Pergamon

Biochemical Pharmacology, Vol. 49, No. 8, pp. 1091–1098, 1995.

0006-2952/95 $9.50 + 0.00

0006-2952(95)00054-2

RESPONSIVENESS OF AN SV40-IMMORTALIZED HEPATOCYTE CELL LINE TO GROWTH HORMONE

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(*Received* 18 *July* 1994; *accepted* 18 *November* 1994)

Abstract—The response of an SV40-immortalized hepatocyte cell line (CWSV-1) derived from adult male rat hepatocytes to human growth hormone (hGH) was investigated. CWSV-1 cells, which have been characterized extensively, retain certain differentiated functions of normal liver (Woodworth and Isom, *Mol Cell Biol* 7: 3740–3748, 1987). This cell line consists of tightly associated polygonal, mononucleated cells that grow as monolayers. These cells showed no significant morphological changes with the addition of hGH. Northern blot analysis showed that continuous treatment of the CWSV-1 cells with hGH induced the expression of insulin-like growth factor I (IGF-I) and 5α-reductase RNAs. In addition, continuous exposure to hGH resulted in the induction of expression of the growth hormone receptor/growth hormone binding protein (GHR/GHBP) genes. This study indicates that the CWSV-1 cells may serve as a valuable *in vitro* model system for studying the signaling pathway of GH.

Key words: growth hormone; hepatocyte cell line; IGF-I; 5α-reductase; GHR; GHBP

GH§, a pituitary polypeptide hormone, physiologically stimulates new bone formation and maintains normal postnatal growth and development, as well as accelerating linear growth. Metabolically, GH induces protein deposition, stimulates lipolysis thereby increasing plasma free fatty acids, and reduces carbohydrate utilization elevating plasma glucose levels [1, 2]. Several of these effects are mediated by IGF-I, which is produced and secreted by the liver in response to GH stimulation [3]. IGF-I mediates the actions of GH especially during the postnatal and adolescent growth and development periods by stimulating tissue growth and inducing terminal differentiation of many cell types [4]. Furthermore, GH is also responsible for regulating the expression of other liver gene products such as 5α-reductase, hormone receptors, major urinary proteins, and certain sex-specific steroid hydroxylase cytochrome P450 enzymes [5, 6].

The cellular effects produced by GH are mediated by the binding of GH to its membrane-bound receptor. Although it has been clearly shown that GH directly influences the expression of specific genes and regulates different cellular events essential for normal cell function, the intracellular signaling mechanisms that mediate these responses are still unclear. There are advantages to investigating the second messengers involved in transducing GH's signal in *in vitro* cell culture systems rather than whole animals. To our knowledge, no GH-responsive hepatocyte or hepatoma cell lines have been identified. Therefore, studies are presently carried out using differentiated primary, non-proliferating adult rat hepatocytes [7] or tissue culture cells engineered to express the GHR through DNA transfection [8–10]. Because primary cells in culture have many limitations, it would be of great value to find a well-differentiated hepatocyte cell line responsive to GH.

The CWSV-1 cell line, an SV40-immortalized cell line derived from adult male rat hepatocytes, has been studied extensively [11–14]. This cell line consists of replicating epithelial cells and grows in a chemically defined medium without any matrices or serum. In addition, CWSV-1 cells retain some differentiated functions characteristic of the normal liver in that they secrete albumin at levels comparable to those of normal liver, show liver-like expression of other liver-specific proteins, and express albumin, α_1-antitrypsin, and transferrin mRNAs at levels approaching those found in normal liver. The goal of this study was to determine if the CWSV-1 cells respond to GH. Specifically, the effects of GH on IGF-I, 5α-reductase and GHR RNA expression by CWSV-1 cells were measured.

Studies carried out using the adult rat liver [15, 16] and cultures of primary male and female rat hepatocytes [17, 18] have shown that IGF-I mRNA expression is GH dependent. Likewise, Miller and Colas [19] showed that GH causes a dose-dependent increase of 5α-reductase activity in adult female hepatocytes. In addition, the continuous infusion of GH to normal male rats increases the activity of 5α-reductase [20, 21]. The GH-regulated expression of 5α-reductase provides another tool with which to investigate the responsiveness of the CWSV-1 cell line to GH.

Conflicting reports have made the issue of whether

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§ Abbreviations: GH, growth hormone; hGH, human growth hormone; pGH, porcine growth hormone; GHR, growth hormone receptor; GHBP, growth hormone binding protein; IGF-I, insulin-like growth factor I; SSC, 0.15 M sodium chloride + 0.015 M sodium citrate; PRL, prolactin; and PRL-R, prolactin receptor.

or not a sex difference exists with the GHR and its regulation by GH confusing. Looking at the effect of GH on the expression of GHR/GHBP RNA in the CWSV-1 cell line may help to clear up some of these issues.

If GH treatment results in the induction of certain GH-responsive genes in the CWSV-1 cell line, it might serve as a potential *in vitro* system in which to study the elements of the intracellular signaling mechanism of GH. In addition, issues concerning sex differences associated with the GHR and its regulation by GH can be investigated further.

MATERIALS AND METHODS

Cell lines. The SV40-hepatocyte cell line CWSV-1 [12] was maintained on 100-mm plastic tissue culture dishes and fed 1 × RPCD medium every other day. RPCD medium is a supplemented RPMI 1640 medium containing 0.28% bicarbonate, 0.36% 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid, bovine serum albumin (0.08%), linolenic acid (2 μg/mL), 2-aminoethanol (4.1 μg/mL), glucagon (0.04 μg/mL), dexamethasone (0.4 μg/mL), transferrin (100 μg/mL), trace metals [22], penicillin (100 μg/mL), and streptomycin (100 μg/mL). When the cells reached confluency, they were trypsinized and plated at a predetermined dilution in 1 × RPCD medium containing 3% fetal bovine serum to aid in cell attachment. Approximately 4 hr after plating, fresh 1 × RPCD medium lacking fetal bovine serum was added. Experiments with GH were carried out by adding the desired amounts of hGH (Sigma Chemical Co., St. Louis, MO) or pGH (Sigma) to 1 × RPCD medium. Cell cultures were treated for the indicated time periods after which poly $(A)^+$ mRNA was extracted.

Poly $(A)^+$-enriched mRNA isolation. Poly $(A)^+$-enriched mRNA was extracted from livers of normal male and female Fisher 344 rats and the CWSV-1 cell line using the PolyAtract System 1000 kit mRNA isolation kit (Promega, Madison, WI). Concentrations were determined spectrophotometrically.

Recombinant plasmids. The plasmid pRat 1-20, which encodes the entire extracellular region that is common to both the GHR and GHBP messages [23], was provided by Dr. William R. Baumbach (American Cyanamide Co., Princeton, NJ). This probe recognizes a 4.75 kb (GHR) and 1.2 kb (GHBP) transcript [23]. The rat IGF-I cDNA clone was a gift from Dr. Graeme I. Bell of the Howard Hughes Medical Institute, The University of Chicago. It recognizes the following transcripts: 7.0, 1.8, and 1.0 kb [24]. The plasmid pB5α-RED1, which contains the full-length rat cDNA encoding 5α-reductase, was a gift from Dr. David Russell of The University of Texas, Southwestern Medical Center at Dallas, and recognizes a 2.47 kb transcript [25].

Northern blot analysis. For Northern blot hybridization, 1–3 μg of formamide-denatured poly $(A)^+$-enriched mRNA were separated electrophoretically on a 1.4% agarose gel. Equal concentrations were verified by visualization of the rRNA concentrations on ethidium bromide-stained gels. The RNA was transferred to NitroPlus 2000 membranes (Micron Separations, Inc., Westboro, MA), baked at 80°, and prehybridized at 46° for 48 hr in a prehybridization buffer [50% deionized formamide, 5× SSC, 5× Denhardt's, 50 mM NaH_2PO_4 (pH 6.5), 0.1% SDS, and 0.5 mg/mL sonicated salmon sperm DNA]. Plasmid cDNA probe was nick-translated for 4 hr [26] and then denatured. The hybridization reaction was carried out in hybridization buffer (prehybridization buffer with the addition of 1.0 mM EDTA) at 46° for 48 hr in the presence of 5×10^7 cpm of the specific cDNA probe. The nitrocellulose filters were washed twice in 2× SSC with 0.1% SDS at room temperature for 10 min each, three times in 0.1× SSC with 0.1% SDS for 30 min each at 55°, and three times in 0.1× SSC at room temperature for 5 min each. The filters were partially dried and exposed to Kodak XAR-5 film (Eastman Kodak Co., Rochester, NY) at −70° for variable exposure times. The Molecular Dynamic Laser Densitometer 100A (Sunny Vale, CA) and Protein Data Bases, Inc. (Huntington, NY) software package were used to quantitatively measure the relative signal intensities of the autoradiographs.

Rocket immunoelectrophoresis. The amount of albumin produced and secreted into the culture medium was measured by rocket immunoelectrophoresis, as reported by Laurell [27].

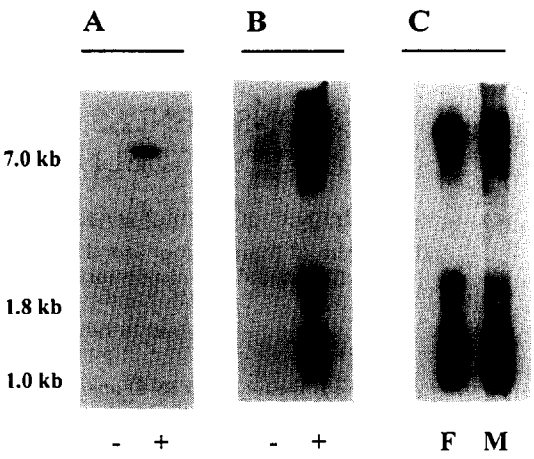

Fig. 1. Effect of hGH on IGF-I RNA expression. (A) Expression of IGF-I RNA in the CWSV-1 cell line, untreated and treated with GH (2 μg/mL, 4 days). Poly $(A)^+$-enriched RNA was extracted from CWSV-1 cells, separated electrophoretically, transferred to filters, and hybridized with ^{32}P-labeled IGF-I cDNA. (B) Expression of IGF-I RNA in the CWSV-1 cell line, untreated and treated with hGH, in an independent experiment. The autoradiogram was overexposed to show IGF-I transcripts in untreated CWSV-1 cells. (C) Expression of IGF-I in female (F) and male (M) rat livers. All lanes contained equal amounts of RNA, as determined by ethidium bromide staining.

RESULTS

Effect of hGH on IGF-I RNA expression. Since the expression of IGF-I has been shown to be GH

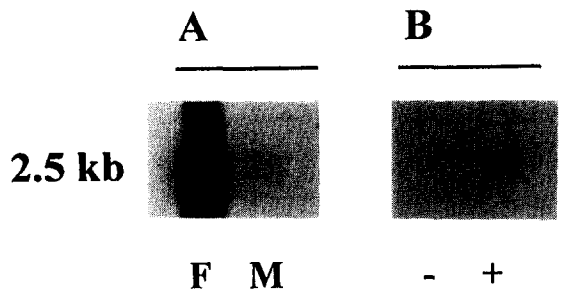

Fig. 2. Effect of hGH on 5α-reductase RNA. (A) Expression of 5α-reductase in female (F) and male (M) rat livers. Poly (A)$^+$-enriched RNA was extracted from female and male rat livers, and Northern blot analysis was carried out. (B) Expression of 5α-reductase in the CWSV-1 cell line, untreated and treated with hGH (2 μg/mL, 4 days). Poly (A)$^+$-enriched RNA was extracted from the CWSV-1 cells, and Northern blot analysis was carried out.

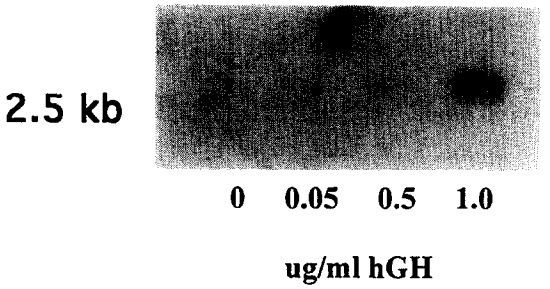

Fig. 3. Effect of concentration of hGH on 5α-reductase RNA expression. Total RNA was extracted from CWSV-1 cells, untreated (0) and treated with 0.05, 0.5, and 1.0 μg/mL hGH for 24 hr. Northern blot analysis was carried out.

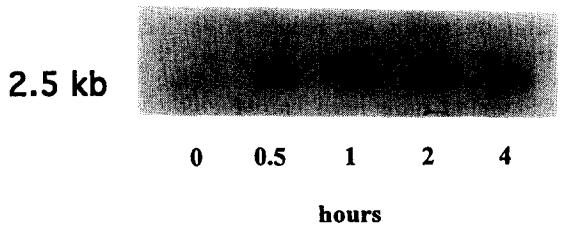

Fig. 4. Effect of hGH on 5α-reductase RNA expression after various treatment times. Total RNA was extracted from CWSV-1 cells, untreated (0) and treated with 2 μg/mL hGH for 0.5, 1, 2, and 4 hr. Northern blot analysis was carried out.

dependent, Northern blot analysis was carried out to determine whether hGH induced IGF-I expression in CWSV-1 cells. Poly (A)$^+$-enriched RNA was prepared from CWSV-1 cells that were treated with 2 μg/mL hGH for 4 days. The expression of IGF-I mRNA increased in hGH-treated CWSV-1 cells (Fig. 1A). In an independent experiment, the autoradiogram was overexposed to determine if the CWSV-1 cells expressed detectable IGF-I mRNA in the absence of GH (Fig. 1B). Treatment with hGH (4 days) increased the 7.0, 1.8 and 1.0 kb IGF-I transcripts approximately 20-, 8- and 11-fold, respectively (Fig. 1B).

Poly (A)$^+$-enriched RNA from female and male rat liver was also analyzed for IGF-I mRNA levels. The 7.0 kb IGF-I message was expressed at a 4-fold higher level in the female than in the male liver (Fig. 1C). To our knowledge, this finding has not been reported elsewhere in the literature. In a separate experiment carried out under the same conditions, the male liver expressed the 7.0 kb transcript 27-fold higher than the hGH-treated CWSV-1 cells (data not shown).

Effect of hGH and pGH on 5α-reductase RNA expression. The expression of 5α-reductase, a female-specific enzyme, is also regulated by GH. The continuous presence of GH in male rats has been shown to induce the expression of this female-specific enzyme. It was of interest to determine if continuous exposure to hGH could increase the expression of 5α-reductase in CWSV-1 cells. The CWSV-1 cell line was derived from a culture of primary adult male rat hepatocytes. GH-treated CWSV-1 cells showed a 4-fold increase in the expression of the 2.5 kb 5α-reductase RNA, as indicated by Northern blot analysis (Fig. 2B).

Poly (A)$^+$-enriched RNA from normal male and female liver was also analyzed for 5α-reductase RNA levels so that the constitutive and induced levels in CWSV-1 cells could be compared with those from the whole animal. The level of expression of 5α-reductase mRNA was 36-fold higher in male liver than in untreated CWSV-1 cells and 10-fold higher than in CWSV-1 cells treated with hGH (data not shown). Quantitative analysis of an autoradiogram exposed for a shorter period of time revealed that the female liver expressed the 2.5 kb 5α-reductase message approximately 14 times higher than the male (Fig. 2A). Our data parallel previous findings, which demonstrate that 5α-reductase activity is higher in the female than in the male rat liver [19].

When a concentration–response curve was carried out, the induction of 5α-reductase was observed when CWSV-1 cells were treated with 0.5 μg/mL hGH for 24 hr (Fig. 3). A 4.5-fold further increase in the level of expression was observed at 1.0 μg/mL hGH, as quantitated by densitometric scanning of the 2.5 kb 5α-reductase transcript.

A time-course study of hGH effects on 5α-reductase RNA by 2 μg/mL hGH was also performed (Fig. 4). CWSV-1 cells were treated with hGH for the indicated times, and total RNA was prepared and analyzed by Northern hybridization to the 5α-reductase cDNA probe. The autoradiogram showed that after 0.5 hr of hGH treatment, increased levels of 5α-reductase RNA expression were already seen in the CWSV-1 cells. Densitometric quantification showed this increase to be 3-fold over the untreated cells. The stimulatory effect of hGH on the 5α-reductase message increased to 5 to 6 fold by 1 hr

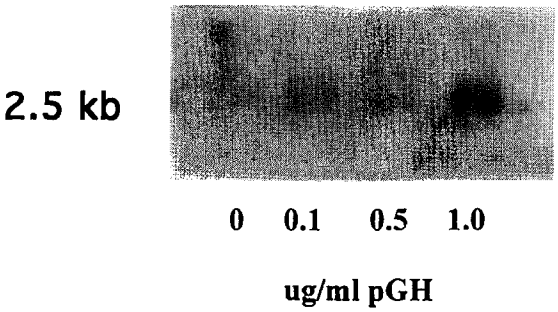

Fig. 5. Effect of concentration of pGH on 5α-reductase RNA expression. Total RNA was extracted from CWSV-1 cells, untreated (0) and treated with 0.1, 0.5, and 1.0 μg/mL pGH for 24 hr. Northern blot analysis was carried out.

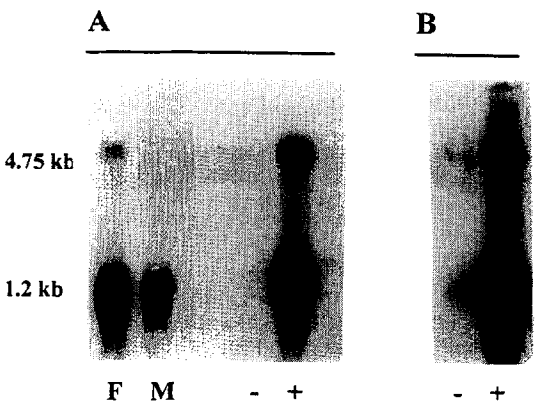

Fig. 6. Effect of hGH on GHR/GHBP RNA expression. (A) Expression of GHR/GHBP in female (F) and male (M) rat livers and the CWSV-1 cell line, untreated and treated with hGH (2 μg/mL, 4 days). Poly $(A)^+$-enriched RNA was extracted and Northern blot analysis was carried out as described in the legend to Fig. 1. (B) Expression of GHR/GHBP in the CWSV-1 cell line, untreated and treated with hGH (2 μg/mL, 4 days), as in panel A, except that the autoradiogram was overexposed to allow for detection of GHR/GHBP bands in untreated CWSV-1 cells.

after treatment and remained at that level when treatment was continued for 2 or 4 hr.

Previous studies on the regulation of IGF-I expression have been carried out primarily with hGH. Studies have shown that hGH possesses both lactogenic and somatogenic activities in the rat liver. It has been reported that hGH binds to PRL-Rs and GHRs in rat liver membrane preparations [28, 29]. In addition, hGH has also been shown to induce PRL-Rs [30]. pGH, on the other hand, possesses no lactogenic properties since it does not bind to PRL-Rs [31–33]. Therefore, pGH was used to determine if the effects of hGH observed on the induction of 5α-reductase RNA were due to hGH binding to the GHR and not the PRL-R. Northern blot analysis showed that exposure of the CWSV-1 cells to 0.1 or 0.5 μg/mL pGH for 24 hr resulted in a 2-fold increase in the level of 5α-reductase RNA (Fig. 5). A 7-fold increase was observed at 1.0 μg/mL pGH.

Effect of hGH on GHR/GHBP RNA expression. We next wanted to determine whether GH regulated the expression of GHR/GHBP RNAs in the CWSV-1 cell line. Poly $(A)^+$-enriched RNAs extracted from normal female and male rat liver, untreated CWSV-1 cells, and hGH-treated CWSV-1 cells were analyzed by Northern blot analysis for the 4.75 kb GHR and 1.2 kb GHBP transcripts. The 1.2 kb GHBP RNA bands were easily detected in female and male liver and in hGH-treated CWSV-1 cells (Fig. 6A). The 4.75 kb GHR transcript was readily detectable in female liver and hGH-treated CWSV-1 cells. The GHBP and GHR transcripts were not detectable in RNA from untreated CWSV-1 cells, and the GHR transcript was not detectable in RNA from normal male liver. Overexposure of the blot showed that untreated CWSV-1 cells do constitutively express both the GHR and GHBP transcripts (Fig. 6B). Quantitative analysis of the autoradiogram indicated that treatment with hGH increased expression of the GHR and GHBP transcripts approximately 15-fold.

Both the GHR and GHBP transcripts were expressed at considerably lower (approximately 10-fold) levels in male than in female liver. In untreated CWSV-1 cells, which were derived from male hepatocytes, the levels of both transcripts were markedly lower than in liver from normal male rats. However, the levels of both the 4.75 and 1.2 kb RNA bands were more than 40-fold higher in hGH-treated CWSV-1 cells than in normal male rats.

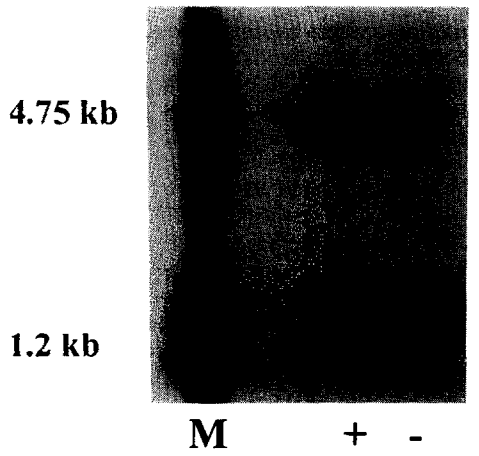

Fig. 7. Effect of insulin on GHR/GHBP RNA expression. Expression of GHR/GHBP RNA in male rat liver (M) and the CWSV-1 cell line, treated and untreated with insulin (0.06 μg/mL, 4 days). Poly $(A)^+$-enriched RNA was extracted from male rat liver and the CWSV-1 cell line and Northern blot analysis was carried out as described in the legend to Fig. 1. The autoradiogram was exposed for a longer period of time than that in Fig. 6B to allow for detection of the GHR/GHBP RNA in untreated CWSV-1 cells.

We next tested the effect of insulin on GHR/

Fig. 8. Morphology of CWSV-1 cells in the presence and absence of hGH. Photomicrographs of the untreated (A) and hGH-treated (B) CWSV-1 cells after 4 days in culture.

GHBP RNA expression in CWSV-1 cells since it has been reported previously that insulin can mimic the effects of GH [34]. Poly $(A)^+$-enriched RNA was extracted from untreated CWSV-1 cells and CWSV-1 cells treated with insulin. Northern blot analysis (Fig. 7) showed that treatment with 0.06 μg insulin/mL medium for 4 days had no effect on the level of expression of either the 4.75 kb GHR or 1.2 kb GHBP transcripts.

Effect of hGH on albumin secretion. Media from untreated CWSV-1 cells and hGH-treated cells were analyzed for rat albumin levels by radioimmunoassay. CWSV-1 cells transcribe albumin RNA at liver-like levels [12, 14] and secrete high levels of albumin into the culture medium [11, 12]. Our results indicated that untreated CWSV-1 cells secreted an average of 13.49 ($\pm$2.36) pg albumin per cell per 24 hr. Likewise, CWSV-1 cells treated with hGH (2 μg/mL, 4 days) produced 13.46 ($\pm$2.42) pg albumin per cell per 24 hr (data not shown). These data were based on albumin measurements made on medium collected from cells at the several different passage levels used for the experiment. Since hGH had no effect on albumin secretion, these data indicate that the stimulatory effects on hGH of IGF-1, 5α-reductase, and GHR/GHBP gene expression were specific.

Morphology. CWSV-1 cells are small, tightly associated polygonal epithelial cells that grow as monolayers (Fig. 8A) in the absence of hGH [11]. Treatment of CWSV-1 cells with hGH for 4 days (Fig. 8B) did not alter significantly the morphology of these cells with regard to cell size and shape.

DISCUSSION

It has been shown previously that primary cultures of non-proliferating rat hepatocytes can be used to study the actions of GH *in vitro*. Primary cultures of hepatocytes isolated from adult male rats have been used to investigate the role of protein kinase C in the regulation of P450IIC12 and IFG-I mRNA expression by GH [7]. Maintenance of primary hepatocytes on a substratum of matrigel, a reconstituted basement membrane, has enabled investigators to study the effects of GH on the expression of cytochromes P450h and P450i and on phenobarbital-induced P450 b/e gene transcription [34, 35]. However, there are several disadvantages of using primary hepatocytes to study GH effects on liver cells. Since primary hepatocytes do not divide, there is a constant need to isolate fresh cells from animals. In addition, the need for a matrigel substratum adds to the complexity of the *in vitro* culture system. The availability of a continuous cell line that grows on plastic cell culture dishes and retains differentiated functions of liver and the ability to respond to GH would be of value. In this study we have shown that the CWSV-1 cell line, an SV40-immortalized hepatocyte cell line derived from adult male rat hepatocytes, is responsive to GH, and, as such, appears to be the first hepatocyte cell line exhibiting this property. GHR/GHBP gene expression increased in hGH-treated CWSV-1 cells and hGH induced the expression of IGF-I and 5α-reductase, two GH-responsive genes. The effect of hGH on the CWSV-1 cells was specific since hGH did not cause a change in the amount of albumin produced per cell. In addition, exposure to hGH did not alter the epithelial morphology of the cells.

CWSV-1 cells were derived from a colony of replicating albumin-secreting cells in a culture of primary adult rat hepatocytes that had been transfected with SV40 DNA [11, 12, 36]. The primary hepatocytes used for these studies were not maintained in medium supplemented with GH, and CWSV-1 cells are grown routinely in medium lacking GH. The CWSV-1 cells have been used previously to study regulation of albumin expression [12, 13], molecular mechanisms of albumin expression [14, 37], and the expression of other liver specific genes [13, 38]. The CWSV-1 cells express liver-like levels of albumin and other liver-specific genes while replicating in serum-free chemically defined medium in the absence of extracellular matrix or epidermal growth factor. The CWSV-1 cells are immortal and not transformed [39–41]; they do not grow in soft agar; they have an organized actin cytoskeleton; and they are not tumorigenic in animals.

We have demonstrated previously that albumin expression in CWSV-1 cells is regulated by dexamethasone [12], and expression of seven acute phase genes expressed by CWSV-1 cells is regulated in the manner expected when the cells are treated with a mixture of cytokines present in conditioned medium from stimulated lymphocyte cultures [38]. In this study we have shown that CWSV-1 cells retain the ability to respond to GH. These studies show that CWSV-1 cells not only maintain expression of genes indicative of hepatic differentiation but also retain the ability to respond to external stimuli in a liver-like fashion.

The highest level of GHR mRNA expression has been shown in the adult rat liver. GHR RNA expression is low at birth and elevates to adult levels in 5 weeks [42]. The same pattern was observed for GH binding [43]. It still remains unclear as to whether a sex difference exists in the expression of GHR and whether this expression is regulated by GH. It has been reported that female rats appear to have a higher number of hepatic GHRs than males [43, 44]. However, other investigators reported no sex differences in the rat liver [28, 45, 46]. Mathews *et al.* [42] reported that GHR mRNA expression is the same in female and male rat livers. Similarly,

the issue of whether GH regulates GHR gene expression needs to be further addressed. A single injection of GH resulted in the decrease of hepatic GHRs in hypophysectomized female rats [47], whereas long-term GH exposure increased the number of GHRs in intact female rats [30]. In contrast, Mathews *et al.* [42] reported that there was no change in the hepatic expression of GHR mRNA in hypophysectomized or GH-treated rats. In this study, we showed that continuous exposure of the CWSV-1 cells to GH markedly increased the expression of GHR/GHBP RNA. Although it has been reported previously that insulin can imitate the effects of GH [34], our data with CWSV-1 cells showed that insulin did not induce GHR/GHBP RNA levels.

IGF-I is a prominent mediator of GH action, with the liver being the primary source of circulating IGF-I [48]. Its expression and production by the liver are dependent on GH stimulation [15–18]. Studies carried out using the adult rat liver [15, 16] and cultures of primary male and female rat hepatocytes [17, 18] have shown that IGF-I mRNA expression is GH dependent. In this study, we showed that CWSV-1 cells express low levels of IGF-I mRNA and that hGH treatment of CWSV-1 cells markedly increased the expression of IGF-I. We also showed that (1) all three IGF-I transcripts were expressed in CWSV-1 cells, (2) all three transcripts were induced by hGH treatment, and (3) the 7.0 kb band, which is more strongly expressed in female liver than in male liver, was the most prominent transcript expressed and induced in CWSV-1 cells.

Growth hormone has been reported to also regulate the expression of 5α-reductase, a female-specific liver enzyme. The higher activity level of 5α-reductase in the female than in the male rat liver is the result of the sexual dimorphic pattern of GH secretion from the anterior pituitary. In the adult male rat, high intermittent episodes of GH secretion occur every 3.3 hr, with low to undetectable trough levels occurring between the large GH pulses. This pattern of release results in GH being present in the plasma intermittently. In contrast, GH peaks appear more frequently and have lower amplitudes with higher baseline levels in the adult female rat. This results in the continuous presence of GH in the circulation [49]. It is the constant exposure to GH that results in the increased activity of 5α-reductase. GH infused constantly into male rats increases the activity level of this enzyme [20, 21]. The ability of GH to induce 5α-reductase in cells *in vitro* has been demonstrated previously; specifically, Miller and Colas [19] showed that GH caused a dose-dependent increase of 5α-reductase activity in adult female hepatocytes. Since the CWSV-1 cell line was derived from a primary culture of adult male rat hepatocytes, it was of interest to determine if exposing the CWSV-1 cells continuously to hGH could increase the expression of the 5α-reductase gene. Indeed, the expression of 5α-reductase RNA increased in the hGH-treated CWSV-1 cells. We also showed that 0.5 μg/mL hGH was sufficient to induce the expression of 5α-reductase and that RNA induction occurred as early as 0.5 hr after hGH treatment.

Human GH has been reported to bind both PRL-Rs and GHRs in rat liver [28, 29]. In addition, hGH has also been shown to induce PRL-Rs in female rat livers [30]. Therefore, in order to address the question of whether the induction of 5α-reductase RNA by hGH was due to GHR binding and not PRL-R, pGH was used. pGH is known to possess no lactogenic properties in rat liver [31, 32], since nonprimate hormones do not bind to the PRL-R [32, 33]. In addition, studies have shown that lactogenic receptors are present at much higher concentrations in female than in male rat liver membranes [50, 51]. Our data showed that pGH induced the expression of 5α-reductase RNA at physiological concentrations. This suggested that the effect seen by hGH on the induction of this gene was through the GHR and not the PRL-R. We have also demonstrated that treatment of CWSV-1 cells with recombinant hGH induced 5α-reductase, indicating that the response elicited by hGH and pGH from pituitaries was not due to contaminating impurities (data not shown).

Overall, we conclude that the well-differentiated immortal rat hepatocyte cell line, CWSV-1, responds to GH and, as such, can be used to study the GH response at the molecular level. Specifically, the CWSV-1 cells and other SV40-immortalized hepatocyte cell lines may be able to be used as *in vitro* hepatic model systems to analyze the interaction of GH and the GHR, functional and structural regulation of GH-regulated genes, and the GH signaling pathway.

Acknowledgements—This work was supported, in part, by CA23931 (H.C.I.), GM40737, and a gift from the Ciba–Geigy Corp., Agricultural Chemicals Division.

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