Regulation of Types I, III, and IV Procollagen mRNA Synthesis in Glucocorticoid-mediated Intestinal Development*

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Administration of dexamethasone (0.8 mg/kg) to 9-day-old rats once daily for 3 consecutive days caused precocious induction of adult specific disaccharidase activity in the small intestine. Maturation-specific disaccharidase activity was accompanied by decreased amounts of types I and III collagen and decreased procollagen type I and III mRNA levels. Conversely, type IV procollagen, fibronectin, and laminin amounts and their respective mRNA levels were increased. In vitro transcription of nuclei isolated from small intestine and colon of suckling rats indicated a decreased rate of synthesis of procollagen types I and III mRNAs and an increased rate of synthesis of procollagen type IV mRNAs and laminin mRNAs after dexamethasone treatment. The data suggest that glucocorticoids mediate a differential regulation of intestinal and basement membrane collagen gene expression in the developing rat intestine.

Glucocorticoids are responsible for enzymatic and structural changes in the rat small intestine at the time of weaning (1-4). Administration of glucocorticoids within the first 2 weeks of the postnatal period causes a precocious induction of the adult specific pattern of small intestinal enzyme activities as well as structural changes which normally occur at weaning (3). Glucocorticoids are also responsible for maturation of colonic epithelium (5).

Development of rat intestinal epithelium is closely associated with structural changes in the extracellular matrix. The basolateral surface of intestinal epithelial cells rests upon a basement membrane which is a distinct extracellular matrix structure composed of type IV collagen, laminin, and fibronectin as well as other glycoproteins and proteoglycans. Rat intestinal epithelial cells synthesize fibronectin, a major component of the basement membrane, as well as type I and presumably type IV procollagen (6, 7). Directly underlying the basement membrane of the intestinal epithelium are pericycral fibroblasts, associated extracellular matrix, and other mesenchymal cells. Rat small intestinal mucosal (primarily pericycral) fibroblasts synthesize types I and III procollagen (7) and apparently have a role in differentiation since, when cultured with embryonic chick stomach endoderm, they can elicit expression of disaccharidase activity by the chick endoderm indicative of intestinal epithelium which does not occur with fibroblasts from other tissue (8). However, the exact nature of the interaction between the basement membrane, and underlying extracellular matrix, and pericycral fibroblasts and their relationship to intestinal epithelial cell differentiation are not fully understood.

Steroid hormones, including glucocorticoids, have significant effects on extracellular matrix protein synthesis in fibroblasts and various tissues (9). Therefore, synthetic parameters of extracellular matrix proteins were assessed with respect to intestinal development in the presence and absence of low levels of glucocorticoid. Our data indicate that the glucocorticoid-induced precocious maturation of suckling rat intestinal disaccharidase activity is associated with a differential regulation of the gene expression of procollagen types I, III, and IV, fibronectin, and laminin. These components of the intestinal extracellular matrix are synthesized by the epithelial cells, the underlying fibroblasts, and possibly smooth muscle cells, the presumed targets of glucocorticoid-mediated cellular events.

EXPERIMENTAL PROCEDURES

Animals—Pregnant rats and their offspring were cared for as previously described (1). At 24 h postpartum, the litters were reduced to 9 pups. At 9, 10, and 11 days of age, rat pups were administered dexamethasone (0.8 mg/kg) intraperitoneally. Both control and treated pups were killed 24 h after the last injection of dexamethasone (day 12). The small intestine and colon were removed at 0-4°C and thoroughly flushed with normal saline.

Protein Determination—Protein concentration of the rat intestinal homogenate was determined by the method of Lowry et al. (10).

Maltase, Sucrase, and Lactase Activities—Disaccharidase activities were determined for the assessment of maturation-specific developmental changes in the rat small intestine by the method of Dahlqvist (11). One unit of disaccharidase activity equals 1 pmol of disaccharide hydrolyzed/min (11).

Immunodot Analysis—Rat intestine was homogenized in Tris-buffered saline, pH 7.5, and equal amounts of homogenate protein from control and dexamethasone-treated samples were applied to nitrocellulose (0.43 μm pore size, Schleicher & Schuell) previously washed with Tris-buffered saline. After drying, the remaining protein-binding sites were blocked with 1% bovine serum albumin (Boehringer Mannheim) in Tris-buffered saline. The first antibody, diluted in 1% bovine serum albumin in Tris-buffered saline, was then applied to the nitrocellulose. The samples were air-dried, followed by washing with Tris-buffered saline. For the immunodot assay, second antibody (goat-anti-rabbit IgG linked to horseradish peroxidase, Bio-Rad), also in 1% bovine serum albumin in Tris-buffered saline, was then applied to the nitrocellulose. The samples were air-dried, followed by washing with Tris-buffered saline. For the immunodot assay, second antibody (goat-anti-rabbit IgG linked to horseradish peroxidase, Bio-Rad), also in 1% bovine serum albumin in Tris-buffered saline, was subsequently applied to the samples. The samples were washed and the nitrocellulose membrane was removed from the minifold apparatus for color development by incubation with 4-chloro-1-naphthol and hydrogen peroxide. There was no color development in blank filters which had no sample protein and/or no first antibody.

Radioimmunodot Analysis—The radioimmunodot assay was identical to the procedure for the enzyme-linked immunodot assay with the exception that Protein A, N-[propionyl-14C]Hapropionylated, 73 Ci/
mmol (Amersham Corp.), in 1% bovine serum albumin in Tris-buffered saline was added in place of the second antibody. The samples were thoroughly washed with Tris-buffered saline, air-dried, cut out, and counted in Filtron X (National Diagnostics). The radioactive counts/min bound nonspecifically to blank filters were subtracted from the radioactive counts/min bound to sample filters.

Quick Blotting—The "quick blot" procedure was that described by Bresser et al. (12). Briefly, 12-day rat small intestine was homogenized in phosphate-buffered saline containing vanadyl ribonucleosides and cycloheximide. The homogenate was digested with proteinase K and solubilized further with detergent and saturated NaCl. The resulting mixture was applied to nitrocellulose under conditions which preferentially bind poly(A)+ mRNA. Interference from contaminating protein was minimized by acetylation with acetic anhydride in ethanolamine. The bound RNA was hybridized to 32P-labeled DNA probes as described below.

RNA isolation—Total RNA from rat intestine was prepared by the guanidine isothiocyanate procedure described by Chirgwin et al. (13). Isolation of Nuclei—Nuclei were isolated from rat intestine by the method of Mulvihill and Palmiter (14). Nuclei were stored in 20 mM Hepes, pH 7.9, 5 mM MgCl2, 0.1 mM EDTA, 20% (v/v) glycerol, 4 mM dithiothreitol at −80 °C.

Electrophoretic Fractionation and Hybridization of RNA—Twenty micrograms of total RNA were separated in 1.4% formaldehyde gels and transferred to nitrocellulose paper BA85 (Schleicher & Schuell), according to Maniatis et al. (15). The RNA filters were prehybridized for 12–16 h at 42 °C in 50% formamide, 5 × SSC, 50 mM sodium phosphate, pH 6.8, 0.1% SDS, 5 × Denhardt's reagent, and 100–200 μg/ml sonicated, denatured salmon sperm DNA (Sigma) (16). The hybridization with nick-translation DNA was carried out under the same conditions with 10% dextran sulfate added for 24 h. The concentration of denatured 32P-labeled DNA fragment (specific activities > 1.8 × 109 cpm/μg of DNA) was 5 ng/ml. The RNA filters were washed in 2 × SSC, 0.1% SDS at 25 °C for 3 × 5 min and in 0.1 × SSC, 0.1% SDS at 65 °C for 3 × 20 min. The filters were autoradiographed with Kodak XAR-5 film and intensifying screens for 2 days at −70 °C.

In Vitro Nuclear Transcription—The isolated intestinal nuclei were transcribed in vitro as described (17). Nuclear RNA labeled in vitro with [α-32P]UTP, 3000 Ci/mmol (Amersham Corp.), was isolated as described (18), and its integrity established by UV absorbance and formaldehyde-agarose gel electrophoresis. Equal counts/min of the labeled RNA extracted from the nuclei isolated from control and dexamethasone-treated tissue was hybridized to DNA filters as described in the legend to Figs. 2, 3, and 4.

Plasmid DNA—All plasmid DNAs were prepared on low-melting agarose to separate the genomic and cDNA inserts from bacterial DNA. The cloned DNA probes were nick-translated as described (19).

Assay of in Vitro Gene Transcription—Unlabeled DNA probes and RNA polymerase (5 ng/ml) were hybridized to nick-translated DNA (0.5 ng/ml) in a mixture containing 20 μg/ml salmon sperm DNA (Sigma), 100 μg/ml yeast RNA, 250 μg/ml salmon sperm RNA, 100 μg/ml yeast RNA, and hybridized in DNA excess for 24 h at 42 °C.

The labeled RNA (equal counts/min for control and treated) extracted from the nuclei transcribed in vitro was added to the filters in 50% formamide, 5 × SSC, 1 × Denhardt's reagent, 0.1% SDS, 20 μg/ml yeast RNA, 0.5 μg/ml salmon sperm RNA, and hybridized in DNA excess for 24 h at 42 °C.

The filters were washed with four times with 2 × SSC, 0.1% SDS at room temperature for 5 min and two times at 0°C in 0.1 × SSC, 0.1% SDS for 15 min. The filters were autoradiographed at −80 °C with Cronex intensifying screens. Quantitative results were obtained by scanning densitometry of the autoradiographs with a Shimadzu densitometer.

RESULTS

Enzyme-linked and radioimmunodot assays of type I and type III collagen accumulation indicated a decrease of these interstitial collagens (Fig. 1). In contrast, type IV procollagen, fibronectin, and laminin accumulation was increased in the small intestine of glucocorticoid-treated 12-day-old rats (Fig. 1). Procollagen type I and collagen type III were decreased by approximately 50%, whereas type IV procollagen was increased by 100% as determined by radioimmunodot assay (Fig. 1). Fibronectin and laminin were also increased by 70 and 80%, respectively (Fig. 1).

Twelve-day-old dexamethasone-treated rats showed a dramatic increase of maltase and sucrase activities expressed as units/g of tissue, wet weight, or units/g of homogenate protein (Table I). In contrast, lactase activity was significantly decreased after dexamethasone treatment (Table I). This observed pattern of disaccharidase enzyme activities in glucocorticoid-treated 12-day-old rats was indicative of precocious induction of maturation-specific intestinal enzyme activity, i.e. premature intestinal development.

Small intestinal type IV procollagen, fibronectin, and laminin mRNA levels were significantly increased, whereas procollagen types I and III mRNA levels were significantly decreased as determined by quick blot hybridization analysis with cloned DNA probes for these proteins (Fig. 2). The levels of β-actin mRNA were not significantly affected by dexamethasone treatment (Fig. 2). The percentage decrease for procollagen type I and III mRNA levels was generally similar to the percentage decrease in their respective tissue protein level in the small intestine of glucocorticoid-treated rats. The percentage increase in procollagen type IV, fibronectin, and laminin mRNA levels was also generally similar to the percentage increase in their respective tissue protein levels.

1 The abbreviations used are: Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; SDS, sodium dodecyl sulfate; kb, kilobase pair.

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**Fig. 1.** A, enzyme-linked immunoassay of 12-day-old rat intestinal extracellular matrix proteins. Antiserum was provided to the following proteins: procollagen type I from Dr. J. Kelly, University of Vermont, procollagen type III from Dr. K. Cutroneo, University of Vermont, procollagen type IV from Dr. G. Martin, National Institutes of Health, fibronectin from Dr. R. Hynes, Massachusetts Institute of Technology, and laminin from Dr. S. Palm and Dr. L. Furcht, University of Minnesota. B, radioimmunoadsorption of 12-day-old rat intestinal extracellular matrix proteins. The values represent the mean plus or minus the standard error from three or four animals. An asterisk indicates significantly different from the control value at p ≤ 0.05 as determined by Student's nonpaired t test.

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**Type 1** Type 3** Type IV** Fibronectin **Laminin**

**Control**

**Dexamethasone**

**Fig. 2.** A, enzyme-linked immunodot assay of 12-day-old rat intestinal extracellular matrix proteins. Antiserum was provided to the following proteins: procollagen type I from Dr. J. Kelly, University of Vermont, procollagen type III from Dr. K. Cutroneo, University of Vermont, procollagen type IV from Dr. G. Martin, National Institutes of Health, fibronectin from Dr. R. Hynes, Massachusetts Institute of Technology, and laminin from Dr. S. Palm and Dr. L. Furcht, University of Minnesota. B, radioimmunoadsorption of 12-day-old rat intestinal extracellular matrix proteins. The values represent the mean plus or minus the standard error from three or four animals. An asterisk indicates significantly different from the control value at p ≤ 0.05 as determined by Student's nonpaired t test.
Glucocorticoid Regulation of Intestinal Procollagen mRNAs

Proximal and distal small intestinal disaccharidase activity in 12-day-old rats

Maltase, sucrase, and lactase activities were determined in the proximal and distal small intestine of control and dexamethasone-treated rats for the purpose of establishing intestinal epithelial cell differentiation mediated by glucocorticoid administration. Enzyme activities were expressed as units/g of tissue or units/g of protein. The values represent the mean plus or minus the standard error from five or six animals.

<table>
<thead>
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<th>Group</th>
<th>Maltase</th>
<th>Sucrase</th>
<th>Lactase</th>
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<td>Control proximal</td>
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<td>0.03 ± 0.01</td>
<td>0.43 ± 0.03</td>
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<tr>
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<td>0.00</td>
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<td>Dexamethasone distal</td>
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<td>0.13 ± 0.02</td>
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*Significantly different from each group’s control at p ≤ 0.05, as determined by Student’s nonpaired t test.

In vitro transcription of isolated nuclei from the small intestine and colon of glucocorticoid-treated and control 12-day-old rats indicated that glucocorticoids attenuate procollagen α1(1), α2(1), and α1(III) gene transcription (Figs. 4 and 5). Procollagen α1(IV), α2(IV), and laminin B1 gene transcription was increased with glucocorticoids (Figs. 4 and 5). Transcription of the glucocorticoid receptor gene was decreased in glucocorticoid-treated small intestine and colon (Figs. 4 and 5). The apparent decrease in procollagen type I, type III, or glucocorticoid receptor gene transcription and the apparent increase in procollagen type IV and laminin gene transcription as determined by the in vitro transcription assay were both reproducible (in six separate transcription assays). However, the respective decrease for procollagen types I and type III or glucocorticoid receptor transcription varied from approximately 3- to 10-fold or greater, and the respective increase for procollagen type IV or laminin transcription rate varied from approximately 3- to 10-fold or greater, as observed from the respective autoradiographic signals from each separate transcription assay.

DISCUSSION

Our data indicate that glucocorticoid-induced precocious development of rat intestinal epithelium is accompanied by a differential accumulation of interstitial and basement membrane collagenases of the intestine. The glucocorticoid-induced alteration of interstitial and basement membrane collagen accumulation is apparently primarily mediated at the level of gene transcription resulting in either decreased or increased tissue mRNA concentrations and ultimately protein. In comparison, dexamethasone administration to adult rats resulted in insignificant changes in intestinal steady state levels of types I, III, and IV procollagen and laminin mRNAs by Northern hybridization analysis (data not shown). The results of this study indicate that glucocorticoid-induced alterations of interstitial and basement membrane procollagen mRNA synthetic rates and steady state levels occur during hormone-mediated postnatal intestinal development. However, our data do not preclude regulation of procollagen and other extracellular matrix protein gene expressions at the post-transcriptional, translational, and/or post-translational levels (e.g. mRNA or protein processing or stability). Fibronectin and laminin, which are also major components of the basement membrane and are closely associated with these procollagens, are increased in amount and mRNA level as well. Laminin B1 gene transcription was increased with glucocorticoid and

FIG. 2. Quick blot hybridization analysis of 12-day-old rat small intestinal extracellular matrix mRNA levels. Plasmid DNA to the following probes was provided by Dr. D. Rowe, University of Connecticut, for procollagen α1(I) and α2(I) (p alpha-1R1 and p alpha-2R2), Dr. B. de Crombrugghe, National Institutes of Health, for procollagen α1(III) (pMCS-1), Dr. M. Kurkinen, Robert Wood Johnson Medical School, for procollagen α1(IV) (pPE123), procollagen α2(IV) (pPES86) and for laminin B1 (pPE18), Dr. R. Hynes for fibronectin (p1F1), Dr. R. Miesfeld, University of San Francisco, for glucocorticoid receptor (pSG-1), and Dr. D. Cleveland, Johns Hopkins University, for β-actin (PA1). DNA inserts were purified and labeled with [32P]dCTP (19). The values represent the mean plus or minus the standard error from three or four animals. An asterisk indicates significantly different from the control value at p ≤ 0.05, as determined by Student’s nonpaired t test.

FIG. 3. Northern hybridization analysis of control and dexamethasone-treated neonatal rat small intestine and colon total RNA. All 32P-labeled DNA are as described in the legend of Fig. 2. A, RNA from control tissue; B, RNA from dexamethasone-treated tissue; Si, small intestine; C, colon. Units are expressed as kilobase pairs.

Northern hybridization analysis of total RNA of the small intestine and colon from glucocorticoid-treated and control 12-day-old rats indicated a decrease in the amount of procollagen α1(I), α2(I), and α1(III) mRNAs with glucocorticoid (Fig. 3). The procollagen α1(I) mRNAs were 5.4 and 4.4 kb. The procollagen α2(I) mRNAs were 5.1 and 5.7 kb. The procollagen α1(III) mRNAs were 5.4, 2.8, and 1.6 kb, the major species being the 5.4-kb mRNA, which is the only large enough to code for a full-length procollagen α1(III) peptide chain. Conversely, procollagen α1(IV) mRNAs were increased in the small intestine and colon with glucocorticoid (Fig. 3). Procollagen α1(IV) mRNAs were 6.8 and 6.2 kb. Northern hybridization of the β-actin cDNA to the total RNAs isolated indicated little, or no, degradation by the presence of a major band at 2.0 kb (data not shown).
Glucocorticoid Regulation of Intestinal Procollagen mRNAs

FIG. 4. In vitro transcription analysis of nuclei isolated from control and dexamethasone-treated neonatal small intestine. Equal counts/min of 32P-labeled input RNA extracted from nuclei transcribed in vitro was hybridized to the nitrocellulose-bound plasmids palR1, palR2, pMCS-1, pPE123, pPE18, pPE386 and pSG1, which contain the DNA inserts indicated. The hybridization reaction was carried out in DNA excess. Top panel, relative transcription rates of the designated genes for control versus dexamethasone-treated nuclei. These values were derived from peak areas obtained from densitometer scanning of the original autoradiographs of the representative hybridization signals shown directly below. Representative autoradiographs (chosen from three separate assays done under identical conditions) of 32P-labeled RNA transcribed in vitro from control and dexamethasone-treated nuclei hybridized in DNA excess to the indicated nitrocellulose-bound DNA are shown below. Each pair of hybridization signals (control versus dexamethasone) was from the same assay. Bottom panel, total incorporation of [α-32P]UTP into trichloroacetic acid-precipitable RNA of control and dexamethasone-treated nuclei transcribed in vitro.

FIG. 5. In vitro transcription analysis of nuclei isolated from control and dexamethasone-treated 12-day-old rat colon. See legend to Fig. 4 for details.

may account in part or in whole for the increase in laminin B1 mRNAs.

The intestinal mesenchyme has significant inductive properties with respect to the morphological development of non-intestinal endoderm into intestinal epithelium (for review see Ref. 20). In addition, homotypic and heterotypic intestinal endoderm-mesenchyme recombination experiments not only revealed the requirement for intestinal mesenchyme but also the requirement for glucocorticoid in the induction of sucrase (21-23).

Evidence that epithelial-mesenchymal interactions mediate glucocorticoid induction of enzyme activity has been demonstrated in developing chick liver (24). Specifically, it was shown that liver hepatocyte/fibroblast cultures in the presence of glucocorticoid produced a soluble factor which caused hepatocytes alone in culture to express aminotransferase activity (24). Suckling rat intestinal intramucosal fibroblasts, but not rat skin or lung fibroblasts, have been shown to induce chick embryonic gizzard endoderm to differentiate into intestinal epithelium and express sucrase and maltase enzyme activity (8). Also, embryonic chick intestinal fibroblasts are necessary for embryonic chick intestinal epithelial cell differentiation in culture (25).

A possible role for extracellular matrix protein in intestinal development and differentiation has been implied by the observation that changes in the distribution and localization of laminin, fibronectin, type III, and type IV collagen occurs during development of the rat small intestine (26). Also, the possible role for collagen of the extracellular matrix in the mesenchymal-dependent differentiation of intestinal endoderm was indicated by homologous graft experiments with fetal intestinal endoderm and mesenchyme (27). Fetal intestinal endoderm and mesenchyme, which were disrupted by collagenase and subsequently recombined and grafted to the kidney, took a significantly longer period of time to express sucrase activity compared to non-disrupted intestinal endoderm-mesenchyme grafts (27).

Fetal rat intestinal mesenchyme has also been shown to induce differentiation of cultured crypt epithelial cells in recombinants of the two when grafted under the kidney capsule of adult rats (27). This system provides sufficient glucocorticoid to the grafted recombinants (27). It was also suggested that extracellular matrix components play a role in the differentiation of the fetal intestinal endoderm or cultured intestinal crypt epithelial cells (27).

In the rat embryo at 5–7 days before birth, the epithelium of the proximal small intestine is separated from the mesenchyme by a continuous basement membrane (28). Just prior to birth and 7–10 days thereafter the basement membrane becomes discontinuous, with large numbers of epithelial cell processes projecting through the gaps underlying extracellular matrix in close association with mesenchymal cells (e.g. pericytial fibroblasts) (28). After 10 days postpartum, up to the time of weaning and maturation-specific differentiation of the small intestinal epithelium, there is a gradual decrease in the

$\frac{\text{Control}}{\text{Dexamethasone treatment}}$

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basement membrane “gap” (28). The sealing of these gaps in the basement membrane require de novo synthesis of basement membrane components. Furthermore, the sealing of the basement membrane (de novo synthesis of the basement membrane components) temporally coincides with a normal increase of circulating concentrations of corticosterone (29). Thus, increased synthesis of basement membrane components (e.g. type IV procollagen and laminin) during the normal process of intestinal epithelial cell differentiation is associated with a normal increase of glucocorticoids.

Glucocorticoid administration to adult rats has no effect on the differentiation-specific enzymes (30). We observed no effect on procollagen type I, type III, type IV, and laminin mRNA levels in the adult rat, indicating a relationship between glucocorticoids, intestinal procollagen synthesis, and intestinal epithelial cell differentiation specific for neonatal rat intestine.

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