Regulation of Insulin-Like Growth Factor Binding Protein Synthesis and Secretion in Human Retinal Pigment Epithelial Cells

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Cultured human retinal pigment epithelial cells (RPE) secrete insulin-like growth factor binding proteins (IGFBPs), a family of polypeptides which modulate the actions of the insulin-like growth factors. RPE cells secrete two IGFBPs with Mr estimates of 34,000 and 46,000, respectively. Treatment of RPE cells with IGF-I markedly stimulated the secretion of the 46,000 Mr form. This stimulation occurred via an IGF-I receptor independent mechanism because both [QAYL]IGF-I (an IGF-I analogue with decreased affinity for the IGFBPs but normal affinity for the IGF-I receptor) and α-IR₃ (a blocking monoclonal antibody against the IGF-I receptor) had no effect on IGF-I stimulated increases in IGFBPs. Additionally, [QAYL]IGF-I enhanced RPE cell proliferation to the same magnitude as IGF-I. Treatment with IGF-I, [QAYL]IGF-I, or α-IR₃ had no effect on steady-state levels of the 2.5 kb IGFBP-3 or the 1.3 kb IGFBP-6 mRNA transcripts as measured by Northern blotting and quantitative autoradiography. Forskolin and a group of candidate growth factors, including platelet-derived growth factor, epidermal growth factor, and acidic and basic fibroblast growth factor, modestly increased IGFBP secretion when compared to untreated cells, but these effects were small when compared to IGF-I treatment. Fetal calf serum enhanced the presence of the 2.5 kb IGFBP-3 mRNA transcript in a dose-dependent fashion but had no effect on the 1.3 kb IGFBP-6 mRNA transcript. IGF-I, forskolin, and the candidate growth factors had no effect on either IGFBP-3 or IGFBP-6 mRNA. These data suggest that the production of IGFBPs in human RPE cells is regulated by distinct mechanisms which include (1) an IGF-I receptor independent interaction of IGF-I with secreted IGFBPs and (2) de novo synthesis of IGFBPs by serum-containing factors.
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Insulin-like growth factors I and II (IGF-I and -II) are polypeptides which play an important role in cellular growth and differentiation (Sara and Hall, 1990). The actions of IGF-I and -II are modulated, in part, by a family of structurally related proteins, the insulin-like growth factor binding proteins (IGFBPs) (Sara and Hall, 1990; Clemmons, 1991a). Six distinct IGFBPs have been identified and cloned (Clemmons, 1991a; Drop, 1992). These IGFBPs transport IGFs (Binoux et al., 1991), modulate IGF tissue availability (Clemmons, 1991b), and alter IGF receptor binding (McCusker et al., 1991a). IGFBP expression varies by cell type (Clemmons, 1991a; Drop, 1992) and is regulated not only by nutritional status (Clemmons and Underwood, 1991; Mc Cusker et al., 1991b) but also by hormones (Ooi et al., 1990; Torring et al., 1991; Unterman et al., 1991) and growth factors (Barreca et al., 1992; Corps and Brown, 1991; Loret et al., 1991; Martin and Baxter, 1991), especially IGF-I (Martin et al., 1992b; Conover, 1991; Clemmons, 1991a). In vivo, IGF-I increases serum levels of IGFBP-2 (Clemmons, 1991a,b) and mediates growth hormone coupled increases in IGFBP-3 (Clemmons and Underwood, 1991). In vitro

IGF-I enhances the levels of IGFBPs, particularly IGFBP-3, in cultured fibroblasts (Bale and Conover, 1992), myocytes (McCusker and Clemmons, 1988), and osteoblasts (Schmid et al., 1989).

Growth factor mediated increases in IGFBPs may be an important determinant in the growth and maintenance of tissues (Corps and Brown, 1991; Loret et al., 1991). IGFBPs contain an Arg-Gly-Asp sequence near their carboxyl termini (Clemmons, 1991b) which is required for cellular attachment of matrix proteins. By binding to extracellular surfaces, secreted IGFBPs could target IGFs to specific cell types, increasing the local bioavailability of IGFs to these cells. Indeed, in fibroblasts, IGF-I action is augmented by incubation with IGFBP-3, implying a direct interaction of exogeneous IGFBP-3 with the cell surface (Conover, 1992).

As an initial approach towards understanding growth factor regulation of IGFBP function, we exam-

ined the regulation of IGFBP gene and protein expression in human retinal pigment epithelial (RPE) cells. RPE cells provide a good in vitro model system for examining growth factor regulation of IGFBP production (Waldbillig et al., 1991, 1992). Cultured human RPE cells express IGFBP-3 and IGFBP-6 mRNA and secrete two IGFBPs with estimated molecular weights of 46,000 and 34,000 (Randolph et al., 1993). These cells synthesize mRNA for the type I and type II IGF receptors (Martin et al., 1991) and display an IGF-I mediated increase in DNA synthesis (Leschey et al., 1990). It has been suggested that epithelial secretion of IGFBPs may allow targeting of IGFs to epithelial cells with concomitant growth (Clemmons, 1991a; Laron et al., 1991).

In the present study, we examined the relative roles of serum, IGF-I, and other growth factors in the regulation of human RPE IGFBP-3 and IGFBP-6 mRNA and protein. We present evidence that serum, but not IGF-I, regulates IGFBP-3 mRNA expression while IGF-I, but not other growth factors, increases IGFBP-3 protein via a receptor independent mechanism.

MATERIALS AND METHODS
Chemicals

Minimum essential medium (MEM), Hank's balanced salt solution, trypsin-EDTA, PDGF, and basic FGF were obtained from Grand Island Biological (Grand Island, NY). Insulin and forskolin were purchased from Sigma (St. Louis, MO), EGF and acidic FGF from Bachem (Torrence, CA). IGF was purchased from BioSource International (Camarillo, CA) and des (1-3)IGF-I from GroPep (Adelaide, Australia). [Gln^{3}Ala^{4}Tyr^{15}Leu^{16}] IGF-I([QAYL]IGF-I) was a gift from Dr. Margaret A. Cascieri, Merck, Sharp and Dohme Research Laboratories (Princeton, NJ). IGF-I receptor antibody (α-IR$_3$) was obtained from Oncogene Science (Uniondale, NY). Bovine calf serum was purchased from Hyclone Labs (Logan, UT). All tissue culture supplies were from Costar (Cambridge, MA) and Corning Glass Works (Corning, NY). Restriction enzymes were purchased from Bethesda Research Laboratories (Gaithersburg, MD). Molecular biology grade reagents were obtained from Sigma, and remaining chemicals were of reagent grade and purchased from Baker Chemical (Phillipsburg, NJ) or Fisher Scientific (Fairlawn, NJ).

Cell culture

Retinal pigment epithelia were isolated from human postmortem eyes obtained from the Michigan Eye Bank. Primary RPE cell cultures were established and maintained as previously described (Feldman et al., 1991). Cells were subcultured at a density of 1.5–2 × 10^6 cells per 75 cm^2 culture flask in MEM containing 20% CS and grown at 37°C in a humidified 95% air–5% CO$_2$ atmosphere. The medium was changed three times weekly. Experiments were performed on passage numbers 10–20 of RPE cell lines established from two separate patients. Prior to dosing, cells were rinsed three times in serum free MEM, followed by an incubation at 37°C in 10 ml serum free MEM for 1.5–2 h.

Cell proliferation assay

Cells were plated at a density of 6 × 10^4 cells/cm^2 in 96-well plates in 100 μl MEM with or without IGF-I, des(1-3) IGF-I or [QAYL]IGF-I. Cell proliferation was measured using the MTT colorimetric assay (Hansen et al., 1989). MTT (3,(4,5-dimethylthiazol-z-yl)2,5-diphenyltetrazolium bromide) (25 μl) was added (1 mg/ml) for a 2 h incubation at 37°C in a humidified atmosphere with 5% CO$_2$ followed by an overnight incubation in 100 μl lysis buffer (50% N,N-dimethyl formamide, 20% w/v SDS, pH 4.7). The optical density (OD) of each well was measured at a wavelength of 570 nm by a Bio-Rad Model 2550 EIA reader (Bio-Rad Laboratories, Melville, NY).

Northern analysis

Total cellular RNA was isolated from cells using the guanidinium thiocyanate-phenol extraction (Chomczynski and Sacchi, 1987). Northern analysis was performed as previously reported (Schmallbruch, 1991; Martin et al., 1992a). Membranes were hybridized with ^{32}P-dCTP labelled (1–10 × 10^8 cpm/mg) cDNA probes for human IGFBP-3 (535 bases excised with Hind III and EcoRI), human IGFBP-6, (267 bases excised with PstI) (Yee et al., 1991), and chicken β-actin (2.1 kb excised with PstI) (Cleveland et al., 1980). For each hybridization, OD values were measured densitometrically by averaging multiple exposures in the linear range of the film. Each experimental and β-actin OD value was expressed as a percentage of the untreated control value. Relative OD values were obtained by dividing the experimental OD by the respective β-actin (%).

Western ligand blotting

In some experiments, conditioned medium was obtained and concentrated twentyfold using a Centriprep concentrator (Amicon, Beverly, MA) with a 10,000 Dalton molecular weight cutoff. The protein content of the concentrates was determined using the Bio-Rad DC Protein Assay (Bio-Rad, Richmond CA). Protein (25 μg) in 35–50 μl of concentrated media and an equal volume of two times concentrated sample buffer (0.1 M Tris-HCl, pH 6.4, 4% sodium dodecyl sulfate (SDS), 0.2% bromophenol blue, 20% glycerol) was boiled for 3 m, loaded into a 3% acrylamide stacking gel, and electrophoresed through a 10% polyacrylamide resolving gel at 200–300 V under nonreducing conditions. Rainbow molecular weight standards (Amersham, Arlington Heights, IL) were used to assess relative molecular weights. Proteins were transferred to nitrocellulose membranes (0.2 μm) (Schleicher & Schuell, Keene, NH) overnight at 20 V in Towbin transfer buffer (0.05 M Tris, 0.4 M glycine, 0.075% SDS) using a Bio-Rad Trans-blot unit (Richmond, CA). Membranes were dried at 37°C for 5 m. The following consecutive washes in Western ligand blot saline (0.15 M NaCl, 0.01 M Tris HCl, pH 7.4) were done at 4°C: 30 m in 3% Nonidet P-40, 2 h in 1% bovine serum albumin, and 10 min in 0.1% Tween 20. Membranes were incubated overnight with 1 × 10^5 cpm/ml of ^{125}IGF-II (Amersham, Arlington Heights, IL) and 1% BSA, 0.1% Tween 20. After rinsing the membranes two times for 15 m at 4°C with
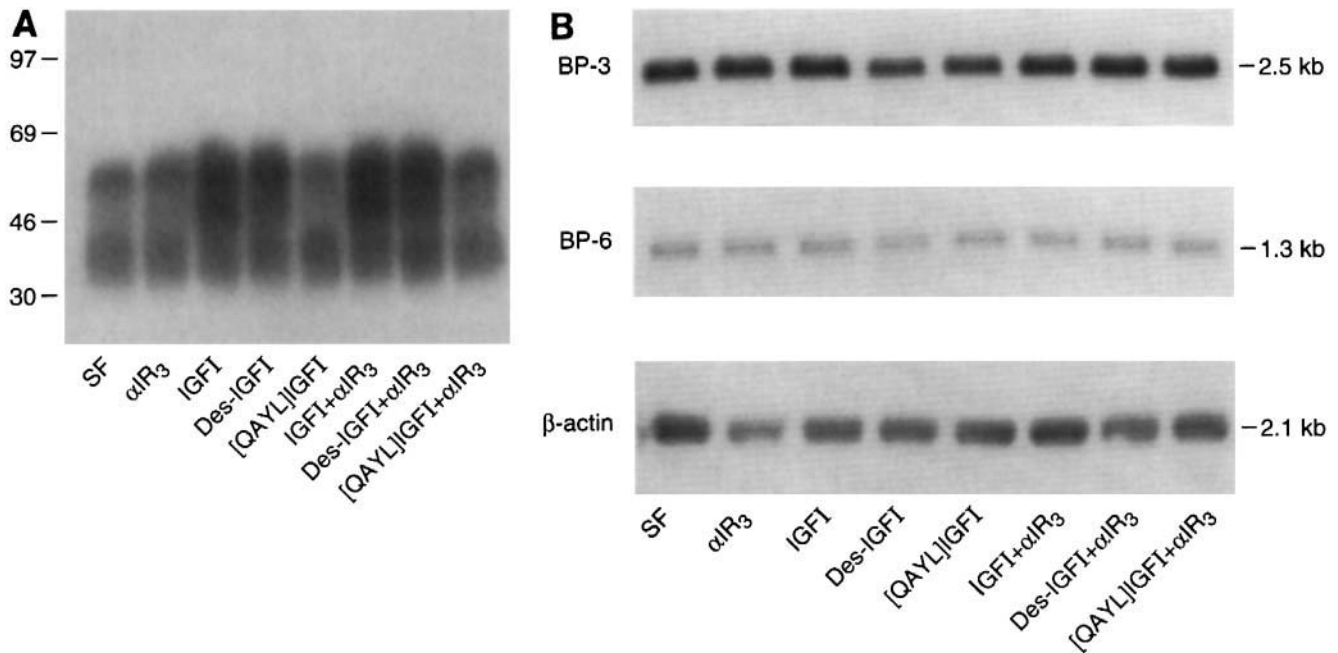
Fig. 1. IGFBP profile after IGF-I or IGF-I analogue treatment. RPE cells were plated at 2×10^6 cells/cm^2, grown for 7 d in MEM + 20% CS, rinsed in MEM as outlined in Materials and Methods and treated with either 1 μg/ml α-IR$_3$ alone, 3 nM IGF-I, 3 nM des(1-3) IGF-I, or 3 nM [QAYL]IGF-I ± 1 μg/ml α-IR$_3$. (A) Western ligand blot of RPE conditioned media. 24 h after treatment, immediately prior to RNA isolation, conditioned media was collected, concentrated and Western ligand blots were performed as outlined in Materials and Methods. Molecular weight markers are indicated on the left. 1 μg/ml α-IR$_3$ had no effect on the 46,000 Mr band in the serum free (SF) control or in the presence of IGF-I or IGF-I analogues. 3 nM IGF-I and 3 nM des(1-3) IGF-I enhanced the presence of the 46,000 Mr band while 3 nM [QAY-

L]IGF-I had no effect over that of the SF control. (B) Autoradiograph from a Northern blot of IGFBP-3, IGFBP-6 and β-actin mRNA. RNA was isolated 24 h after treatment. Northern analysis was performed with ^{32}P-labeled cDNA probes for IGFBP-3, IGFBP-6 and β-actin, as indicated in Materials and Methods. RNA transcript sizes in kb are listed at the right of each autoradiograph. Autoradiographs exposed for 1 day (IGFBP-3), 2 days (IGFBP-6), and 1 day (β-actin). Densitometric analyses of autoradiographs from multiple exposures of the experiments shown in A and at least one additional experiment were quantitated using relative OD as described in Materials and Methods. There were no differences among treatment groups (data not shown).

0.1% Tween 20 and two times for 15 m at 4°C in Western ligand blot saline, membranes were dried in air and exposed to X-Omat film (Eastman Kodak, Rochester, NY) at −70°C for 1–7 days in the presence of an intensifying screen.

RESULTS
Effects of IGF-I and IGF-I analogues on IGFBP gene and protein expression

IGF-I can control IGFBP gene and protein expression in multiple cell types (Clemmons, 1991a). To initially examine the effect of IGF treatment on IGFBP protein expression, RPE cell conditioned medium was analyzed by Western ligand blotting. Ligand blots of conditioned media from cells treated with IGF-I showed increased intensity in the 46,000 Mr band (presumed to be IGFBP-3) when compared to untreated cells (Fig. 1A). To determine if binding of IGF-I to IGFBP-3 was required for these effects, cells were treated with IGF-I analogues. Des(1-3)IGF-I has a sixfold increased affinity for the IGF-I receptor and, at concentrations ⩾1 nM, an equal affinity to IGFBP-3 as IGF-I (Forbes et al., 1988). [QAYL]IGF-I binds and activates the IGF-I receptor with normal affinity but has a 600-fold reduced affinity for IGFBP-3 (Cascieri et al., 1991). Des(1-3)

IGF-I produced the same pattern of IGFBP band intensities as IGF-I. In contrast, treatment with [QAYL]IGF-I reduced the band intensities to the level of untreated cells (Fig. 1A). Addition of α-IR$_3$, the blocking antibody against the IGF-I receptor, had no effect on the increased intensity of the 46,000 Mr band observed after IGF-I or des(1-3)IGF-I treatment (Fig. 1A).

Northern blots were prepared from RPE cells treated for 24 h in serum-free media alone or with the addition of 3 nM IGF-I, des(1-3)IGF-I, or [QAYL]IGF-I, in the presence or absence of α-IR$_3$. There were no observed changes in the 2.5 kb IGFBP-3 transcript or the 1.3 kb IGFBP-6 transcript with these treatments (Fig. 1B).

To examine if changes in cell number were contributing to the differences in IGFBP patterns observed with Western ligand blotting (although equal amounts of protein were electrophoresed for each treatment), cells were plated in MEM alone or with the addition of IGF-I, des (1-3)IGF-I, or [QAYL]IGF-I. Cell proliferation was determined using the MTT assay (Hansen et al., 1989). After 48 h, 3 nM IGF-I, des(1-3)IGF-I and [QAYL]IGF-I each increased cell number when compared to serum-free treatment (21, 22, and 30%, respectively); however, there were no statistically significant differences in the magnitude of the increases between growth factor treatments.

Serum dependent changes of IGFBP gene and protein expression

Because treatment of cultures with IGF-I did not affect the abundance of IGFBP-3 mRNA, we speculated that other growth factors might potentially modulate IGFBP-3 mRNA. As an initial step, we examined the effect of increasing concentrations of calf serum on IGFBP-3 mRNA production. We reasoned that if serum treatment had an effect on IGFBP-3 mRNA, then candidate growth factors, other than IGF-I, might also modulate IGFBP-3 mRNA. Serum treatment increased the abundance of the 2.5 kb IGFBP-3 transcript in a dose-dependent manner with a twofold increase in IGFBP-3 mRNA when cells were grown in 20% calf serum (Fig. 2). In contrast, there was no effect on IGFBP-6 mRNA (Fig. 2).

We then surveyed the effects of candidate growth factors and the cAMP agonist, forskolin, on IGFBP-3 and -6 gene expression. Insulin, platelet-derived growth factor, epidermal growth factor, acidic and basic fibroblast growth factor, and forskolin had no effect on IGFBP-3 or IGFBP-6 gene expression (Fig. 3). Western ligand blots of conditioned media of growth factor treated cells revealed the expected 34,000 and 46,000 Mr proteins (Fig. 4). In two separate experiments, comparing the effects of forskolin, the candidate growth factors, and IGF-I on IGFBP secretion, only IGF-I significantly enhanced the presence of the 46,000 Mr protein (Fig. 4). Forskolin and the candidate growth factors, except for insulin, produced a modest increase in the 46,000 Mr protein when compared to untreated cells (Fig. 4).

DISCUSSION

Cell specific expression of IGFBPs may allow individual cell types to influence IGF function in the pericellular microenvironment (Clemmons, 1991a,b; Binoux et al., 1991; Minuto et al., 1991; Rutanen and Pekonen, 1990). In the current study, we examined growth factor regulation of IGFBP gene expression and secretion in human RPE cells. We have previously reported that human RPE cells express IGFBP-3 and IGFBP-6 mRNA and secrete two IGFBPs with Mr estimates of 46,000 and 34,000 (Randolph et al., 1993). The specific identities of these IGFBPs have not been determined, although we speculate that the 46,000 Mr form represents IGFBP-3 and the 34,000 Mr form represents IGFBP-6 (based on its Mr and preferential binding of IGF-II) (Randolph et al., 1993).

In the present study, IGF-I had no effect on constitutive IGFBP-3 or IGFBP-6 mRNA expression. Similarly, the IGF-I analogues, des(1-3)IGF-I and [QAYL]IGF-I, and the IGF-I receptor antibody, α-IR₃, did not alter IGFBP mRNA expression. These results imply that neither sequestration of IGF-I by extracellular IGFBPs nor constitutive IGF-I secretion was responsible for the lack of an IGF-I effect on IGFBP gene expression. Like human RPE cells, cultured bovine kidney epithelial cells also express IGFBP-3 mRNA but, unlike RPE cells, IGF-I treatment results in a twofold increase in IGFBP-3 mRNA (Cohick and Clemmons, 1991). In bovine fibroblasts, treatment with IGF-I also increases IGFBP-3 mRNA by sixfold (Bale and Conover, 1992). In
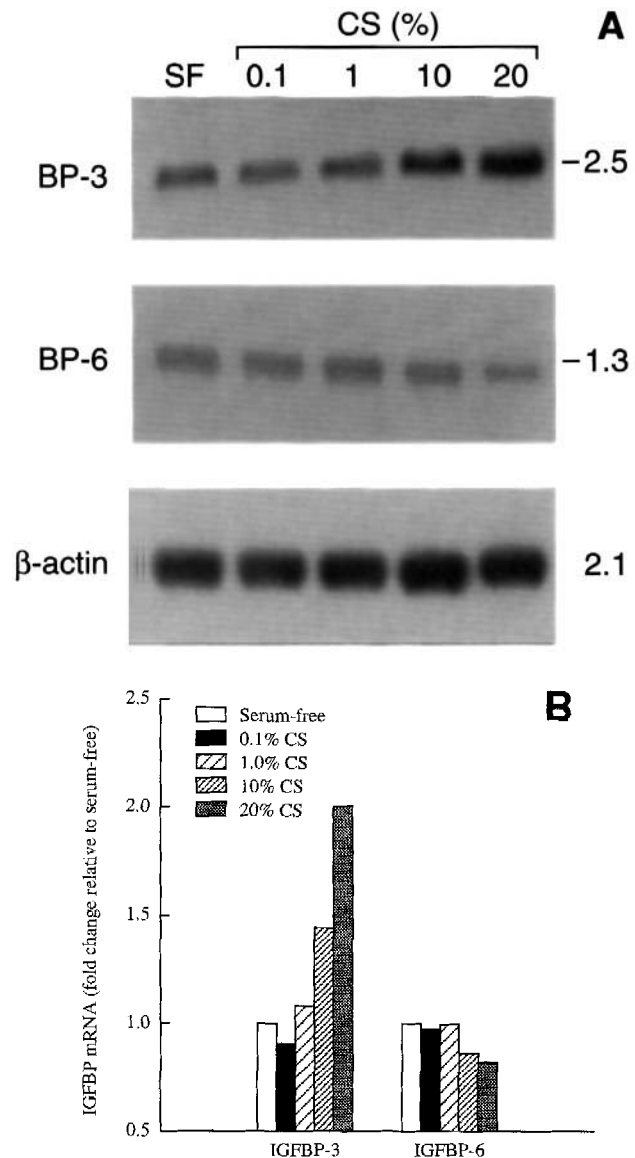

Fig. 2. Effects of serum on IGFBP mRNA. RPE cells were plated at 2 × 10⁶ cells/cm² for 3 d in MEM + 20% CS, rinsed in MEM 3 times, and then treated with the indicated concentrations of calf serum (CS). (A) Autoradiograph from Northern blot of IGFBP and β-actin mRNA. RNA was isolated after 3 d in the indicated serum concentrations. Northern analysis was performed with ³²P-labeled cDNA probes as indicated in Materials and Methods. RNA transcript sizes in kb are listed on the right of each autoradiograph. Autoradiographs exposed 1 day (IGFBP-3, IGFBP-6 and β-actin). (B) Densitometric analysis of IGFBP-3 and IGFBP-6 mRNA. Autoradiographs from multiple exposures of the experiments shown in (A) were quantitated using relative OD, as described in Materials and Methods. Values are the means of the ratio of IGFBP mRNA relative OD to β-actin relative OD, expressed as percentage of the serum free untreated control. A and B are taken from one of three representative experiments.

contrast, IGF-I has no effect on IGFBP-3 mRNA expression in human fibroblasts (Bale and Conover, 1992; Martin et al., 1992b).
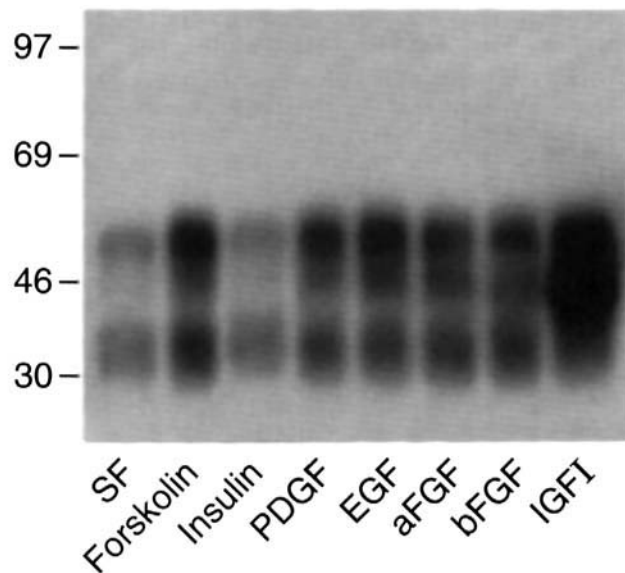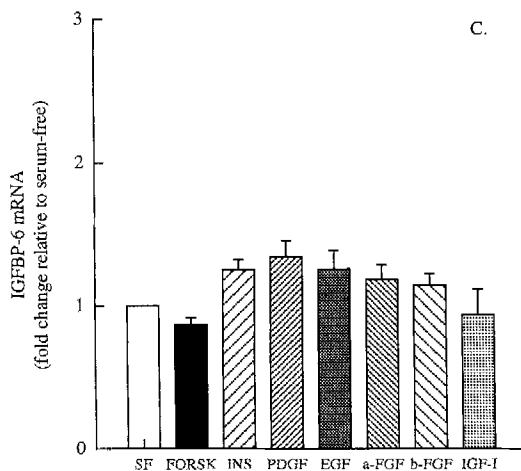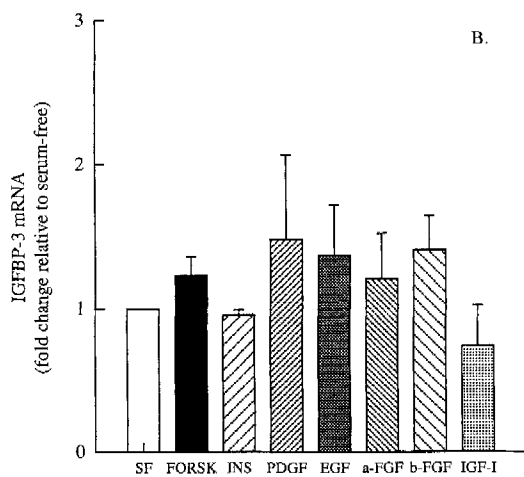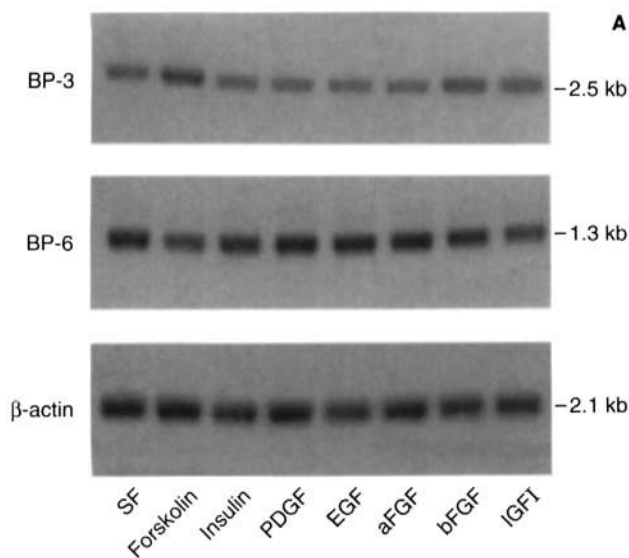
Fig. 4. Western ligand blot of RPE conditioned media after growth factor treatment. Cells were plated, rinsed and dosed as described in Fig. 3. 24 h after treatment, immediately prior to RNA isolation, conditioned media was collected, concentrated and Western ligand blots were performed as outlined in the Materials and Methods. Molecular weight markers are indicated on the left.

The mechanism which accounts for these differential effects of IGF-I on IGFBP-3 gene expression remains unknown. While a species specific difference (human vs. bovine) cannot be discounted, it seems unlikely. Both human fibroblasts and RPE cells are responsive to IGF-I via type I receptors (Sara and Hall, 1990; Martin et al., 1991b; Leschey et al., 1990) and secrete IGFBP-3 into the media (Randolph et al., 1993; Bale and Conover, 1992; Martin et al., 1992b) like their bovine counterparts (Cohick and Clemmons, 1991; Bale and Conover, 1992). Interestingly, different sera are used to maintain human and bovine fibroblasts (calf serum vs. fetal calf serum), suggesting a factor in fetal calf serum might augment the ability of IGF-I to enhance IGFBP-3 gene expression in bovine cells. In support of this idea is the fact that our human RPE cells are maintained in calf serum. However, serum crossover experiments by

Fig. 3. Northern analysis of IGFBP mRNA after growth factor treatment. RPE cells were plated at 2×10^6 cells/cm^2 for 7 days in MEM + 20% CS, rinsed in MEM as outlined in Materials and Methods, and then treated with 5 μM forskolin, 5 μg/ml insulin, 10 ng/ml PDGF, EGF, a-FGF, b-FGF, or 10 nM IGF-I. (A) Autoradiograph from Northern Blot of IGFBP-3, IGFBP-6 and β-actin mRNA. RNA was isolated 24 h after treatment. Northern analysis was performed with ^{32}P-labeled cDNA probes for IGFBP-3, IGFBP-6, and β-actin, as indicated in Materials and Methods. RNA transcript sizes in kb are listed at the right of each autoradiograph. Autoradiographs exposed for 1 day (IGFBP-3), 2 days (IGFBP-6), and 1 day (β-actin). (B), (C). Densitometric analyses of IGFBP-3 (B) and IGFBP-6 (C) mRNA. Autoradiographs from multiple exposures of the experiments shown in A and at least one additional experiment were quantitated using relative OD as described in Materials and Methods. Values are means ± SEM of the ratio of IGFBP-3 mRNA relative OD (B) or IGFBP-6 mRNA relative OD (C) to β-actin relative OD, expressed as percentage of serum free untreated control.

Bale and Conover (1992) (i.e., culturing human fibroblasts in fetal calf serum and bovine fibroblasts in calf serum) did not alter the ability of IGF-I to induce IGFBP-3 mRNA in bovine fibroblasts. Further work is needed to understand the mechanism behind the selective regulation of IGFBP-3 by IGF-I.

Using ligand blot analysis, we have previously shown that RPE cells secrete two IGFBPs, with Mr estimates of 34,000 and 46,000, presumed to represent IGFBP-6 and -3, respectively (Flier et al., 1986). In the current study, IGF-I treatment of human RPE cells increased the media concentration of the 46,000 Mr band. Similar increases in IGFBP-3 in the presence of IGF-I have been reported in multiple cultured cell types, including fibroblasts (Conover, 1991; Bale and Conover, 1992; Martin et al., 1992b), neuroblastoma cells (Neely and Rosenfeld, 1992), and breast cancer cells (Adamo et al., 1992). Our study suggests that IGF-I induced changes in the 46,000 Mr IGFBP are not mediated via the IGF-I receptor. First, [QAYL]IGF-I, an analogue with normal receptor affinity but a marked decreased affinity for IGFBPs, including IGFBP-3 (Cascieri et al., 1991), did not increase RPE IGFBP concentrations. In contrast, des(1-3)IGF-I, an analogue which retains the ability to bind the IGF-I receptor and IGFBP-3 (Forbes et al., 1988) (but not the remaining IGFBPs), increased the concentration of the 46,000 Mr IGFBP to the same degree as IGF-I alone. Thus, an IGF analogue which retains the ability to bind IGFBP-3 mimics IGF-I's effects. Secondly, both IGF-I and the IGF analogues were essentially equipotent at enhancing RPE cell proliferation, eliminating the possibility that the increased concentration of the 46,000 Mr species was simply due to an increase in cell number. Finally, the IGF-I receptor antibody, α-IR$_3$, did not block IGF-I stimulation of the 46,000 Mr IGFBP. Similarly, α-IR$_3$ did not affect the ability of des(1-3)IGF-I to enhance media concentrations of the 46,000 Mr IGFBP. Collectively, these results suggest that the IGF-I coupled increased concentration of the 46,000 Mr IGFBP, presumed to be IGFBP-3, is a receptor independent phenomenon. In agreement with these findings, α-IR$_3$ and [QAYL]IGF-I have no effect on IGF-I coupled increases of IGFBP-3 in human fibroblasts (Conover, 1991). These increases are secondary to the fact that IGF-I can bind to and promote the subsequent release of fibroblast cell surface–associated IGFBP-3 (Martin et al., 1992b). Future experiments directly measuring secreted and cell-associated IGFBP-3 in IGF-I treated RPE monolayers will reveal if a similar mechanism is occurring in human RPE cells.

RPE cells were grown in different concentrations of fetal calf serum to assess whether mitogens other than IGF-I could potentially enhance IGFBP-3 or IGFBP-6 production. A twofold increase in IGFBP-3 mRNA, with no change in IGFBP-6 mRNA, was observed in 20% fetal calf serum. These results led us to treat RPE cells with both the cAMP agonist, forskolin, as well as candidate growth factors known to enhance both IGFBP secretion and gene expression in other cell lines. Forskolin, platelet-derived growth factor, epidermal growth factor, and both acidic and basic fibroblast growth factor produced very modest increases in the secreted 34,000 Mr and 46,000 Mr proteins when com-

pared to untreated RPE cells or RPE cells treated with insulin. These increases, especially in the 46,000 Mr protein, were small when compared to the increases produced by IGF-I treatment. In agreement with these data, fetal calf serum promotes IGFBP-3 production in human skin fibroblasts (Baxter and Martin, 1991). In these cells, not only IGF-I but also at least two other growth factors, epidermal growth factor and transforming growth factor-β, mimic the effects of fetal calf serum (Martin and Baxter, 1991; Martin et al., 1992b). In Swiss 3T3 cells, several heterologous mitogens, including bombesin, vasopressin, platelet-derived growth factor, and epidermal growth factor stimulate IGFBP production. Epidermal growth factor and acid and basic fibroblast growth factors enhance IGFBP-3 secretion in rat astroblast cultures (Loret et al.,1991), while epidermal growth factor alone enhances IGFBP synthesis in adult rat hepatocytes (Barreca et al., 1992).

The exact mechanism which underlies growth factor coupled increases in IGFBPs remains unclear. In RPE cells, the cAMP agonist, forskolin, modestly increased IGFBP secretion but had no effect on steady-state levels of IGFBP mRNA. Agents which elevate intracellular cAMP enhance IGFBP secretion in MDA-231 and HEC-1B cells (Camacho-Hubner et al., 1991). Parathyroid hormone, independent of IGF-I, increases IGFBP secretion in rat osteoblasts through a cAMP dependent pathway (Torring et al., 1991). cAMP also augments IGFBP mRNA in rat hepatoma cells (Unterman et al., 1991). In contrast, forskolin decreases both IGFBP-3 secretion and IGFBP-3 mRNA in porcine granulosa cells (Grimes et al., 1992), as well as Swiss 3T3 cells, where phorbol ester treatment increases IGFBP-3 mRNA (Corps and Brown, 1991). Thus, it is unlikely that a final common pathway will explain growth factor coupled changes in IGFBP secretion and gene expression. Differences likely reflect the availability of growth factor receptors in different cells, the direct interaction of growth factors with IGFs or IGFBPs, or the fact that some growth factors likely activate transcription while others affect mRNA translation or stability.

In summary, our studies suggest that IGFBP production and secretion in human RPE cells is regulated by multiple variables. Most importantly, IGF-I significantly enhances the secretion of the 46,000 Mr IGFBP via a receptor independent mechanism but has no effect on secretion of the 34,000 Mr IGFBP. Serum alone stimulates IGFBP-3 mRNA while multiple candidate growth factors, including IGF-I, and the cAMP agonist, forskolin, have no effect on IGFBP-3 or IGFBP-6 mRNA. Future studies are needed to understand the relationship between IGFBP mRNA regulation and growth factor stimulated IGFBP secretion in human RPE cells.

ACKNOWLEDGMENTS

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