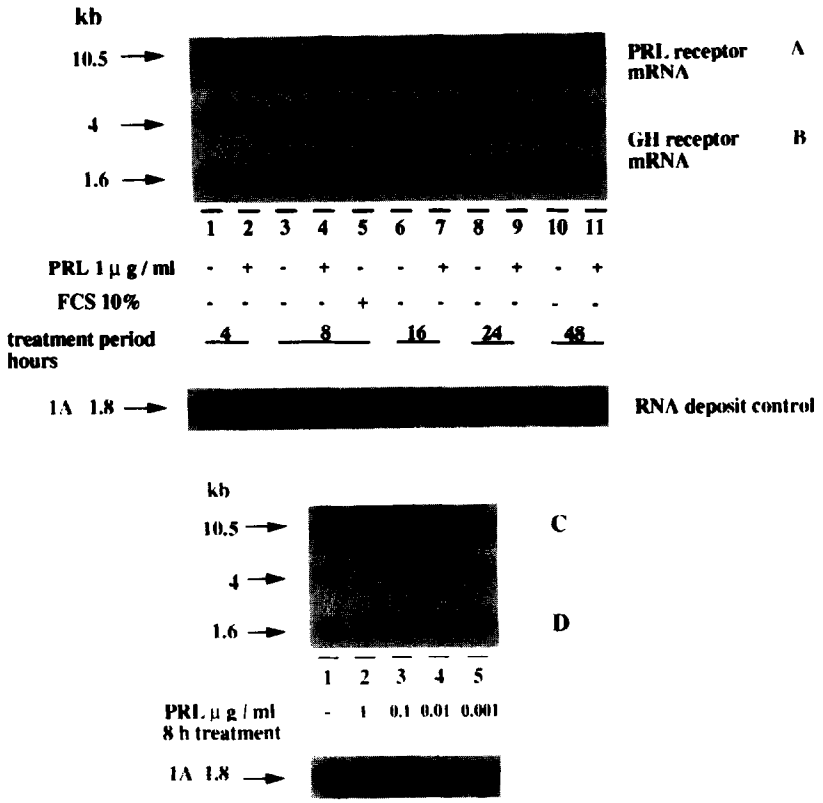

Fig. 3. Regulation of PRL and GH receptor gene expression by rPRL and FCS. Cells were cultured in CM for 48 h. The cultures were then washed with PBS and after 24 h pre-incubation in SFM, the cells were incubated either in SFM containing rPRL or in medium containing 10% FCS. For the time effect, RNA was extracted at indicated time intervals and hybridized to rat PRL (A) or rat GH (B) receptor cDNA probes. For the dose effect, the cells were incubated at various concentration of rPRL and the RNA was extracted after 8 h and hybridized to either of the receptor cDNA probes as indicated above (C,D).

fold 24 h after removal of FCS from the culture medium, as quantified by densitometry of autoradiograms (data not shown). Addition of 1 μg/ml of either bGH or rPRL to the cells for 4 h resulted in a fourfold increase in mRNA levels with a maximum of 5–6-fold above control within 8 h. mRNA remained elevated during the 48-h treatment. The

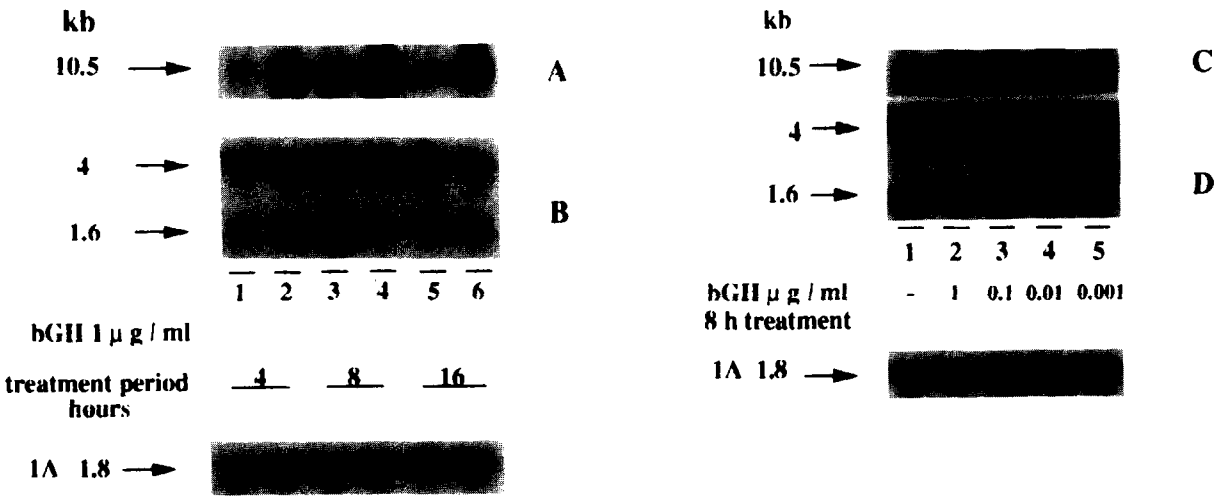

Fig. 4. Regulation of PRL and GH receptors by bGH. Cells were cultured in CM for 48 h. The cultures were treated exactly as described in the legend of Fig. 3, except in this experiment, cells were incubated in SFM containing bGH. For the time effect, RNA was extracted at the indicated time intervals and hybridized to rat PRL (A) or rat GH (B) receptor cDNA probes. Lanes 1, 3, and 5, cells that were incubated in SFM (control); lanes 2, 4 and 6, cells that were incubated in SFM in the presence of 1 μg/ml bGH. For the dose effect, the cells were incubated at various concentrations of bGH and the RNA was extracted after 8 h and hybridized to either of the receptor cDNA probes as indicated above (C,D).

increase in PRL receptor mRNA was detectable in cells treated with 10 ng/ml of either hormone and enhanced at increasing hormone concentrations (Figs. 3C and 4C). FCS increased the mRNA levels (fourfold) of PRL receptor (Fig. 3A).

The regulation of GH receptor was in the completely opposite direction to that of PRL receptor. GH receptor mRNA was down-regulated by rPRL, FCS (Fig. 3B,D) and bGH (Fig. 4B,D). Removal of serum from culture medium resulted in a twofold increase in the 4 kb transcript of GH receptor mRNA levels after 24 h (data not shown), and treatment of cells with 1 μg/ml rPRL, bGH, or 10% FCS reduced the levels to the basal within 4–16 h of treatment. The 1.6 kb transcript of GH receptor was regulated in a manner similar to that of the 4 kb transcript (compare lanes 6 and 7, 8 and 9, 10 and 11 in Fig. 3B); the variation, however, was less pronounced than in the 4 kb transcript.

4. Discussion

In the present paper we have demonstrated the presence of GH and PRL receptors in the rat insulin producing cell line, INS-1, and we have shown the differential regulation of PRL and GH receptor gene expression.

INS-1 cells express hGH binding sites with binding characteristics expected of a receptor, and the binding affinity of the hGH receptor was comparable to that of rat hepatocytes (Ranke et al., 1976; Postel-Vinay and Desbupuais, 1977). As hGH interacts with both GH and PRL receptors, it was important to determine the specificity of the hGH binding sites found in INS-1 cells. For this purpose, in the competitive binding assay, we used ovine PRL and bovine GH, which has been shown in rat liver cells to interact only with PRL and GH receptors, respectively (Postel-Vinay, 1976; Picard and Postel-Vinay, 1984). Our findings are consistent with previous investigations in rat islets, suggesting that the majority of hGH binding sites are of lactogenic specificity (Polak et al., 1990) and differ from those reported for RIN5AH cells in which the hGH binding sites exhibit primarily GH specificity (Billestrup and Martin, 1985).

The GH receptor mRNA species found in INS-1 cells were of 4 and 1.6 kb while in female rat liver cells, in addition to the 4 and 1.6 kb forms, a third mRNA species of about 0.8 kb was detected. According to previous findings, the 4 kb GH receptor mRNA encodes the full length GH receptor, while the 1.6 kb transcript encodes the GH binding protein (Baumbach et al., 1989). The function of the 0.8 kb transcript is not known. The PRL receptor mRNA in INS-1 cells was a predominant species of 10.5 kb detected in either total RNA or poly-A RNA (data not shown) preparations, using either the short or the long form cDNA probe. This was in marked contrast to the mRNA size in female rat liver cells which were of 3.4 and 1.8 kb. The long PRL receptor mRNA in INS-1 cells could be the predominantly expressed precursor to the shorter transcripts. A long (9.7 kb) PRL receptor mRNA, however, has been reported in rat ovaries (Hu and Dufau, 1991). PRL receptor mRNA of 10.5 kb species has been reported in human and in rabbit tissues (Dusanter-Fourt et al., 1991; Ormandy and Sutherland, 1993). The size of PRL receptor mRNA in this cell line is much greater than that reported in RIN5AH cells (3.5 kb) (Moldrup et al., 1990), although both cell lines are derived from the same tumor. In INS-1 cells, both GH and PRL receptors mRNAs were regulated by FCS, rPRL and bGH in a time and concentration-dependent manner but in a completely opposite direction. Removal of serum for 24 h resulted in marked reduction of PRL receptor mRNA and addition of either rPRL, bGH or FCS restored the expression after 8 h of treatment. This is consistent with previous reports on the effects of these hormones on PRL receptors in other cell types (Posner et al., 1975; Baxter et al., 1984; Grichting and Goodman, 1986; Kelly et al., 1991; Orian et al., 1991). The negative effects of rPRL and bGH on GH receptor mRNA levels were more pronounced after 16 h of treatment of INS-1 cells with these hormones.

Depending on the tissue and species examined, the regulation of GH receptors by GH or PRL is different. For example, GH and PRL receptors were elevated in the liver cells of transgenic mice expressing GH (Orian et al., 1991), in adipocytes of hypophysectomized rats treated with bGH (Grichting and Goodman, 1986), and in liver cells treated with GH (Baxter and Zaltsman, 1984; Baxter et al., 1984). GH has been reported, however, to decrease the GH receptor levels in cultured lymphocytes (Lesniak and Roth, 1976), and rat diaphragm (Albertson-Wikland et al., 1980). Also the number of GH binding sites in liver membranes of hypophysectomized male rats (with almost no circulating GH) were elevated (Picard and Postel-Vinay, 1984).

GH and PRL receptors belong to the same superfamily of transmembrane proteins, and the signalling pathways for the two receptors remain to be elucidated. Recent results (Argetsinger et al., 1993), however, have shown that a tyrosine kinase (JAK2) is associated with both GH and PRL receptors. The remarkable parallelism of the effects of GH and PRL in this cell line is further evidence that the two receptors might function through the same signalling pathway. If the same regulatory mechanism applies to the receptor protein, the different levels of activities of the two hormones on β-cells may reside on differential expression and regulation (availability) of the hormone receptors rather than on differences in the intracellular signal transduction. In this case, the presence of either of the two hormones would be sufficient for their respective activities in β-cells. This may explain why in certain GH deficiencies such as a certain form of dwarfism due to serum GH deficiency (deletion of GH gene for example) or GH receptor defects (Laron type syndrome),

the carbohydrate tolerance is within normal range, suggesting normal β-cell development and function.

The regulatory activities of GH and PRL on their receptors in INS-1 cells are very similar to those reported in normal β-cells (Moldrup et al., 1993), therefore, greater effects of PRL and PL on normal β-cell functions reported previously (Brelje et al., 1993), could have been due to the higher levels of PRL receptor gene expression under the conditions that had been tested (i.e. 7 days treatment with the hormones). This condition corresponds well to pregnancy where due to the high levels of circulating PL, PRL receptor should be up-regulated and more available than GH receptors for hormone binding. In this paper, we have demonstrated that INS-1 cells express functional GH and PRL receptors which are differentially regulated by the two hormones. Whether the regulatory effects of the two hormones on the receptors are at transcriptional or post transcriptional levels remains to be determined. INS-1 cells represent a good model to study the mode of action of GH and PRL in β-cells.

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