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EFFECT OF DEXAMETHASONE ON COLLAGEN METABOLISM IN TWO STRAINS OF MICE

PAMELA GEHRON ROBEY

*Laboratory of Developmental Biology and Anomalies, National Institute of Dental Research, National Institutes of Health, Bethesda, MD 20014, and Biology Department, The Catholic University of America, Washington, DC, U.S.A.

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Abstract—The effect of dexamethasone on skin collagen was studied in adult mice from strains with a high frequency (A/J) or low frequency (NIH Swiss Webster) of spontaneous or stress-induced facial malformations. Dexamethasone inhibited synthesis of collagen in both strains of mice. The proportion of collagen in the skin of dexamethasone-treated A/J but not Swiss Webster mice appeared to increase slightly due to a greater loss of noncollagenous proteins in the skin. These results indicate that dexamethasone causes degenerative effects in the skin by altering both the synthesis and degradation of proteins and that A/J mice are more affected by glucocorticoid treatment.

Glucocorticoids are potent compounds with diverse effects. Pharmacological amounts of glucocorticoids can cause facial malformations in the developing embryos of animals [1], and in adults cause degenerative changes in connective tissues [2, 3]. The synthesis of collagen, the major constituent of these tissues, is decreased in animals given glucocorticoids [4–8]. This reduction could be due to the decrease in prolyl hydroxylase activity observed in these animals [5], because this enzyme may be rate-limiting in collagen synthesis [9, 10]. It has also been suggested that glucocorticoids may increase collagen degradation by the induction and/or activation of proteolytic enzymes [11, 12].

Various strains of mice show different frequencies of spontaneous or stress-induced facial malformations. For example, the frequency of spontaneous cleft lip is 10–14 per cent among A/J offspring [13] while the incidence is close to zero among Swiss mice (R. Pratt, personal communication). In addition, A/J mice have a high frequency of stress-induced malformations (69 per cent) [14–16], as compared to Swiss mice (10 per cent) [16]. This may be related to the sharp increase and very slow decrease of serum glucocorticoids observed in these mice after exposure to various forms of stress [17].

The purpose of this study was to investigate the effect of dexamethasone, a synthetic glucocorticoid, on skin composition and collagen synthesis in adult A/J and Swiss Webster mice. In both strains, dexamethasone treatment inhibited synthesis of collagen, but the composition of skin was altered only in the A/J mice. These findings indicate that strain differences exist in the response of skin to glucocorticoids.

MATERIALS AND METHODS

Treatment of mice. Six-week-old female A/J (Jackson Labs) and 6-week-old female Swiss Webster (NIH) mice were divided into two groups. One group was fed a diet containing β-aminoproprionitrile (BAPN) (Aldrich Chemical Co., Milwaukee, WI, 4 g/kg of Purina ground chow) to inhibit the crosslinking of collagen. The other group was fed ground chow alone. After the animals had been on their respective diets for 3 days, half of the animals in each group received daily injection of dexamethasone (Dex) (Sigma Chemical Co., St. Louis, MO). The following groups resulted for each strain: (1) BAPN + Dex; (2) BAPN—no Dex; (3) no BAPN + Dex; and (4) no BAPN—no Dex. Dexamethasone was dissolved in 100% ethanol (2 mg in 1 ml) and diluted to 10 ml with sterile phosphate-buffered saline. Each mouse receiving dexamethasone was injected intraperitoneally daily for 7 days with 0.2 cc of the final solution (40 μg/mouse). On day 8, all mice were injected intraperitoneally with 250 μCi [U-^{3}H]glycine (New England Nuclear, Boston, MA) and 8 hr later were killed by cervical dislocation and skinned.

Extraction of collagen from the skin. Collagen was extracted from the skins according to the method of Rowe *et al.* [18] with 0.5 M acetic acid containing the protease inhibitors phenylmethylsulfonyl fluoride and *p*-hydroxymercuribenzoate. The extract was neutralized and the collagen was partially purified by salt precipitation. The isolated collagen was dialyzed to remove salt, and then lyophilized.

Carboxymethyl (CM)-cellulose column chromatography. A portion of each partially purified sample (40 mg) was chromatographed on a column of CM-cellulose (Whatman CM52) according to the method of Piez *et al.* [19]. The effluent was monitored spectrophotometrically and fractions were collected. Aliquots of every other fraction were assayed for radioactivity.

Molecular sieve column chromatography. Partially purified samples were dissolved and denatured by heating in 1 M $CaCl_2$, 0.05 M Tris–HCl, pH 7.4, and applied to a column containing 6% agarose (Bio-Rad Laboratories, Rockville Center, NY, A-5m, 100–200 mesh) equilibrated in the same buffer. The effluent was monitored and fractions were collected and assayed for radioactivity.

* Address to which correspondence should be sent.

Amino acid analysis. Whole skin samples were prepared from the dorsal areas of the animals. The skins were treated with a dipilatory to remove fur, washed thoroughly and lyophilized. Samples for amino acid analysis were hydrolyzed in 6 N HCl at 100° for 24 hr. The hydrolysates were anaylzed by either a Beckman or Durrum amino acid analyzer.

Uptake of [^{3}H]glycine. In order to determine whether both strains of mice had similar rates of glycine uptake, whole skin samples (dry weight of 0.5 g) were homogenized in cold distilled water and dialyzed vs distilled water. Aliquots of the dialysate were assayed for radioactivity to determine dialyzable radioactivity. The retained material was dissolved by heating in 6 N HCl, neutralized by adding an equal volume of 6 N NaOH, and assayed for radioactivity to determine non-dialyzable labeled protein.

RESULTS

Composition of skin and collagen from A/J and Swiss Webster mice. The collagen content of mouse skin was estimated from its amino acid composition. Collagen is the most abundant protein in the skin and has a distinctive composition. In contains 333 glycines and approximately 110 hydroxyprolines/1000 residues. Other connective tissue proteins contain less glycine and lack hydroxyproline. Therefore, the amounts of these two amino acids are indicative of the collagen content of the skin, and their levels can be compared between the two strains of mice. Table 1 shows the average of five skin samples for each group taken from two different experiments. The analyses of skin from A/J mice revealed 39 residues of hydroxyproline and 209 of glycine/1000 total residues. Analyses of skin from Swiss Webster mice showed a content of 47 residues of hydroxyproline and 242 residues of glycine/1000 residues (Table 1). The lower proportions of hydroxyproline and glycine in the A/J mice indicate that the skin of this strain contains less collagen than that of the Swiss Webster strain.

Skin samples were also analyzed from A/J and Swiss Webster mice that had been treated with dexamethasone (Table 1). The analysis of skin taken from A/J mice treated with dexamethasone was quite different from that obtained from the untreated A/J mouse skin. Dexamethasone treatment appeared to increase the levels of hydroxyproline (54 as compared to 36) and glycine (262 as compared to 209). This finding indicates that the proportion of collagen relative to other proteins was increased in the skin of A/J mice after 1 week of treatment. In contrast, the amino acid composition of the skin of the Swiss Webster mice was not altered by dexamethasone treatment. No differences were found in the composition of collagen extracted from the skins of A/J and Swiss Webster mice that received either BAPN or BAPN and dexamethasone (data not shown).

Collagen synthesis. The incorporation of labeled glycine into collagen chains was used to measure the effect of dexamethasone on collagen synthesis in the two strains of mice. As a control, equal weights of collagen, synthesized by animals not treated with either BAPN or dexamethasone, were chromatographed on CM-cellulose under denaturing conditions. These chromatograms revealed that little radioactivity was present in the extracted collagen and that crosslinked components were more abundant that α1(I) and α2 chains, when observed by the optical profile (not shown). This finding was expected because adult mouse skin is highly crosslinked [18]. In comparison, the collagen extracted from animals receiving BAPN contained more radioactivity than the others and this radioactivity was found predominantly in the α1 and α2 chains separated by

Table 1. Amino acid composition of whole skin*

	A/J		Swiss Webster	
	− Dex	+ Dex	− Dex	+ Dex
Hyp	36 ± 11.7†	54 ± 8.8†	47 ± 11.8‡	52 ± 8.9‡
Asp	67	61	68	61
Thr	38	31	38	35
Ser	68	58	64	61
Glu	112	102	109	105
Pro	79	89	75	80
Gly	209 ± 12.2§	262 ± 10.2§	242 ± 16.0‡	237 ± 7.9‡
Ala	83	91	86	95
Cys	30	14	14	13
Val	41	36	41	41
Met	9	7	6	8
Ile	24	21	27	24
Leu	55	43	54	50
Tyr	24	17	20	18
Phe	21	19	21	20
Hyl	3	4	3	3
His	9	7	8	7
Lys	45	40	46	46
Arg	45	40	50	49

* Residues/1000 ± S.E.M.
† $P \leqslant 0.05$.
‡ Differences not significant at any level.
§ $P \leqslant 0.01$.

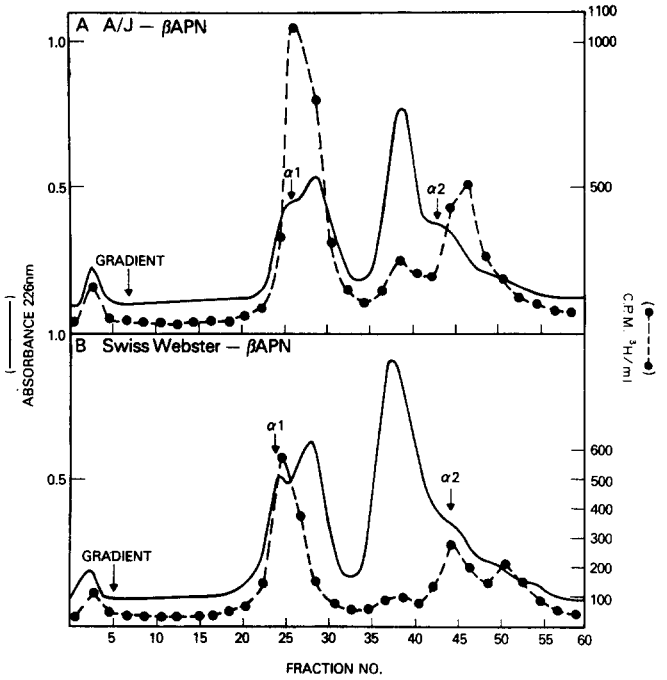

Fig. 1. Separation of collagenous components by carboxymethyl-cellulose column chromatography; (A) A/J—BAPN, (B) Swiss Webster—BAPN. The animals receiving BAPN were injected with tritiated glycine 8 hr prior to death. Their skins were extracted with acid and collagen was partially purified by salt precipitation. A portion (40 mg) of this material was dissolved and heated in 0.04 M sodium acetate, pH 4.8, and applied to a heated column (45°) equilibrated in the same buffer. The collagenous components were eluted with a 700 ml gradient ranging from 0 to 0.1 M NaCl. Ten ml fractions were collected, and 0.5 ml from every other fraction was assayed for radioactivity.

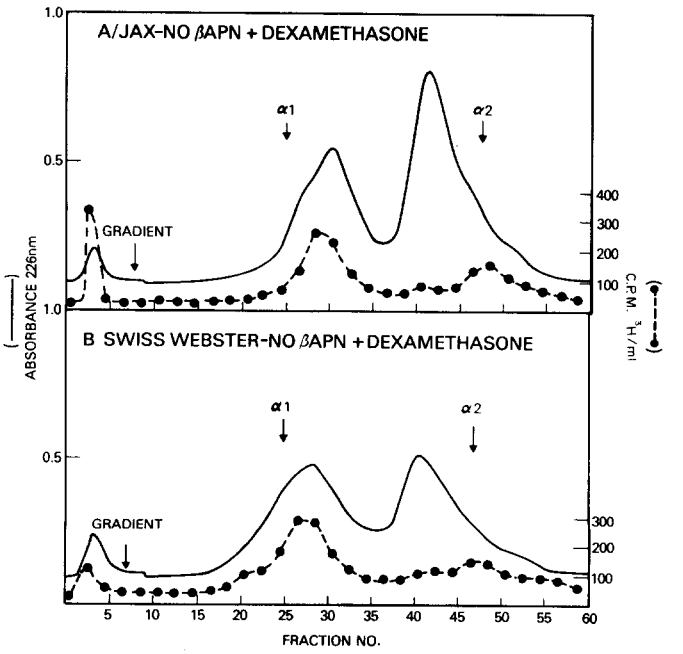

Fig. 2. Separation of collagenous components by carboxymethyl-cellulose column chromatography; (A) A/J—no BAPN + dexamethasone, (B) Swiss Webster—no BAPN + dexamethasone. Animals were treated as described in Fig. 1.

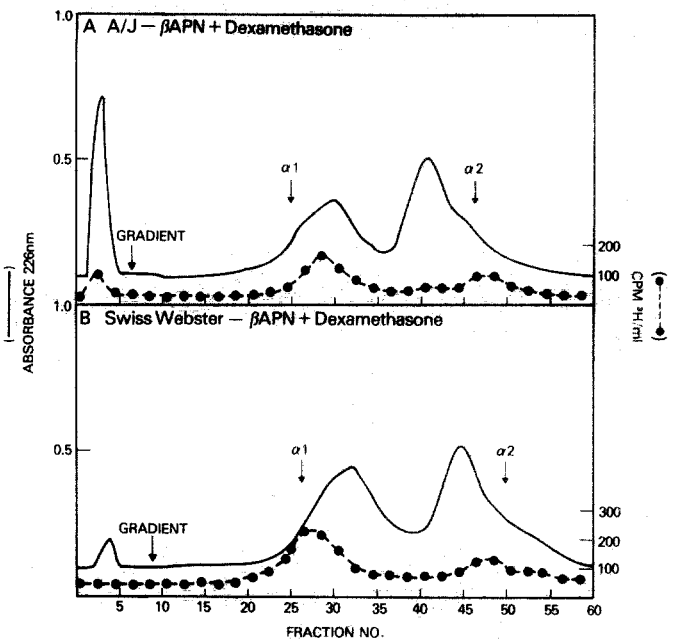

Fig. 3. Separation of collagenous components by carboxymethyl-cellulose column chromatography; (A) A/J—BAPN + dexamethasone, (B) Swiss Webster—BAPN + dexamethansone. The animals were treated as described in Fig. 1.

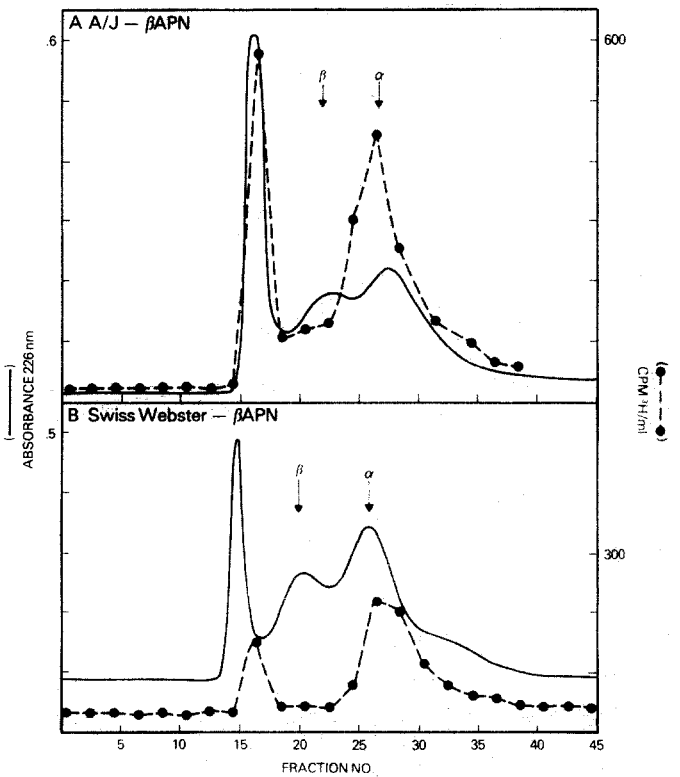

Fig. 4. Separation of collagenous components by molecular sieve column chromatography; (A) A/J—BAPN, (B) Swiss Webster—BAPN. The animals were injected with tritiated glycine 8 hr prior to death. Their skins were extracted with acid and collagen was partially purified by salt precipitation. A portion of the sample (30 mg) was dissolved in 1 M $CaCl_2$, 0.05 M Tris–HCl, pH 7.4, and heated. The sample was applied to a 6% agarose column (100–200 mesh, 4 × 120 cm) equilibrated in the same buffer. Ten ml fractions were collected and 0.5 ml was assayed for radioactivity.

CM-cellulose (Fig. 1). The collagen from A/J mice fed BAPN contained α chains with twice the radioactivity as the collagen from Swiss Webster mice fed BAPN.

Chromatograms of the collagen synthesized by A/J and Swiss Webster mice receiving dexamethasone but not BAPN were similar in appearance to those obtained from untreated animals (Fig. 2). However, the chromatograms of collagen from A/J and Swiss Webster mice receiving both dexamethasone and BAPN differed from those obtained from animals receiving only BAPN (Fig. 3). The optical and radioactive profiles did not show prominent α peaks and resembled the chromatograms of collagen obtained from animals not receiving BAPN. Also, the amount of radioactivity incorporated into α1 and α2 chains was greatly reduced, especially in the A/J sample, relative to the amount of radioactivity without dexamethasone. It is apparent from these studies that dexamethasone inhibits collagen synthesis in both strains.

Collagen extracted from the various groups was also chromatographed on 6% agarose. Samples from A/J and Swiss Webster mice receiving BAPN, but no dexamethasone, produced a typical profile of collagenous components. The γ component, as well as aggregates of high molecular weight, eluted in the exclusion volume and were followed by distinct β and α peaks (Fig. 4). More radioactivity was incorporated into α chains in the A/J mice than in the Swiss Webster mice as shown already by the CM-cellulose chromatograms. The molecular-sieve chromatograms obtained from animals receiving dexamethasone were quite different. Samples prepared from two different batches of A/J mice receiving dexamethasone chromatographed as components considerably smaller than α chains. The samples taken from Swiss Webster mice receiving dexamethasone were only slightly different from controls (Fig. 5). Degradation was also observed in samples taken from BAPN and dexamethasone-treated A/J mice. These studies indicate that there was considerable degradation after extraction and purification of the collagen from the A/J mice receiving dexamethasone. Because the CM-cellulose chromatograms and gel electrophoresis patterns (not shown) were normal, degradation of collagen must have occurred in the $CaCl_2$ buffer prior to or during molecular-sieve chromatography.

Non-dialyzable and dialyzable radioactivities of whole skin samples were measured 8 hr after the administration of glycine. Skin taken from A/J mice not receiving dexamethasone contained about half as much non-dialyzable radioactivity as did skin from Swiss Webster mice. This observation indicates that A/J mice incorporate glycine into total protein at a slower rate than Swiss Webster mice. Skins of dexamethasone-treated A/J and Swiss Webster mice contained one half to two thirds of the level of non-dialyzable radioactivity found in skins taken from untreated A/J and Swiss Webster mice.

DISCUSSION

The analysis of amino acids showed that there was somewhat less collagen in the skin of A/J mice than in Swiss Webster mice. After dexamethasone treatment there was an apparant increase in the collagen content of A/J skin as judged by amino acid analysis. However, since the incorporation of radioactivity into non-dialyzable protein was greatly reduced, it is unlikely that the changes resulted from enhanced collagen synthesis. Rather, it appears that the change in composition is due to the more rapid loss of noncollagenous protein from when dissolved in the buffer used for molecular sieve chromatography, whereas much less degradation was

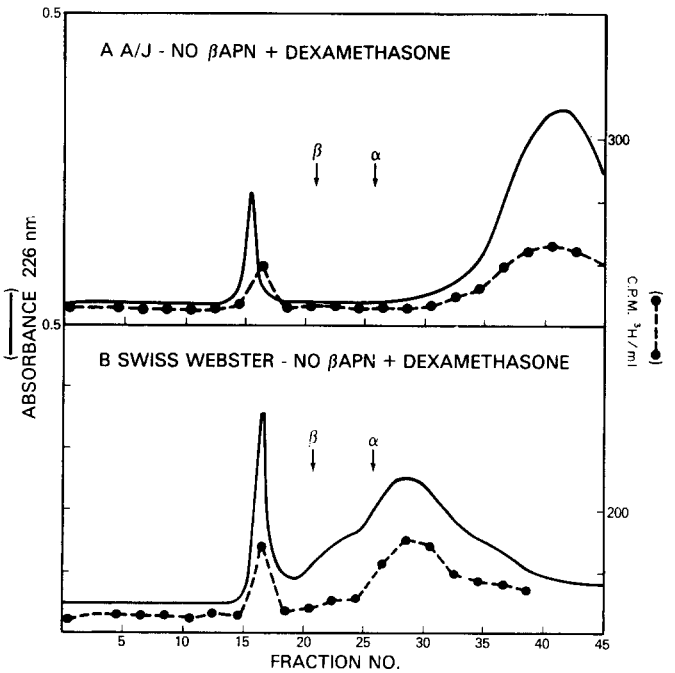

Fig. 5. Separation of collagenous components by molecular sieve column chromatography; (A) A/J—BAPN + dexamethasone, (B) Swiss Webster—BAPN + dexamethasone. The animals were treated as described in Fig. 4.

methasone, can cause an increased breakdown of non-collagenous proteins and thereby increase the relative proportion of collagen [20].

The synthesis of collagen can be estimated by measuring the amount of label incorporated into α chains separated on CM-cellulose in BAPN-treated animals. The radioactive profiles obtained from such samples indicate that the synthesis of collagen by A/J mice was about twice that found in Swiss Webster mice. Because the proportion of collagen in A/J skin is slightly lower than in Swiss Webster skin, it is likely that the turnover of skin collagen is more rapid in A/J than in Swiss Webster mice. The increased incorporation of labeled glycine into A/J α chains is not due to an increased uptake of the labeled compound by the tissue, because A/J skins contained less labeled protein than Swiss Webster mice.

When collagen synthesis was examined, dexamethasone caused a marked inhibition. Because the rate of synthesis was higher in the A/J mice than in Swiss Webster mice before dexamethasone treatment, the reduction of collagen synthesis was greater in the A/J than in the Swiss Webster mice.

Partially purified collagen extracted from A/J mice receiving dexamethasone appeared to be degraded when dissolved in the buffer used for molecular sieve chromatography, whereas much less degradation was observed in the Swiss Webster mice receiving dexamethasone. Degradation could be the result of proteolytic enzymes that were either induced or activated by dexamethasone and carried along during the preparation of collagen. *In vivo*, other investigators have observed increased collagen degradation following glucocorticoid treatment [21, 22].

From these studies, it appears that A/J mice contain slightly less collagen in their skin than Swiss Webster mice and synthesize collagen more rapidly. The decrease in collagen synthesis after dexamethasone treatment shows that both strains are susceptible, but A/J mice are more susceptible at the dosage used in this study. Recent studies employing [^{3}H]dexamethasone binding revealed that mesenchymal cells isolated from midgestation A/J mice have more than twice as many receptors for steroids than cells isolated from C57B1 mice [23]. Cells that produce the matrix of connective tissue may also have more steroid receptors and could possibly cause the altered dexamethasone sensitivity observed in A/J mouse skin.

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