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Developmentally Regulated Primary Glucocorticoid Hormone Induction of Chick Retinal Glutamine Synthetase mRNA

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We have characterized the glucocorticoid hormone induction of glutamine synthetase mRNA in embryonic chick retinal organ cultures by quantitative dot hybridization using a cDNA clone derived from chick retinal RNA. Hydrocortisone (K_{app} = 3–4 nM) and dexamethasone (K_{app} = 1–2 nM) produce an approximate 30-fold increase in glutamine synthetase mRNA after incubation of organ cultures derived from embryonic day 12 retinae with either hormone for 3 hr. Progesterone is a poor inducer. The glucocorticoid-mediated rise is rapid ($t_{1/2}$ = 2–3 hr) and occurs in the presence of either of the protein synthesis inhibitors cycloheximide or puromycin, indicating that the induction is a primary or direct response to the hormone. However, the magnitude of the hormonal response observed in culture increases markedly during retinal development. These observations, coupled with the previously reported absence of a hormonal induction in embryonic liver, raise the possibility of a synergistic mechanism, involving tissue-specific regulatory molecules in addition to the glucocorticoid hormone receptor, to explain the retinal-specific primary glucocorticoid hormone induction of glutamine synthetase mRNA.

Key words: glucocorticoid hormone induction (of glutamine synthetase) during development, induction of glutamine synthetase, responsiveness during development, tissue-specific induction by glucocorticoid hormones, induction of mRNA (by glucocorticoid hormones), glucocorticoid hormone induction (of glutamine synthetase) in retina

Terminal differentiation of the chick retina is accompanied by a several-hundred-fold rise in the specific activity of the enzyme glutamine synthetase (GS) [1], a rise that is confined to the retinal Müller glial cells [2]. Retinal GS enzyme rises several-fold during the second week of development, begins to increase substantially around embryonic day 16 (E16), and plateaus approximately 1 wk later, soon after hatching [3]. However, glucocorticoid hormones can induce a premature increase in retinal GS enzyme activity, either in ovo [4] or in organ culture [4,5]. In contrast to the developmental and hormonal regulation of GS enzyme activity in the chick retina,

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expression of this enzyme in hepatic tissue is constitutive during terminal differentiation and not glucocorticoid hormone inducible [6,7].

We have recently described the isolation of a GS cDNA clone (pCRGS1) derived from chick retinal poly A^+ RNA [7]. Based on Northern blotting, the clone detects a 3.2-kilobase RNA present in chick retina, brain, and liver. Expression of this RNA is developmentally regulated in the neural tissues but is constitutive in hepatic cells. Moreover, these three tissues respond in a different manner to glucocorticoid hormone challenge; ie, injection of hydrocortisone 21-phosphate into yolk sacs at embryonic day 10 (E10) produces a retinal-specific induction of GS mRNA [7].

Do the tissue-specific responses to glucocorticoid hormones contribute to the distinct patterns of hepatic and retinal GS expression observed during development, and if so, how is this tissue-specific hormonal induction achieved? A single gene must exist in a transcriptionally competent conformer in these tissues and give rise to the RNA detected by pCRGS1, based on Southern blotting experiments using pCRGS1 [7]. Accordingly, tissue-specific expression of divergent glutamine synthetase genes does not present a tenable model.

In order further to assess the physiological relevance of the precocious glucocorticoid hormone induction of retinal GS and better to define potential mechanisms, this report quantitates the glucocorticoid hormone induction of GS mRNA in retinal organ cultures. The magnitude of the hormonal effect supports the contention of Moscona and colleagues that glucocorticoid hormones are important physiological mediators of the developmental rise in retinal GS [1]. In addition, the hormonal induction is primary and increases in magnitude during retinal development. These features suggest that either developmental alterations in the glucocorticoid hormone receptor or additional ancillary factors are required to achieve a primary hormonal response in the retina.

MATERIALS AND METHODS

Retinal Organ Cultures and RNA Dot Blotting

Fertile white leghorn chicken eggs were purchased from SPAFAS (Roanoke, IL) and incubated at 38°C. Embryos were staged according to Hamburger and Hamilton [8]. Retinae in flasks containing Dulbecco's modified Eagle's medium (DME) + 10% fetal bovine serum (charcoal stripped and dialyzed) supplemented with hormones and/or protein synthesis inhibitors where indicated, were flushed with 5% CO_2 and incubated at 37°C with swirling at 70 rpm. In all cases, retinae from three separate organ cultures were pooled prior to RNA isolation by the guanidine thiocyanate method [9].

GS mRNA was quantitated by filter hybridization using [^{32}P]-labeled cRNA probe (specific activity, 0.5–1.0 × 10^9 cpm/μg) derived from pCRGS2 using T3 RNA polymerase. Hybrid-selected translation experiments demonstrated that cRNA probe derived from pCRGS1 was complementary to chick retinal GS mRNA [7]. pCRGS2 contains a 1.2-kilobase-pair (kbp) cDNA insert that is homologous to the 0.8-kbp insert contained in pCRGS1. It was selected from a lambda gt10 cDNA library enriched in chick retinal GS mRNA sequences [7] and subcloned into pBSM13+ (Stratagene, San Diego, CA) (A.P.Y., G.P., and Haifeng Pu, unpublished). After RNA concentration was determined from the OD_{260}/OD_{280} of an aliquot, total retinal RNA's were loaded onto Nytran (Schleicher and Schuell, Keene,

NH) using a dot-blotting manifold. Each sample, containing 6 × SSC(1 × SSC is 0.15 M NaCl + 0.015 M Na citrate, pH 7.0), 2.5 M formaldehyde, and 10 μg RNA (a combination of sample RNA and yeast tRNA), was heated to 65°C for 5 min prior to loading. Hybridization and washing of blots were as discussed in reference 10. Autoradiographs were obtained using Kodak X-Omat film exposed at −80°C in the presence of a DuPont Lightning Plus intensifying screen. Controls lacking retinal RNA gave no autoradiographic signal.

Data Analysis

Autoradiographs were scanned using a Biorad Model 1650 densitometer and Model 1321 recorder. All RNAs were analyzed over at least a 32-fold concentration range (by loading six or more successive twofold dilutions). For samples containing the highest amounts of GS mRNA, the initial RNA loadings produced excessive darkening of the film. For these samples, the signal intensity was not proportional to RNA loaded over the entire 32-fold range. For samples containing the lowest amounts of GS mRNA, the final RNA loadings gave signals that were not above background. For all samples, 3–6 loadings (4–32-fold range) yielded a signal suitable for quantitation. Data shown are means and standard deviations of GS mRNA, based on the densitometric signals obtained in the linear range. They are either normalized relative to an indicated control value or are expressed as arbitrary units obtained by densitometric scanning.

The time-course for the dexamethasone-induced increase in GS mRNA was analyzed as a first-order perturbation (Fig. 1, inset). Accordingly, a semilog plot of $1-[(R_t-R_0)/R_{24}-R_0)]$ vs t was produced [11], where t = the time in organ culture; R_t = the relative level of GS mRNA in steroid-treated retinae at time t; R_0 = the relative level of GS mRNA at time 0; and R_{24} = the relative level of GS mRNA in steroid-treated retinae after 24 hr.

Determination of the Effects of Puromycin or Cycloheximide on Protein and RNA Synthesis

To estimate relative levels of protein synthesis, retinae were cultured in methionine-deficient DME + 10% fetal bovine serum for 30 min in the presence or absence of 2 μg/ml cycloheximide or 2 μg/ml puromycin followed by labeling with 5 μCi L-[^{35}S]-methionine for 30 min and quenching by addition of unlabeled methionine (0.67 mg/ml). After the retinae were washed with ice-cold Tyrode's sterile saline two times, radioactivity incorporated into trichloroacetic acid (TCA)-precipitable material was determined by scintillation counting of precipitates on glass fiber disks (Whatman). All determinations were performed in triplicate. SDS-gel electrophoresis and fluorography of labeled proteins were as discussed previously [7].

To estimate relative levels of RNA synthesis, retinae were precultured for 30 min in the presence or absence of inhibitors in DME + 10% fetal bovine serum followed by labeling for 3 hr with 1 μCi/ml [^{3}H]-uridine (28 Ci/mmol) and assay for incorporation of label into RNA using methods discussed previously [12].

RESULTS

Glucocorticoid Hormone Induction of GS mRNA in Retinal Organ Cultures

Data shown in Figure 1 demonstrate the effect of 50 nM dexamethasone on the level of GS mRNA in retinal organ cultures. Retinae obtained at embryonic day 12

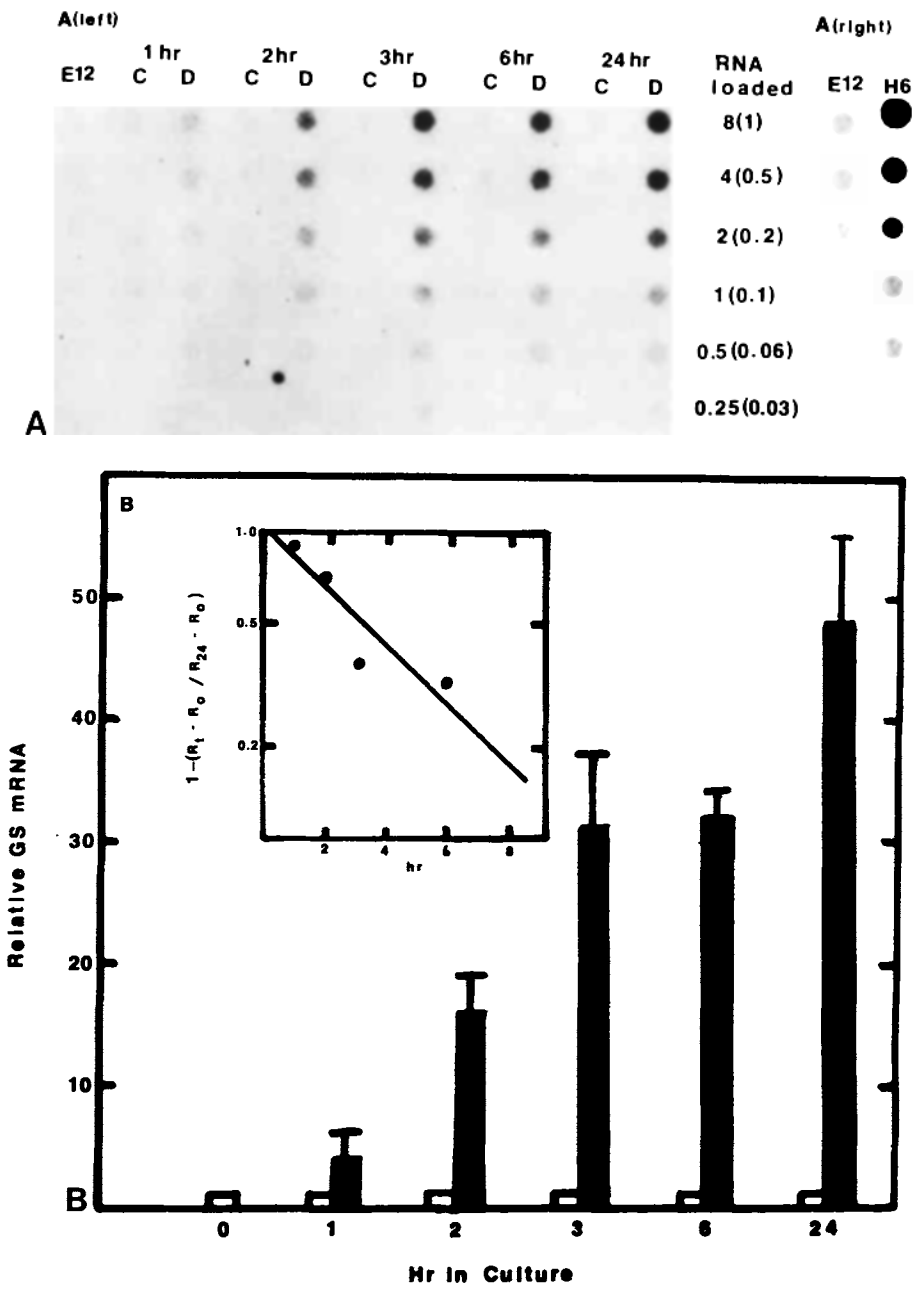

Fig. 1. Dot hybridization of retinal RNA's probed with pCRGS2. (**A**): Autoradiographic data. **Left**: Total cell RNA was isolated directly from E12 retinae or from E12 retinae organ cultured for the indicated time in the absence (C) or presence (D) of 50 nM dexamethasone prior to RNA isolation and dot blotting analysis. **Right**: In a separate experiment, RNA obtained from E12 retinae or from H6 retinae was analyzed. The retinal RNA loaded in each row is indicated (in micrograms). The numbers in parenthesis refer only to the loading of H6 retinal RNA. (**B**): Densitometric analysis. The autoradiographic data were quantitated as discussed in the text. Data shown are means ± standard deviations for the hormone-induced levels of GS mRNA as a function of time of organ culture (black bars) normalized to a value of 1.0 for GS mRNA in E12 retinae. Organ culture without hormone (white bars) did not result in an alteration in the level of GS mRNA. **Inset**: Hormone-induced levels of GS mRNA are plotted as a function of time in organ culture as discussed in the text.

(E12) were cultured in the presence or absence of the glucocorticoid hormone for the indicated times followed by RNA isolation and dot hybridization analysis. Figure 1A shows the autoradiograph obtained after hybridization to [^{32}P]-labeled probe derived from pCRGS2, a chicken glutamine synthetase cDNA clone. The data were quantitated by densitometric analysis and summarized in Figure 1B.

The level of GS mRNA in cultures treated with dexamethasone for 24 hr is approximately 50-fold that of untreated controls. Between E12 and 6 days after hatching (H6), GS mRNA levels rise approximately 130-fold (Fig. 1A). The magnitude of the premature steroid-induced rise in GS mRNA in organ cultures of embryonic retinae approaches that observed during terminal differentiation. These data therefore provide quantitative support for the hypothesis that glucocorticoid hormones are important physiological modulators of the developmental increase in retinal GS, as originally suggested by Moscona and colleagues [3].

The time course for the accumulation of GS mRNA in response to dexamethasone is also of interest. As noted by Berlin and Schimke [13], the kinetics of accumulation of an induced product reflect its turnover rate. Indeed, since cytotoxic inhibitors are not involved [14], such a measurement provides perhaps the most reliable estimation of turnover. In the most straightforward cases, turnover is a first-order process, and its associated half-time can be deduced from semilog plots such as that shown in the inset of Figure 1B [11]. In addition, the period of time required to achieve new steady-state rates of production or turnover after addition of inducer is reflected by the lag phase of this plot.

The replotted data shown in the inset of Figure 1B demonstrate that the lag phase for the glucocorticoid-mediated induction of GS mRNA is roughly 30 min. The lag phase appears too short to accommodate new protein synthesis and suggests that the hormonal effect is direct or primary [15], a finding consistent with previous studies [7] and elaborated below. Moreover, turnover of GS mRNA in steroid-induced E12 retinae has a half-time of 2–3 hr. This rapid time course contrasts sharply with the time course for the rise in GS enzyme activity [3] and mRNA [7] that occurs during development. Therefore, the turnover rate of GS mRNA upon steroid induction does not appear to dictate the time course for appearance of retinal GS enzyme and mRNA in vivo.

We have estimated the relative level of retinal GS mRNA after induction with varying concentrations of hydrocortisone, dexamethasone, or progesterone. Retinae were incubated in the presence or absence of hormone for 3 hr prior to dot hybridization analysis. Although the magnitude of the steroid induction is dependent upon developmental stage (see below), the concentration dependence is similar using E10 or E12 retinae. The results obtained with E12 retinae are summarized in Figure 2. Based on these data, we estimate that K_{app} is approximately 1–2 nM and 3–4 nM for the induction mediated by dexamethasone (Fig. 2A) and hydrocortisone (Fig. 2B), respectively. K_{app} for dexamethasone is in good agreement with the equilibrium dissociation constant for the chick retinal receptor, determined by Scatchard analysis using ^{3}H-dexamethasone (1.3 nM [16]). These data suggest that the hormonal response is proportional to receptor occupancy, a finding that has emerged as a general tenet of steroid hormone action [17]. Scatchard analysis of ^{3}H-hydrocortisone binding to chick retina extracts revealed the presence of two classes of binding sites with dissociation constants of 0.8 and 4.4 nM [16]. Our data suggest that the lower-affinity sites are responsible for the hormonal induction of GS mRNA. In contrast to the

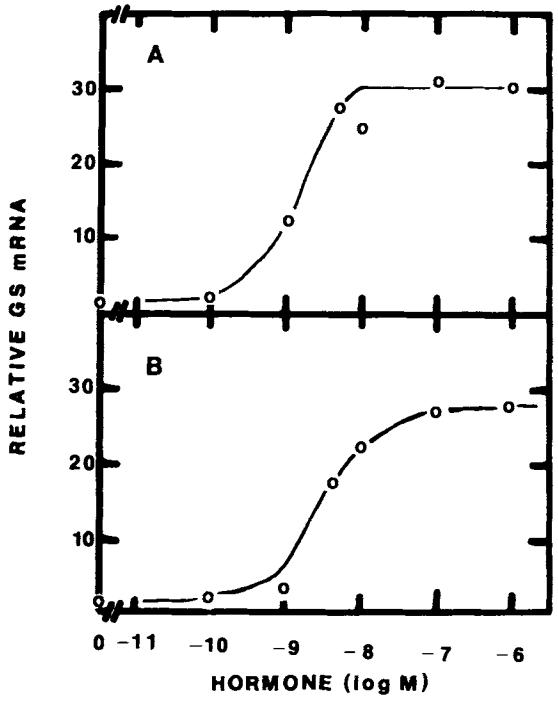

Fig. 2. Dose-response curves for hormonal induction of GS mRNA in E12 retinal organ cultures. Retinae were cultured for 3 hr with indicated concentrations of dexamethasone (**A**) or hydrocortisone (**B**), prior to determination of the relative levels of GS mRNA. Data are normalized relative to the value obtained with retinae cultured in the absence of hormone.

results obtained with dexamethasone and hydrocortisone, progesterone elicited only a modest induction of GS mRNA (1.5–3.5-fold) at concentrations as high as 10 μM (data not shown).

The Glucocorticoid Hormone Induction of GS mRNA Does Not Require Ongoing Protein Synthesis

Hormonal inductions of specific mRNA's can be dependent on ongoing protein synthesis. These secondary effects presumptively involve direct hormonal induction of an ancillary protein that directs accumulation of the measured product mRNA [15]. Because the GS gene is expressed constitutively during liver development and does not appear to be glucocorticoid-hormone inducible in that tissue [6,7], a retinal-specific secondary hormonal induction appeared tenable to us. However, cycloheximide pretreatment of retinal organ cultures did not block the hydrocortisone-mediated induction of GS mRNA, based on Northern blotting [7]. We have extended this initial observation by quantitative analysis of the effects of cycloheximide and puromycin, mechanistically distinct inhibitors of eukaryotic protein synthesis [18], on the dexamethasone-mediated induction of GS mRNA.

Retinae were pre-incubated for 30 min in the presence or absence of 2 μg/ml cycloheximide or 2 μg/ml puromycin. This was followed by induction with or without 50 nM dexamethasone for 2.5 hr prior to dot hybridization analysis. The quantitated data are summarized in Table I. Retinae were also preincubated in the presence or

TABLE I. Effect of Protein Synthesis Inhibitors on the Dexamethasone Induction of Glutamine Synthetase mRNA in Retinal Organ Cultures

Additions to organ culture[a]	E12 retinae				E10 retinae	
	Puromycin		Cycloheximide		Cycloheximide	
	GS mRNA[b]	Fold induction by Dex	GS mRNA[b]	Fold induction by Dex	GS mRNA[b]	Fold induction by Dex
None	1.00 ± 0.25		1.00 ± 0.02		1.00 ± 0.22	
Dex	18.3 ± 4.0	18.3[c]	23.8 ± 4.6	23.8[c]	15.5 ± 2.8	15.5[c]
Protein synthesis inhibitor	0.32 ± 0.07		0.91 ± 0.09		1.26 ± 0.24	
Dex + protein synthesis inhibitor	3.43 ± 1.14	16.4[d]	14.7 ± 2.0	16.1[d]	9.38 ± 2.74	7.4[d]

[a]Retinae were precultured for 30 min in the presence or absence of 2 μg/ml puromycin or 2 μg/ml cycloheximide followed by culture for 2.5 hr in the presence or absence of 50 nM dexamethasone (Dex).

[b]Arbitrary units. Determined by densitometric analysis as discussed in the text. For each experiment, the data are normalized to the level of GS mRNA after culture in the absence of inhibitors or hormone.

[c]Determined by the ratio of GS mRNA (with hormone)/GS mRNA (without hormone) for cultures incubated without protein synthesis inhibitor.

[d]Determined by the ratio of GS mRNA (with hormone)/GS mRNA (without hormone) for cultures incubated with protein synthesis inhibitor.

absence of inhibitors followed by labeling with [^{3}H]-uridine or with L-[^{35}S]-methionine and determination of incorporation of label into RNA and protein, respectively. These data are summarized in Table II.

The relative glucocorticoid hormone induction of retinal GS mRNA was not affected by puromycin, although drug-pretreatment diminished both basal and hormonally induced levels of GS mRNA. Since puromycin pretreatment had a general inhibitory effect on RNA synthesis, based on diminished incorporation of [^{3}H]-uridine into RNA, it is likely that quantitative perturbations produced by puromycin are not restricted to the GS system. These data, therefore, support a primary induction mechanism. However, it is difficult to quantitate the effect of puromycin on the synthesis of functional proteins. Although puromycin treatment did not completely block incorporation of L-[^{35}S]-methionine into trichloracetic acid (TCA)-precipitable material (Table II), the labeled proteins are apt to be truncated [18]. Densitometric scanning of fluorographs obtained after SDS-gel electrophoresis (data not shown) indicates that the amount of labeled high molecular weight proteins is preferentially diminished in puromycin-treated retinae relative to controls. Thus, determination of TCA-precipitable radioactivity appears to overestimate the relative level of synthesis of full-length proteins in puromycin-treated retinae.

Results obtained with cycloheximide provide more definitive support for a primary induction mechanism. A substantial glucocorticoid hormone induction of GS mRNA is observed in the presence of cycloheximide using retinae obtained at E10 and E12. The basal level of GS mRNA is not affected by drug treatment. The diminution in the induced level of GS mRNA after cycloheximide treatment (Table I) is only marginally greater than the general effects on [^{3}H]-uridine incorporation into RNA (Table II). Moreover, cycloheximide, at the concentration used to assay hormonal responsiveness, produced near-complete blockage of protein synthesis (Table II). Taken together, the results obtained with these mechanistically distinct inhibitors confirm that the glucocorticoid hormone induction of GS mRNA is a primary response.

Hormonal Inducibility of GS mRNA Increases During Retinal Development

We have measured the glucocorticoid hormone induction of GS mRNA in retinal organ cultures derived from E8, E10, E12, and E14 eggs. Cultures were incubated for 3 hr in the presence or absence of 50 nM dexamethasone followed by dot hybridization analysis. Quantitation of these data yielded the estimates of GS mRNA shown in Figure 3A, normalized to the level of GS mRNA present in E12 retinae cultured without hormone. Basal levels of GS mRNA increase approximately 3–5-fold between E8 and E12. This might reflect an increase in circulating glucocorticoids during development [19,20] or a hormone-independent increase in GS mRNA. Moreover, during this same period, the glucocorticoid-induced levels of GS mRNA increase nearly 100-fold. In fact, in contrast to the results observed with retinae obtained at E10, E12, or E14, GS mRNA levels are similar after culture of E8 retinae with or without hormone. GS mRNA is also not hormone-inducible in organ cultures of E6 retinae (data not shown).

The onset of hormonal responsiveness occurs during a narrow window of development. GS mRNA is not steroid-inducible in retinae derived from stage 32 embryos (chronologic age ca 7.5 days [8]). However, by stage 34–35 (chronologic age ca 8.5 days), a substantial hormonal induction can be observed (data not shown).

TABLE II. Inhibition of RNA and Protein Synthesis by Puromycin or Cycloheximide in Retinal Organ Cultures

Addition to organ culture prior to labeling[a]	RNA synthesis		Protein synthesis		
	Incorporation of labeled uridine[b]	Relative level of RNA synthesis[c]	Incorporation of labeled methionine[d]		Relative level of protein synthesis[e]
			Experiment 1	Experiment 2	
None	1,359 ± 626		6,350 ± 197	4,102 ± 652	
Puromycin	699 ± 62	0.51	2,383 ± 284		0.38
Cycloheximide	1,012 ± 582	0.74		297 ± 65	0.07

[a]Retinae derived from E12 eggs were precultured for 30 min in the presence or absence of 2 μg/ml puromycin or 2 μg/ml cycloheximide prior to labeling with [^{3}H]-uridine or [^{35}S]-methionine as discussed in the text.

[b]Mean ± standard deviation of perchloric acid precipitable-NaOH soluble cpm/1 μg retinal RNA after subtraction of the background value for three separate determinations.

[c]Ratio of perchloric acid precipitable-NaOH soluble cpm/1 μg retinal RNA obtained in the presence of inhibitor divided by the similar value obtained in the absence of inhibitor.

[d]Mean ± standard deviation of TCA-precipitable cpm/30 μg extract protein after subtraction of the background value for three separate determinations.

[e]Ratio of TCA-precipitable cpm/30 μg extract protein obtained in the presence of inhibitor divided by the similar value obtained in the absence of inhibitor.

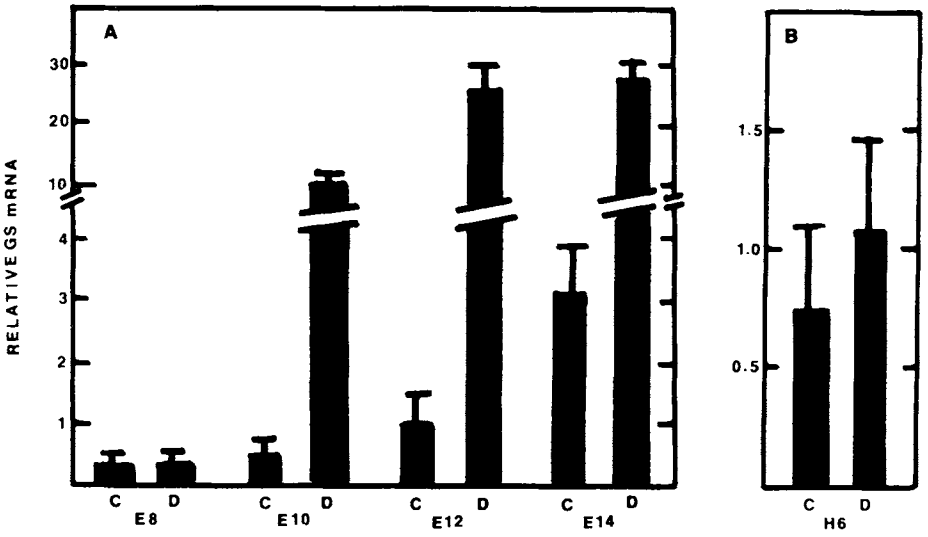

Fig. 3. Increase in hormonal induction of GS mRNA during retinal development. Prior to determination of relative levels of GS mRNA, retinae obtained at the indicated stages of development were cultured for 3 hr without (bars labeled C) or with 50 nM dexamethasone (bars labeled D). Data are expressed as means ± standard deviations, normalized relative to the value obtained with E12 retinae cultured without hormone (**A**) or H6 retinae that had not been cultured (**B**).

A priori, since glucocorticoid receptor proteins are present in retina early in embryogenesis [16,21], it might be expected that the extent of hormonal induction of GS mRNA would be constant throughout retinal development. However, the data shown in Figure 3A are consistent with the observation that hormonal inducibility of GS enzyme activity increases dramatically during the second week of development [5]. Moreover, while the studies reported here were in progress, Moscona and coworkers demonstrated that incubation of retinal organ cultures with hydrocortisone for 24 hr produces an induction of GS mRNA that is dependent upon the embryonic age of the retina, based on Northern blotting analysis [22]. Our results differ from these with regard to quantitative aspects of the extent of hormonal induction in retinae derived from early embryos and the effect of organ culture on the basal level of GS mRNA. However, both studies support the notion that hormonal responsiveness increases during retinal development.

We note that the level of GS mRNA in retinae cultured without hormone is selectively increased between E12 and E14, relative to that observed in retinae cultured with dexamethasone. This supports the notion that the developmental rise in GS mRNA is hormone-mediated in vivo, as discussed below. Additional support is provided by organ culture studies using retinae obtained from 6-day-old chickens (H6), after GS mRNA levels have plateaued [7]. We compared the level of GS mRNA after a 3-hr incubation with or without 50 nM dexamethasone. These results were normalized relative to the level of GS mRNA found in H6 retinae prior to organ culture and are summarized in Figure 3B. If the premature glucocorticoid induction observed with organ cultures derived from embryonic retinae was unrelated to the developmental rise in GS mRNA, one might expect to observe a hormonal induction superimposed upon the developmental increase. This is not the case, since organ

culture in the presence of dexamethasone does not significantly increase the level of GS mRNA, relative to that observed in H6 retinae prior to organ culture. Therefore, glucocorticoid hormones either act via a mechanism that prohibits an induction superimposed upon the developmental rise, or (more likely) they are responsible for the rise in GS mRNA observed during development.

DISCUSSION

A large body of data suggests that glucocorticoid hormones are physiological inducers of the dramatic rise in GS enzyme that occurs during terminal differentiation of the chick retina [1,3]. The results presented here support this contention and indicate that glucocorticoids mediate this effect by a direct induction of GS mRNA. However, hormonal responsiveness increases during retinal development, and we question whether this developmental program embraces complexities of general interest with regard to steroid hormone action.

It is conceivable that developmental regulation is directed at expression or modification of the glucocorticoid receptor protein. Although retinal tissue contains glucocorticoid receptors very early in development [16,21], it is not certain that Müller cells, the cells in which GS is hormonally inducible [2], contain these receptors prior to the onset of hormonal responsiveness. Accordingly, if the GS gene is masked in neuronal cells but transcriptionally competent in Müller cells, a developmentally timed appearance of the glucocorticoid receptor in Müller cells might explain these data.

It is also tenable that the developmental program is targeted at glucocorticoid receptor modification. A rationale for such a model is provided by the report that expression of either the viral mos or the activated human H-ras oncogene can repress glucocorticoid hormone-mediated transcription directed by the mouse mammary tumor virus long terminal repeat (MMTV LTR [23]). Therefore, it is possible that developmentally regulated post-translational modification of the glucocorticoid receptor might influence the hormonal responsiveness of the retinal GS system.

However, although developmental regulation directed at the glucocorticoid receptor is clearly possible, we suggest that additional ancillary factors, whose production or activity [24] is developmentally regulated, might contribute to the primary hormonal induction directly. Recent studies have implicated glucocorticoid-dependent nuclear factor binding to the promoter region of the MMTV LTR in the hormonal induction of transcription [25]. Developmentally regulated factors might act in concert with the activated glucocorticoid receptor to stimulate GS gene expression. An additional tenable mechanism is prompted by observations suggesting that unligated steroid hormone receptors might preexist in the nucleus [26–28]. If so, a developmentally regulated anchoring protein might position the unligated glucocorticoid receptor near the GS gene and be required for efficient hormonal induction. These latter mechanisms imply a transcriptional basis for the hormonal induction of GS; studies aimed at ascertaining whether this is the case are in progress.

We have previously demonstrated that expression of GS mRNA is constitutive during liver development and does not appear to be glucocorticoid hormone-inducible [7]. A requirement for an ancillary factor to achieve the primary hormonal induction in retina would lead directly to a likely mechanism to explain the absence of hormonal induction in liver, provided this putative factor was expressed in a retinal-specific

manner. If this proves to be the case, elucidation of the molecular basis by which glucocorticoid hormones regulate GS expression during retinal development is apt to broaden our general appreciation of how steroid hormones exert their effects in a tissue-specific manner.

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