Gene Expression of Insulin-Like Growth Factor-I, Its Receptor and Binding Proteins in Retina Under Hypoxic Conditions

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Hypoxia is the main stimulus for neovascularization in the retina. Insulin-like growth factor-I (IGF-I) is thought to be one of the mediators of this process. Severe persistent hypoxia, as occurs in central retinal artery occlusion, is associated with less retinal neovascularization than relative hypoxia. To study the influence of different types of hypoxia on the IGF system, we used a model of neonatal rat retina that responds with neovascularization to a relative hypoxic stimulus produced by alternating oxygen concentrations in the respired air. We studied the influence of 24-hour hypoxia (10% oxygen), 48-hour hyperoxia (75% oxygen), and relative hypoxia (shifting from 48 hours in 75% oxygen to 24 hours in room air) on the gene expression of IGF-I, IGF-I receptor (IGF-IR), and IGF binding protein-1 (IGFBP-1), IGFBP-2, and IGFBP-3 in retina using a solution hybridization RNase protection assay. Hypoxia induced a significant increase in retinal IGF-IR (178%), IGFBP-2 (227%), and IGFBP-3 (317%) mRNA; however, retinal IGF-I mRNA was reduced, as well as serum growth hormone (GH). Relative hypoxia caused a similar but less pronounced trend in the gene expression of IGF-IR and the binding proteins, whereas retinal IGF-I mRNA was unchanged and serum GH was elevated. Both hypoxia and relative hypoxia may cause IGF system stimulation in the retina through upregulation of IGF-IR and IGFBPs. This stimulation may result in neovascularization. However, during hypoxia, low levels of tissue oxygenation and reduced local production of IGF-I may impede the neovascularization process.

HYPOXIC STIMULI cause neovascularization in many tissues. In the eye, hypoxia induces retinal neovascularization, which is associated with the development of several pathologic eye conditions. Severe hypoxia, as occurs during central retinal artery occlusion, is associated with less neovascularization than relative or transient hypoxia. The chain of events leading to new vessel formation remains to be established, but one of the factors thought to be related to this process is insulin-like growth factor-I (IGF-I).

The angiogenic effect of IGF-I has been demonstrated in the retina[1] and other ocular tissues.[2] Evidence suggesting a role for IGF-I in the pathogenesis of ischemia-induced proliferative diabetic retinopathy (PDR) is based on the well-established association between growth hormone (GH) and PDR.[3] In the early fifties, before the currently standard laser treatment for PDR became widespread, the accepted therapy for PDR was hypophysectomy.[4-6] Subsequently, a long-acting somatostatin analog was used to retard the development of PDR.[7] Since IGF-I mediates most of the tissue actions of GH, it was assumed that this growth factor may play a role in the cascade of events leading to neovascularization.[8] Most diabetic patients have normal serum levels of IGF-I; however, elevated serum levels of IGF-I occur in subjects with rapidly accelerating retinopathy.[9] In one large study of 928 diabetic patients, higher serum levels of IGF-I were associated with an increased frequency of PDR.[10] Local tissue levels of IGF-I may be more important than serum levels. The vitreal IGF-I concentration was found to be increased in diabetic patients undergoing therapeutic vitrectomy,[11] as well as in other conditions that cause retinal ischemia.[12] IGF-I tissue action is closely related to the whole IGF system, which includes IGF-I receptor (IGF-IR), IGF binding proteins (IGFBPs), and proteases of IGFBPs.[13] Vitreous levels of IGFBP-2 and -3 were shown to increase fourfold and 13-fold, respectively, in patients with rubeosis related to retinal ischemia,[12] and to a similar extent in animals with experimental diabetes and retinopathy.[14] However, the source of the excess IGFBPs and IGF-I in the vitreous, whether local or serum-derived, was not studied.

Alternations of the oxygen concentration (relative hypoxia) are associated with retinopathy of prematurity (ROP) in neonates. This association was well-established both in premature infants and in term experimental animals exposed to alternating levels of oxygen in the first days after birth, before the development of normal retinal vasculature.[15,16] Recently, the essential role of GH and IGF-I in the development of retinal neovascularization in a ROP model in neonatal mice has been demonstrated.[17]

We studied the influence of hypoxia, relative hypoxia, and hyperoxia on IGF-I, IGF-IR, and IGFBP-1, -2, and -3 gene expression in the retina of 8-day-old newborn rats. We chose a model of neonatal rat retina because it is known to produce neovascular proliferation under altnerating oxygen concentrations.[18]

MATERIALS AND METHODS

Animals

Newborn Hebrew University Sabra rats were studied. Each litter of 10 newborn rats with a lactating rat was kept separately. Lactating rats had free access to standard rat chow and tap water. The animals were maintained and treated in accordance with the Association for Research in Vision and Ophthalmology Statement for the Use of Animals in Ophthalmic and Vision Research.

We studied the gene expression of IGF-I, IGF-IR, and IGFBP-1, -2, and -3 in the retina of newborn rats under the following experimental settings: hyperoxia, normoxia, hypoxia, and relative hypoxia. In each experiment, a lactating rat with a litter of 10 newborn rats were placed in

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Submitted October 2, 1997; accepted April 17, 1998.

Supported by a grant from the Pauline and Joseph Fried Family and a grant from the Chief Scientist, Ministry of Health, Israel.

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0026-0495/98/4711-0006$03.00/0

a sealed chamber with a controlled oxygen concentration. A mixture of oxygen and room air was used to achieve hyperoxia (75% ± 5% oxygen) starting at day 6 after birth until day 8 (48 hours). A mixture of room air with nitrogen was used to achieve hypoxia (10% ± 1% oxygen) starting at day 7 until day 8 (24 hours). Relative hypoxia was induced by exposing animals to 75% ± 5% oxygen for 48 hours (days 5 to 7) and then changing to room air for 24 hours (days 7 to 8). A minimal flow of 2 L/min was used during the experiments. The oxygen concentration was monitored using a Hudson oxygen analyzer (catalog no. 5577; Bemed, Israel). The newborn rats were killed under ether anesthesia on day 8 after birth. Blood was drawn from the abdominal aorta. The eyes were enucleated, and the retinas were separated within 1 minute in a dish with phosphate-buffered saline and then promptly placed in a Tri Reagent solution (Molecular Research Center, Cincinnati, OH), homogenized by a polytron, and stored at −70°C till further processing. Blood samples were centrifuged for 15 minutes at 2,000 rpm, and the serum was separated and stored at −70°C for measurement of GH, IGF-I, and IGFBPs levels.

Serum GH and IGF-I Assays

Serum rat GH (rGH) levels were measured by radioimmunoassay (RIA) using a specific polyclonal rabbit rGH antibody and rGH as standard. Reagents for the RIA were obtained from Amersham (Amersham International, Bucks, UK). Intraassay and interassay coefficients of variation were less than 5% and 10%, respectively. The serum IGF-I level was measured after extraction with acid-methanol (30 μL serum and 750 μL acid methanol).[19] The mixture was incubated for 2 hours at room temperature and centrifuged, and 25 μL of the supernatant was diluted 1:200 before analysis. Serum IGF-I was determined by RIA using a polyclonal rabbit antibody (Nichols Institute Diagnostics, San Juan Capistrano, CA) and recombinant human IGF-I as standard (Amersham International). Mono-iodinated IGF-I, ^{125}I-(Tyr31)-IGF-I, was obtained from Novo-Nordisk (Bagsvaerd, Denmark). When exposing the serum extract to Western ligand blotting (WLB), no IGFBPs could be identified, and furthermore, semilogarithmic linearity of biosynthetic IGF-I and serum extracts was observed, indicating antigen similarity and no IGFBP interference in the RIA. Intraassay and interassay coefficients of variation were less than 5% and 10%, respectively.

WLB for Serum IGFBPs

Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) and WLB were performed according to the method of Hossenlopp et al[20] as previously described.[21] Two microliters of serum was subjected to SDS-PAGE (10% polyacrylamide) under nonreducing conditions. The electrophoresed IGFBPs were transferred by electroelution onto nitrocellulose paper (Schleicher & Schuel, Munich, Germany), and the membranes were incubated overnight at 4°C with approximately 500,000 cpm ^{125}I-IGF-I (specific activity, 2,000 Ci/mmol) in 10 mL 10-mmol/L Tris hydrochloride buffer (TBS) containing 1% bovine serum albumin and 0.1% Tween (pH 7.4). The membranes were washed with TBS, and after drying overnight, the nitrocellulose sheets were autoradiographed with Kodak (Rochester, NY) X-AR film and exposed to a Du Pont-New England Nuclear (Boston, MA) enhancing screen at −80°C for 3 to 7 days. Specificity of IGFBP bands was ensured by competitive coincubation with unlabeled IGF-I purchased from Bachem (Bubendorf, Switzerland). The WLB method yielded five different bands of IGFBPs in serum with an apparent molecular weight of 38 to 42 kd (doublet), 33 kd, 30 kd, and 24 kd. The doublet band corresponds to the acid-stable IGF-binding subunit of IGFBP-3 and the 24-kd band to IGFBP-4, while the 30- to 33-kd band may represent IGFBP-1, IGFBP-2, and IGFBP-5. When the serum was immunoprecipitated with specific rat IGFBP-1, IGFBP-2, and IGFBP-5 antibodies kindly provided by Dr N. Ling (Neurocrine Biosciences, San Diego, CA), IGFBP-2 appeared as a single 33-kd band and IGFBP-1 as a single 30-kd band, whereas no band could be visualized for IGFBP-5.

Solution Hybridization RNase Protection Assay

Gene expression of IGF-I, IGF-IR, IGFBP-2, and IGFBP-3 was analyzed by a solution hybridization RNase protection assay performed as follows. Total RNA was prepared from the retinal tissue of individual rats with Tri Reagent according to the method of Chomczynski[22] and quantified by absorbance at 260 nm. The integrity of the RNA and accuracy of the spectrophotometric determinations were assessed by visual inspection of the ethidium bromide–stained 28S and 18S ribosomal RNA bands after agarose formaldehyde gel electrophoresis of 5-μg aliquots as described previously.[23] The pT7 RNA 18S (Ambion, Austin, TX) was used as the antisense control to ensure that equal amounts of RNA were loaded in each lane of the gel. The size of the protected fragment was 80 bases. IGF-I, IGF-IR, IGFBP-1, IGFBP-2, and IGFBP-3 riboprobes were a generous gift from Derek LeRoith (National Institutes of Health [NIH], Bethesda, MD).

The antisense RNA probe used to detect IGF-IR mRNA has been described previously.[24] This transcript contains 40 bases of vector sequence and 265 bases complementary to 15 bases of 5′-untranslated sequence and to the region encoding the signal peptide and the first 53 amino acids of the IGF-IR-a subunit. On hybridization of this RNA probe with IGF-IR mRNA and subsequent RNase digestion, a protected band of 265 bases was obtained.

The riboprobe used to measure IGF-I mRNA levels has been described previously.[25] This probe allows detection of both IGF-I mRNA species encoding the IGF-Ia and IGF-Ib prohormones. Only IGF-Ia mRNA levels that constitute greater than 90% of the total IGF-I message and correlate with the levels of IGF-Ib mRNA were measured in this study.

IGFBP-1 mRNA was measured using an antisense probe derived from a rat IGFBP-1 cDNA clone isolated from a dexamethasone-treated H-4 11-E-C3 hepatoma cell library (Derek LeRoith, NIH, Bethesda, MD). The size of the protected band obtained by hybridizing this antisense RNA probe with IGFBP-1 mRNA was 203 bases. IGFBP-2 mRNA was measured using an antisense probe of 585-basepair fragments corresponding to nucleotide 502 to 1087 from the coding region.[26] The IGFBP-3 mRNA measurement has been previously described.[27] The size of the protected band obtained by hybridizing this antisense RNA probe with IGFBP-3 mRNA was 493 bases.

Solution hybridization RNase protection assays were performed as previously described.[23] Briefly, 10 μg total RNA was hybridized with 1×10^6 dpm ^{32}P-labeled antisense RNA probes. One nanogram of total RNA was hybridized with 18S antisense RNA probe. The hybridization was performed at 45°C for 16 hours in a buffer containing 80% formamide. After hybridization, RNA samples were digested with RNase A and T1, and the protected hybrids were extracted with phenol-chloroform, ethanol-precipitated, and electrophoresed on 8% polyacrylamide–8-mol/L urea denaturing gel. Multiple autoradiograms from each gel were scanned by a densitometer connected to an Apple (Cupertino, CA) Macintosh computer.

Statistics

Changes in the signal were expressed as a percent of the mean control (normoxia) value for each gel. We used a one-tail Student's t test assuming unequal variance to calculate P values. A P value less than .05 was regarded as significant. Results are given as the mean ± SEM.

RESULTS

A total of 144 8-day-old rats were evaluated (Table 1). Due to the proximity of the bands, it was possible to evaluate IGF-I and IGF-IR mRNA on the same gel (Fig 1), so the number of

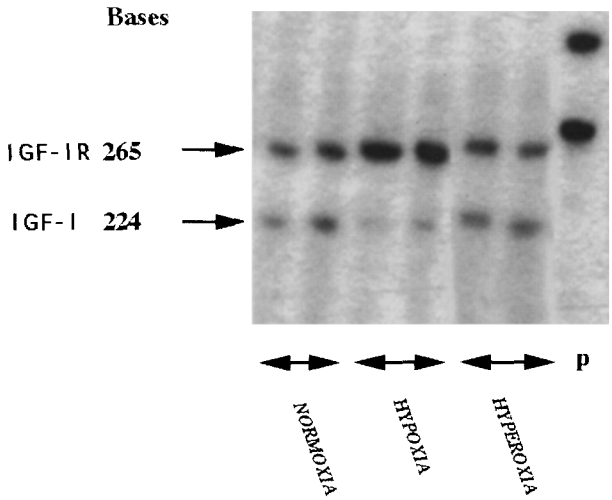

Fig 1. IGF-I and IGF-IR gene expression in the retina by solution hybridization RNase protection assay. Results are representative of 2 animals from each group: normoxia, hypoxia, and hyperoxia. Arrows at left denote position and size of the protected probe bands; p indicates the native probes.

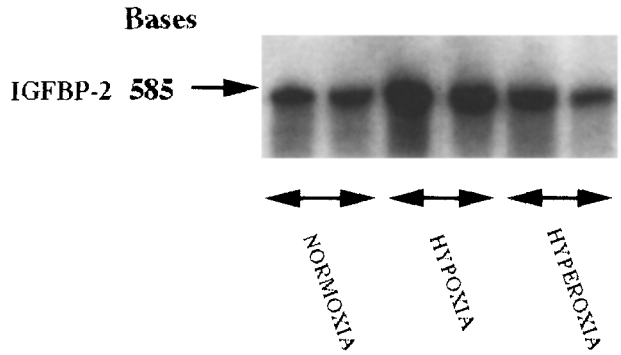

Fig 2. IGFBP-2 gene expression in the retina by solution hybridization RNase protection assay. Results are representative of 2 animals from each group: normoxia, hypoxia, and hyperoxia. Arrow at left denotes position and size of the protected probe band.

samples in Table 1 rows 1 and 2, is overlapping and noncumulative. We were able to extract 25 to 40 μg retinal RNA per animal (two retinas). This amount was generally sufficient for two gels and RNA quality control.

Figure 1 shows gel electrophoresis results for IGF-I and IGF-IR mRNA. Quantification densitometry analysis of the gel showed that IGF-I mRNA in the hypoxic retina was significantly reduced compared with that found in normoxia. In contrast to its influence on IGF-I mRNA, 24-hour hypoxia caused a significant increase of IGF-IR, IGFBP-2, and IGFBP-3 gene expression (Figs 1 to 3 and Table 1). IGFBP-1 remained undetected under various oxygen concentrations. Relative hypoxia produced a similar but less marked increase in IGF-IR, IGFBP-2, and IGFBP-3 gene expression; however, IGF-I mRNA was unchanged. Hyperoxia for 48 hours did not change IGF system gene expression compared with the control. Retinal 18S mRNA was similar in normoxia (2,060 ± 150 densitometry units [DU]), hypoxia (1,950 ± 150 DU), relative hypoxia (1,890 ± 120 DU), and hyperoxia (1,850 ± 110 DU) as measured by densitometry.

Serum IGF-I levels were not significantly changed under the different experimental conditions. A trend toward slightly higher levels of IGF-I after exposure to hypoxia and to relative hypoxia was observed. GH levels were significantly reduced after 24 hours in a hypoxic environment and were significantly elevated 24 hours after exposure to relative hypoxia (Table 2).

Serum levels of IGFBPs are summarized in Table 2, and a representative WLB of serum IGFBPs is shown in Fig 4. IGFBP-2 was the most abundant of the serum IGFBPs evaluated, while IGFBP-3 was the least abundant. The increase in IGFBP-3 during hypoxia was statistically significant ($P = .006$). A similar trend, albeit not statistically significant, was found in IGFBP-2 during hypoxia ($P = .09$). IGFBP-4 was elevated during relative hypoxia, and the IGFBP-1 level was not affected by the oxygen concentration.

DISCUSSION

We have demonstrated significant changes in IGF system gene expression in the retina after exposure to hypoxia and to relative hypoxia. These changes point to the role of the IGF system in the retinal response to hypoxia, and indicate that different types of hypoxia produce similar patterns of IGF system response.

We showed a decrease in retinal IGF-I production during hypoxia. Similarly, Lee et al[28] reported down-regulation of IGF-I gene expression 24 hours after ischemia in neonatal rat brain. However, high vitreal levels of IGF-I were reported in diabetic and nondiabetic patients with ischemic retinal conditions.[12,14] The discrepancy may be related to the fact that the increased permeability of blood vessels associated with ischemic retinopathy causes leakage of serum IGF-I into the vitreous (normal serum IGF-I levels are about 50 times higher than in the vitreous).[29] It was previously shown that hypoxic-ischemic brain injury may enhance IGF-I movement into the tissues.[30] The accumulation of IGF-I in the eye may be enhanced due to an increased binding of IGF-I by its receptor and binding proteins, which are upregulated locally. These

Table 1. IGF System Gene Expression in the Retina Under Different Experimental Conditions

	Normoxia		Hypoxia			Relative Hypoxia			Hyperoxia		
Gene	Mean ± SE (%)	No.	Mean ± SE (%)	No.	*P*	Mean ± SE (%)	No.	*P*	Mean ± SE (%)	No.	*P*
IGF-I	100 ± 9	21	84 ± 8	16	.004	106 ± 12	9	.22	99 ± 14	11	.44
IGF-IR	100 ± 11	21	178 ± 29	16	.00005	132 ± 19	9	.007	115 ± 14	11	.06
IGFBP-2	100 ± 19	13	227 ± 53	15	.0002	173 ± 45	10	.006	125 ± 40	9	.14
IGFBP-3	100 ± 22	11	317 ± 81	9	.001	177 ± 27	15	.0001	109 ± 43	5	.37

NOTE. The results are the mean value as compared with the mean value for controls (normoxia = 100%). *P* values were calculated by Student's *t* test assuming unequal variance.

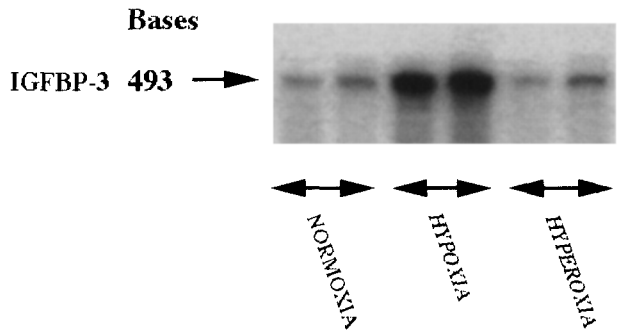

Fig 3. IGFBP-3 gene expression in the retina by solution hybridization RNase protection assay. Results are representative of 2 animals from each group: normoxia, hypoxia, and hyperoxia. Arrow at left denotes position and size of the protected probe band.

changes may lead to an increase in the IGF system concentration and activity in the retina even while local production of IGF-I is decreased during hypoxia. Moreover, during relative hypoxia, IGF-I gene expression was not decreased. Serum IGF-I levels were not significantly changed under the different experimental conditions, although a trend toward slightly higher levels of IGF-I was observed after exposure to hypoxia and to relative hypoxia.

We found no IGFBP-1 mRNA in the retina, in accordance with other studies.[31] IGFBP-2 and -3 gene expression in the retina was significantly upregulated during hypoxia. This finding is consistent with major elevations of IGFBP-2 and -3 levels in the vitreous of diabetic patients and patients with other neovascular eye disease induced by local retinal ischemia.[12,14] Ischemia was previously shown to upregulate the expression of IGFBP-2[32] and other IGFBPs[33] in rat brain after transient hypoxic-ischemic injury produced by carotid artery ligation. The exact role of the different IGFBPs is not known, although it has been shown that via their binding of IGF-I, they can either inhibit or enhance its interaction with the IGF-IR.[13] Insight into the function of IGFBP-2 and -3 may be inferred from their different rates of prevalence in diverse biological settings. The presence of IGFBP-2 in the retina at the time of growth of new blood vessels,[34] as opposed to its absence in the adult rat retina,[31] suggests its role in promoting the local anabolic action of IGF-I during development of new vessels in the retina. IGFBP-3 has been shown to enhance the effect of IGF-I on cellular proliferation.[35] The role of IGFBP-3 in mediating angiogenesis was suggested by its 13-fold elevation in the vitreous of patients with PDR and rubeosis as opposed to no significant elevation in patients with PDR without rubeosis.[12] Inhibition of IGF-I action by addition of binding proteins was demonstrated in several studies; however, most used a one-compartment model, which poorly simulates the in vivo situation. IGFBP-3 in pericellular fluid is thought to inhibit IGF-I action by forming high-affinity soluble complexes that prevent IGF-I from binding to signaling IGF-IR. However, cell-associated IGFBP-3 was suggested to potentiate IGF-I action by blocking down regulation of the IGF-IR.[36] Other studies have suggested that binding of IGF-I to IGFBP-3 may prolong its half-life significantly,[37] producing a large storage of IGF-I. High levels of expression of transgenic IGFBP-3 in mice were shown to inhibit apoptotic involution of mammary glands after cessation of lactation.[38]

The elevated serum level of IGFBP-3 during hypoxia may be the result of increased production in different tissues, similar to our findings in the retina. However, the significance of this elevation is unclear.

The twofold increase in IGF-IR gene expression under hypoxia is of particular interest. Keeping in mind the increased, probably serum-derived, IGF-I levels, as reported in similar conditions,[12] this upregulation of IGF-IR may produce a significant overstimulation of the IGF system in the retina. Analogous to our findings, in a model of streptozotocin-induced diabetes in the rat, IGF-I mRNA levels in the kidney tend to decrease, while IGF-IR and IGFBP-1 mRNA are increased in parallel with an increase in IGF-I protein and activity as evidenced by renal hypertrophy.[39,40]

We found no significant changes in the IGF system during hyperoxia. This may indicate that gene expression of the IGF system is not regulated by oxygen tension per se, but by a second messenger related to hypoxia. It is well known that hyperoxia causes an arrest in the development of the normal retinal vasculature in neonates.[41] Since we found no downregulation of IGF-I, IGF-IR, and IGFBP-1, -2, and -3 under hyperoxia, we assume this arrest is related to a change in other growth factors (such as vascular endothelial growth factor) or other components of the IGF system, not examined herein (eg, IGF-II and IGFBP-4, -5, and -6) that are important in the process of angiogenesis. Vascular constriction in response to high oxygen levels has also been proposed as a mechanism underlying the arrest in normal vascular growth.[42]

We found that the effect of relative hypoxia, ie, a change from

Table 2. Serum Levels of GH, IGF-I, and IGFBP-1, -2, -3, and -4 Under Different Experimental Conditions

	Normoxia		Hypoxia			Relative Hypoxia			Hyperoxia		
Parameter	Mean ± SE (%)	No.	Mean ± SE (%)	No.	*P*	Mean ± SE (%)	No.	*P*	Mean ± SE (%)	No.	*P*
GH	44 ± 6	10	32 ± 3	20	.002	61 ± 14	7	.03	48 ± 8	20	.21
IGF-I	94 ± 10	10	111 ± 19	20	.07	102 ± 3	7	.09	90 ± 5	18	.22
IGFBP-1	115 ± 40	8	123 ± 54	17	.40	116 ± 31	7	.49	95 ± 20	16	.21
IGFBP-2	851 ± 168	8	1,003 ± 144	17	.09	848 ± 146	7	.49	831 ± 170	16	.44
IGFBP-3	38 ± 32	8	94 ± 14	17	.006	68 ± 12	7	.06	47 ± 22	16	.33
IGFBP-4	222 ± 26	8	188 ± 49	17	.12	306 ± 64	7	.02	216 ± 32	16	.39

NOTE. IGF-I and GH levels are μg/L; IGFBP levels are arbitrary absorption U/mm. *P* values were calculated by Student's *t* test assuming unequal variance.

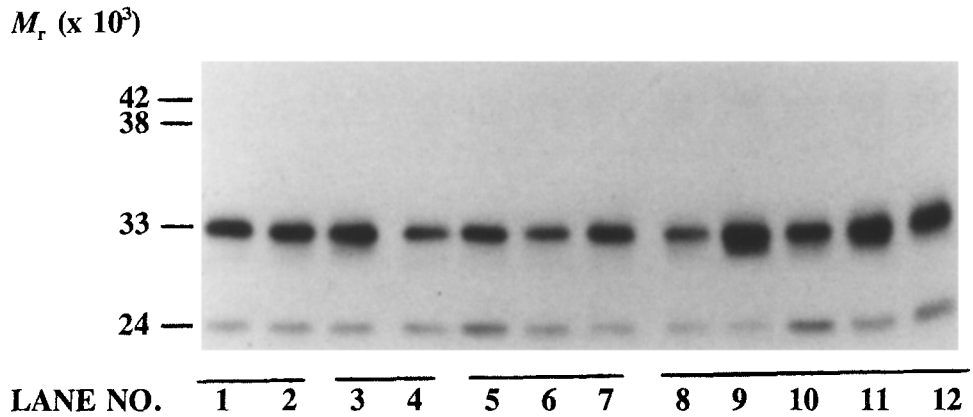

Fig 4. Representative WLB autoradiogram of serum samples under different experimental settings: normoxia (lanes 1 to 2), hyperoxia (lanes 3 to 4), relative hypoxia (lanes 5 to 7), and hyperoxia (lanes 8 to 12). The different IGFBPs appear in the following order: 38- to 42-kd IGFBP (IGFBP-3 [doublet]), 30- to 33-kd IGFBPs (IGFBP-2 and IGFBP-1), and 24-kd IGFBP (IGFBP-4). Note that in newborn rat serum, 30- to 33-kd and 24-kd IGFBPs are quantitatively dominating and IGFBP-3 is the least abundant.

high to normal oxygen tension, is similar to that of true hypoxia. IGF-IR, IGFBP-2, and IGFBP-3 gene expression was increased, although to a lesser degree. However, IGF-I gene expression was not reduced in true hypoxia. Constant hypoxia produces less stimulus for neovascularization in the retina than an alternating oxygen level.[43] It is possible that downregulation of IGF-I gene expression during hypoxia prevents a neovascular response despite the increase in IGF-IR, IGFBP-2 and IGFBP-3 gene expression and the presence of serum-derived IGF-I in the vitreous. We cannot exclude the possibility that local retinal IGF-I has better access to the receptor than serum-derived IGF-I. Normal expression of the IGF-I gene during transient or relative hypoxia in the presence of increased IGF-IR, IGFBP-2, and IGFBP-3, may have a permissive effect on the neovascularization process. It is also possible that reoxygenation or reperfusion is essential to allow actual growth of the new blood vessels, and without a minimal necessary oxygen supply, there will be no neovascularization. In our relative hypoxia experiment, animals were examined following a 24-hour stay in room air, ie, 24 hours after the actual hypoxic stimulus for neovascularization. IGF-IR and IGFBP levels were still high, while the oxygen supply was apparently normal. It is possible that this combination is of critical importance for neovascularization. Thus, the increase in IGF-IR, IGFBP-2, and IGFBP-3 during relative hypoxia will result in stimulation of the IGF system and neovascularization.

Serum GH levels were significantly reduced in the hypoxic environment and significantly elevated after exposure to relative hypoxia. This finding may be related to the fact that the hypoxic stimulus was a severe and sustained 24-hour hypoxia, whereas the relative hypoxia was a transient stimulus. Similarly, it was reported that GH is reduced after severe hypoxia and elevated after transient hypoxia in human neonates.[44] The elevated GH level was not followed by a similar elevation in the IGF-I level. However, the duration of this change was probably too brief. For example, in animals treated with pharmacological doses of GH (ie, 2 mg/kg body weight/d), only modest increases in serum IGF-I were demonstrated after 3 to 5 days of treatment.[45] In contrast, we observed a decrease in retinal IGF-I production following a decrease in serum GH during hypoxia.

In conclusion, we have demonstrated an increase in IGF-IR, IGFBP-2, and IGFBP-3 in the retina during hypoxia and relative hypoxia at the time when these conditions induce neovascularization in the retina. These findings suggest that the IGF system may play an important role in angiogenesis induced by relative tissue hypoxia and ischemia. An increase in IGF-IR, IGFBP-2, and IGFBP-3 gene expression during relative retinal hypoxia may be a stimulus for neovascularization.

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