

# Metabolism of Corticosterone in Mammalian and Avian Intestine

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11 $\beta$ -hydroxysteroid dehydrogenase (11 $\beta$ HSD) catalyzes the conversion of the glucocorticoids, corticosterone and cortisol, to the respective derivatives 11-dehydrocorticosterone and cortisone. The recent findings underline the importance of this enzyme in excluding glucocorticoids from mineralocorticoid receptors. In the present study, 11 $\beta$ HSD activity was compared in the intestine of herbivorous (guinea pig), omnivorous (rat), and granivorous (hen) animals, i.e., in animals in which the Na<sup>+</sup> transport either is or is not regulated by aldosterone under normal conditions and in which the plasma levels of individual glucocorticoids are different. Slices of various intestinal segments were incubated in the presence of corticosterone or 11-dehydrocorticosterone, and the steroids were extracted and analyzed by HPLC. In the mammalian intestine, the activity of 11 $\beta$ HSD was very low (approaching zero) in aldosterone-insensitive segments (duodenum, jejunum) but significant activity was revealed in aldosterone-sensitive segments (ileum, cecum, and proximal and distal colon). In comparison with the rat, the guinea pig large intestine exhibited significantly higher activity of 11 $\beta$ HSD. There was no detectable reductase activity (conversion of 11-dehydrocorticosterone to corticosterone) in any intestinal segments of either species. Unexpectedly, no 11 $\beta$ HSD activity was observed in the avian intestine. It was found that, in contrast to the mammalian intestine, corticosterone was metabolized to 20-dihydrocorticosterone while 11-dehydrocorticosterone was converted to 11-dehydro-20-dihydrocorticosterone. The distribution of 20-hydroxy-

steroid dehydrogenase (20HSD) activity in the avian intestine was homogenous along the intestine and did not correlate with the mineralocorticoid sensitivity of intestinal segments. To trace different cosubstrate dependence of 11 $\beta$ HSD and 20HSD, homogenates of ileum and distal colon were incubated with NAD<sup>+</sup>/NADH or NADP<sup>+</sup>/NADPH, respectively. In accordance with slice experiments mammalian intestine displayed only oxidation of corticosterone to 11-dehydrocorticosterone and NAD<sup>+</sup> preference. In avian intestine, the metabolite formed from corticosterone was 11-dehydrocorticosterone in the presence of NAD<sup>+</sup> or NADP<sup>+</sup> whereas in the presence of NADPH 11-dehydro-20-dihydrocorticosterone and 20-dihydrocorticosterone were formed. Given the wide similarity between mineralocorticoid regulation of epithelial transport in mammals and birds, the unexpected finding of differences in the metabolism of corticosterone suggests that role of 20HSD is to allow aldosterone occupancy of mineralocorticoid receptors. © 1998 Academic

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The enzyme 11 $\beta$ -hydroxysteroid dehydrogenase (11 $\beta$ HSD) interconverts active glucocorticoids (cortisol, corticosterone) to their 11-dehydro forms (cortisone, 11-dehydrocorticosterone), which normally exhibit very weak corticosteroid activity *in vivo*. There is now considerable evidence suggesting that 11 $\beta$ HSD decreases the concentration of intracellular glucocorticoids to levels that allow aldosterone to occupy nonselective mineralocorticoid receptors (Funder *et al.*, 1988;

Edwards and Stewart, 1991; Monder, 1991). Because aldosterone is not a substrate for  $11\beta$ HSD, it is available to occupy mineralocorticoid receptors in spite of the fact that its levels are much lower than those of corticosterone or cortisol. If  $11\beta$ HSD activity is decreased, glucocorticoids are able to act as mineralocorticoids (Leckie *et al.*, 1995) which results in excessive  $\text{Na}^+$  retention and  $\text{K}^+$  secretion (Souness and Morris, 1989). The biochemical and molecular biological evidence indicates that there exist at least two distinct isoforms of  $11\beta$ HSD. One isoform ( $11\beta$ HSD1) has a high  $K_m$  ( $\mu\text{M}$ ) for its glucocorticoid substrates, is  $\text{NADP}^+$  ( $\text{NADPH}$ ) dependent, and exerts both dehydrogenase and reductase activities (Lakshmi and Monder, 1988; Agarwal *et al.*, 1989). It is localized in various organs such as liver, heart, and testis (Lakshmi and Monder, 1988; Walker *et al.*, 1992). The other isoform is an  $\text{NAD}^+$ -dependent enzyme ( $11\beta$ HSD2), which exerts dehydrogenase activity only and has a  $K_m$  in the nanomolar range (Rusvai and Naray-Fejes-Toth, 1993; Albiston *et al.*, 1994; Agarwal *et al.*, 1994). It is predominantly expressed in the placenta (Brown *et al.*, 1993) and in mineralocorticoid target epithelia such as renal distal tubules and collecting ducts (Walker *et al.*, 1992; Rusvai and Naray-Fejes-Toth, 1993; Krozowski *et al.*, 1995), the colon (Walker *et al.*, 1992; Whorwood *et al.*, 1994; Kyossev *et al.*, 1996), and toad bladder (Brem *et al.*, 1993).

The principal action of aldosterone in mineralocorticoid target epithelia is to increase the absorption across epithelial membranes; increased  $\text{Na}^+$  absorption is mediated by the electrogenic amiloride-sensitive  $\text{Na}^+$  pathway, i.e., via amiloride-sensitive  $\text{Na}^+$  channels in the apical membrane and  $\text{Na,K-ATPase}$  in the basolateral membrane. This transport pathway is present not only in renal but also in intestinal epithelia, however, with large species differences. In herbivores (rabbits, guinea pigs), electrogenic amiloride-sensitive  $\text{Na}^+$  transport is present as a constitutive pathway in the colon (Clauss *et al.*, 1985; Binder and Sandle, 1994), whereas in omnivores, such as rats, there is no evidence of a conductive pathway in normal circumstances and  $\text{Na}^+$  absorption is an electroneutral  $\text{Na}^+\text{-Cl}^-$  process which is regulated by glucocorticoids (Binder and Sandle, 1994). Only severe, nonphysiological salt depletion, which stimulates plasma levels of aldosterone, is capable of inducing electrogenic  $\text{Na}^+$

transport in the rat distal colon (Pácha and Pohlová, 1995). Although the effect of aldosterone in the rat colon is similar to that in herbivorous animals, it is not a simple amplification of the existing transport pathway but its induction *de novo*. Phenomena similar to those in the rat were observed in the lower intestine of seed-eating birds such as the domestic fowl. Colonic  $\text{Na}^+$  transport in hens is electrogenic and amiloride-sensitive, but increasing dietary  $\text{Na}^+$  intake abolishes electrogenic  $\text{Na}^+$  transport and induces  $\text{Na}^+$ -hexoses and  $\text{Na}^+$ -amino acid cotransport systems (Clauss and Skadhauge, 1988).

So far the presence of  $11\beta$ HSD in intestinal epithelia was studied only in the rat and human intestine, predominantly in the colon (Pácha and Mikšík, 1994, 1996; Whorwood *et al.*, 1994; Kyossev *et al.*, 1996), i.e., in omnivorous species which express mineralocorticoid regulation of  $\text{Na}^+$ -transporting epithelia only under conditions of severe salt depletion. To address whether the conversion of corticosterone to 11-dehydrocorticosterone is a common feature of the intestine and whether there are potential interspecies differences in intestinal  $11\beta$ HSD activity of animals that either do or do not exert mineralocorticoid regulation of intestinal epithelium in normal circumstances, we measured the distribution of  $11\beta$ HSD in mineralocorticoid-sensitive and -resistant segments of the intestine in omnivorous (rat), herbivorous (guinea pig), and seed-eating (hen) species.

## MATERIALS AND METHODS

### Animals

Experiments were performed on male Wistar rats (200–250 g), Hartley guinea pigs (350–400 g), and White Leghorn hens (1.1–1.3 kg). The rats received a standard diet containing 126  $\mu\text{equiv Na}^+/\text{g}$  diet, guinea pigs were fed green feed, and hens cereal grains (wheat and barley) for 10 days before the experiment and all animals had free access to distilled water. It has been demonstrated previously that hens and guinea pigs fed these diets possess electrogenic amiloride-sensitive  $\text{Na}^+$  transport in the large intestine (Clauss *et al.*, 1985; Clauss and Skadhauge, 1988), whereas rats do not (Pácha and Pohlová, 1995).

### **Transformation of Corticosterone and 11-Dehydrocorticosterone**

**Assay in intestinal slices.** The animals were killed by decapitation and intestinal segments were rapidly removed, washed in ice-cold 0.9% saline, dissected free from fat and connective tissue, and opened longitudinally. The intestinal segments of the duodenum, jejunum, ileum, cecum, and proximal and distal colon (middle part of the colon and coprodeum in hens) were cut with a razor blade into small strips 1- to 2-mm wide and rinsed in an ice-cold incubation solution containing (in mM): NaCl, 119.0; CaCl<sub>2</sub>, 1.2; MgCl<sub>2</sub>, 1.2; NaHCO<sub>3</sub>, 21.0; K<sub>2</sub>HPO<sub>4</sub>, 2.4; KH<sub>2</sub>PO<sub>4</sub>, 0.6; glucose, 10.0; glutamine, 2.5;  $\beta$ -hydroxybutyrate, 0.5; and mannitol, 10.0, previously gassed for 10 min with 95% O<sub>2</sub>/5% CO<sub>2</sub> (v/v), pH 7.4.

Transformation of corticosterone and 11-dehydrocorticosterone was measured according to Pácha and Mikšík (1996). Incubation of the intestinal tissue was carried out in sealed vessels containing 10 ml of oxygenated incubation solution and unlabeled corticosterone (1.45  $\mu$ M) or 11-dehydrocorticosterone (1.45  $\mu$ M) for 80 min at 37°C. Using this substrate concentration, the transformation of corticosterone was linearly proportional to the time of incubation up to 150 min in both rat and guinea pig distal colon and in hen colon (data not shown). At the end of incubation, an internal standard of desoxycorticosterone (5  $\mu$ g/10 ml, 1.52  $\mu$ M) was added and the vessels were mixed and placed on ice. The incubation medium was centrifuged for 10 min at 3000g. The supernatant was loaded onto a C18 reversed-phase Sep-Pak column (Waters, Milford, MA) and the steroids were extracted by 2 ml methanol, evaporated to dryness under nitrogen, and stored at -20°C.

**Assay in intestinal homogenates.** Ileum or distal colon (middle part of the colon in hen) were homogenized in ice-cold 0.2 M sucrose (1:9 w/v) by a Polytron homogenizer. The homogenate was centrifuged at 1000g for 10 min and the supernatant was assayed for protein concentration using the method of Bradford (1976). Transformation of corticosterone was assayed in tubes containing 250  $\mu$ l of intestinal homogenate (rat, hen: 1 mg of protein; guinea pig: 0.1 mg of protein), 750  $\mu$ l of buffer (100 mM KCl, 50 mM Tris-HCl, pH 9.0), and 40  $\mu$ l NAD<sup>+</sup>, NADP<sup>+</sup>, NADH, or NADPH (final concentration 400  $\mu$ M). After a

10-min preincubation at 37°C 1.3  $\mu$ Ci of [<sup>3</sup>H]corticosterone (final concentration 14.5 nM) was added. The incubation was selected on the basis of preliminary experiments to fit the linear part of the time dependence (incubation time versus percentage substrate conversion plot). The reaction was terminated by cooling of the samples, which were then centrifuged for 20 min (3000g). The supernatant was loaded onto C18 reversed-phase Sep-Pak columns and the steroids extracted and stored as described above. Steroids were obtained from Sigma (St. Louis, MO) and Steraloids (Wilton, NH).

### **Analytical Techniques**

The metabolites of corticosterone and 11-dehydrocorticosterone were estimated by high-performance liquid chromatography (HPLC) and micellar electrokinetic chromatography (MEKC).

**HPLC technique.** The samples were reconstituted in 300  $\mu$ l of methanol and 90  $\mu$ l was loaded onto a Lichrospher 100 RP-18 (125  $\times$  4 mm) column (Merck, Darmstadt, Germany) and eluted using a linear methanol-water gradient (Waters automated gradient controller and Model 510 pump) from 45:55 (v/v) to 65:35 (v/v) over 15 min followed by isocratic washing with 100% methanol for another 10 min. The flow rate was 1.0 ml/min and the column temperature was held at 45°C. Ultraviolet absorbance at 254 nm (Waters absorbance detector Model 441) was recorded to monitor unlabeled corticosteroids. Calibration was made for the range 0.06–3.0  $\mu$ g per injection; the limit of detection was 0.02  $\mu$ g. The elution of <sup>3</sup>H-labeled steroids was monitored by online radioactive detection using a radioisotope detector with solid cell (Beckman Type 171, Fullerton, CA). After subtraction of the background radioactivity, integrated counts within peaks were analyzed by Apex Version 3.1 software (DataApex, Prague, Czech Republic).

**MEKC technique.** MEKC was performed on a Beckman P/ACE 5500 Instrument with a diode-array detector controlled by System Gold software. An unmodified fused-silica capillary 47 cm long (40 cm to the detector), 75  $\mu$ m i.d. was used with a background buffer consisting of 50 mM sodium deoxycholate and 50 mM borate buffer adjusted to pH 10.0 with 1 M NaOH. The capillary was first rinsed for 2 min with 1 M NaOH, then for 2 min with water, and then

washed for 2 min with the background buffer. Separations were routinely run at 15 kV at 30°C and monitored at 250 nm.

### Statistical Analysis

Experimental values are reported as means with their standard errors. Further statistical analysis was performed using BMDP programs (Dixon, 1988). For a comparison of transformation of unlabeled corticosterone or 11-dehydrocorticosterone in slices of intestinal segments two-way analysis of variance (ANOVA) was used (species versus segment). For multiple comparison of transformation of [<sup>3</sup>H]corticosterone in intestinal homogenates three-way ANOVA was used (cosubstrate versus segment versus species). Simple effects in this model were revealed from two-way ANOVA under conditions of a fixed level of the third factor. The

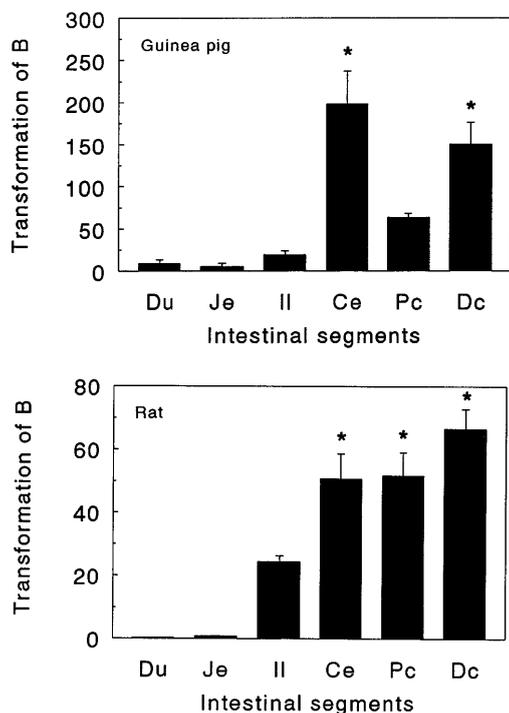


FIG. 1. Transformation of unlabeled corticosterone (B; 1.45  $\mu$ M) along the intestine of guinea pig and rat via 11 $\beta$ HSD. 11 $\beta$ HSD activity is based on the formation of 11-dehydrocorticosterone in pmol per mg of dry weight per hour. Bars indicate SEM ( $n = 5-6$ ). Du, duodenum; Je, jejunum; Il, ileum; Ce, cecum; Pc, proximal colon; Dc, distal colon. Analysis of variance demonstrated significant differences among intestinal segments ( $P < 0.001$ ). \*Significantly higher than in other segments ( $P < 0.05$ ).

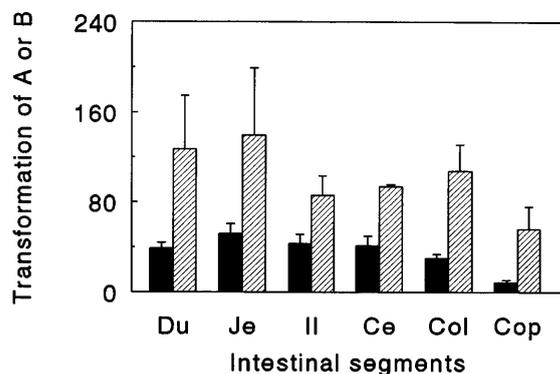


FIG. 2. Transformation of unlabeled corticosterone (B; 1.45  $\mu$ M) and 11-dehydrocorticosterone (A; 1.45  $\mu$ M) in avian intestine. The transformation is expressed as the formation of 20-dihydrocorticosterone (substrate: B) or 11-dehydro-20-dihydrocorticosterone (substrate: A) in pmol per mg of dry weight per hour. Solid, transformation of corticosterone; hatched, transformation of 11-dehydrocorticosterone. Du, duodenum; Je, jejunum; Il, ileum; Ce, cecum; Col, colon; Cop, coprodeum. Analysis of variance did not demonstrate any significant differences among the intestinal segments.

Newman-Keuls multiple range test was used to determine significant differences among individual means. Statistically significant changes were accepted at the 5% level.

## RESULTS

### Intestinal Slices

Results obtained with slices from different intestinal segments are presented in Figs. 1 and 2. In rats and guinea pigs, corticosterone (1.45  $\mu$ M) was metabolized into 11-dehydrocorticosterone predominantly in the large intestine (cecum, proximal and distal colon) and less in the ileum. No or very weak conversion was detected in the duodenum and jejunum (Fig. 1). This oxidation indicates the presence of 11 $\beta$ HSD in the distal part of the small intestine and in all segments of the large intestine. Even if the patterns of activity in rat and guinea pig were not identical, a clearcut difference in the distribution of this activity was absent. The only difference was significantly higher activity of 11 $\beta$ HSD in the distal colon and cecum of the guinea pig than of the rat ( $P < 0.01$ ). In order to test the reverse reaction, designated as 11-oxoreductase activity, tissue slices

were incubated with 11-dehydrocorticosterone (1.45  $\mu\text{M}$ ). No detectable corticosterone in the incubation medium of all intestinal segments was found regardless of the incubation time (80 or 160 min).

Glucocorticoid metabolism in the avian intestine was quite different. As shown in Fig. 3A, corticosterone was not converted into 11-dehydrocorticosterone but into a different metabolite. This metabolite has been tentatively designated as 20-dihydrocorticosterone: the retention times of the hitherto unknown metabolite and 20 $\beta$ -dihydrocorticosterone were identical and when authentic extract was spiked with 20 $\beta$ -dihydrocorticosterone, this compound coeluted with the unknown analyte (HPLC analysis). MEKC proof for the identity of the metabolite with 20-dihydrocorticosterone is presented in Fig. 4. These findings document that in contrast to the mammalian intestine, the

segments of avian intestine possess 20-hydroxysteroid dehydrogenase (20HSD) operating in the reduction mode. The distribution of this activity is not significantly different among various intestinal segments (Fig. 2). The avian intestine also metabolizes 11-dehydrocorticosterone. However, the metabolite was not corticosterone but again an unknown compound, which has been designated 11-dehydro-20-dihydrocorticosterone (Figs. 3B and 4B). In support of this conclusion, we have prepared the same compound by incubation of 20 $\beta$ -dihydrocorticosterone (1.45  $\mu\text{M}$ ) with the rat distal colon, which possesses high activity of 11 $\beta$ HSD. As is shown in Fig. 2, there is no difference in reduction of 11-dehydrocorticosterone among the intestinal segments. To test whether 20HSD activity reflects a physiological phenomenon we have further investigated the metabolic activity in immature 8-day-old

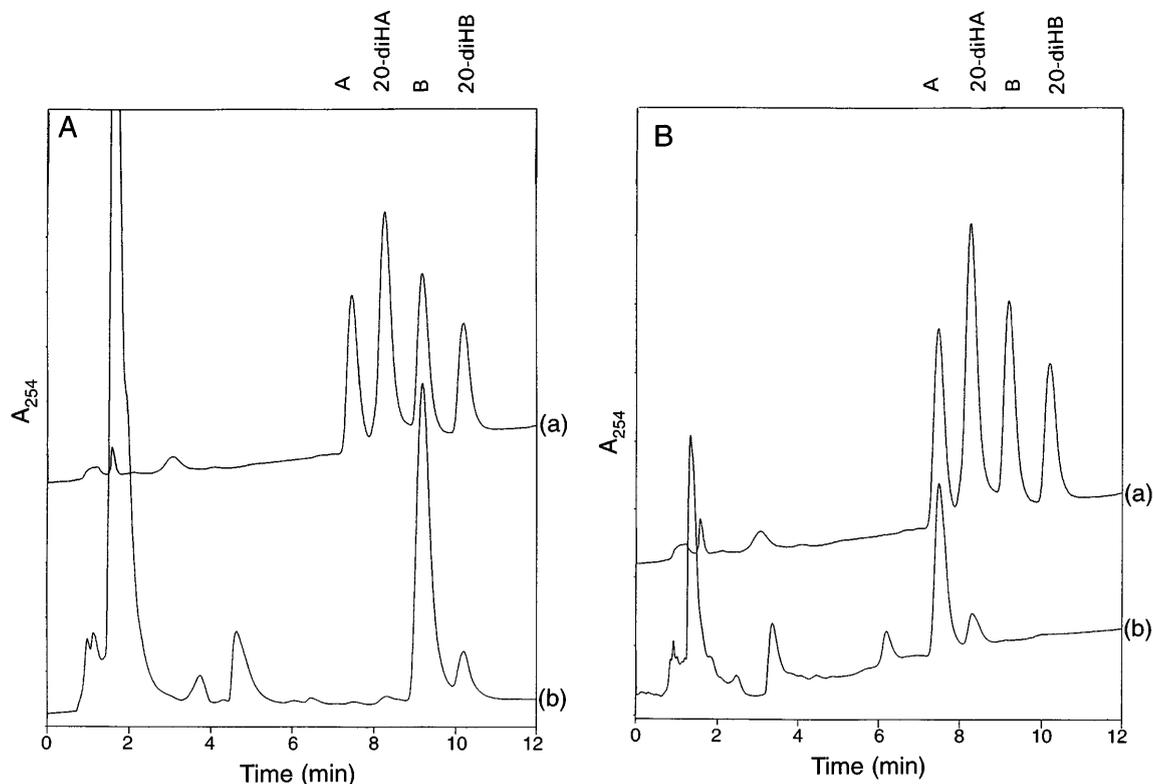


FIG. 3. (A) Metabolism of corticosterone in the hen. Representative HPLC chromatograms demonstrate the identification of the corticosterone metabolite. The HPLC chromatograms are (a) standards of steroids: corticosterone (B), 11-dehydrocorticosterone (A), 20 $\beta$ -dihydrocorticosterone (20-diHB), and 11-dehydro-20-dihydrocorticosterone (20-diHA); (b) jejunum incubated with unlabeled corticosterone (1.45  $\mu\text{M}$ ). (B) Metabolism of 11-dehydrocorticosterone by the hen jejunum. The representative chromatograms are (a) standards of steroids: abbreviations are the same as in A; (b) jejunum incubated with unlabeled 11-dehydrocorticosterone (1.45  $\mu\text{M}$ ).

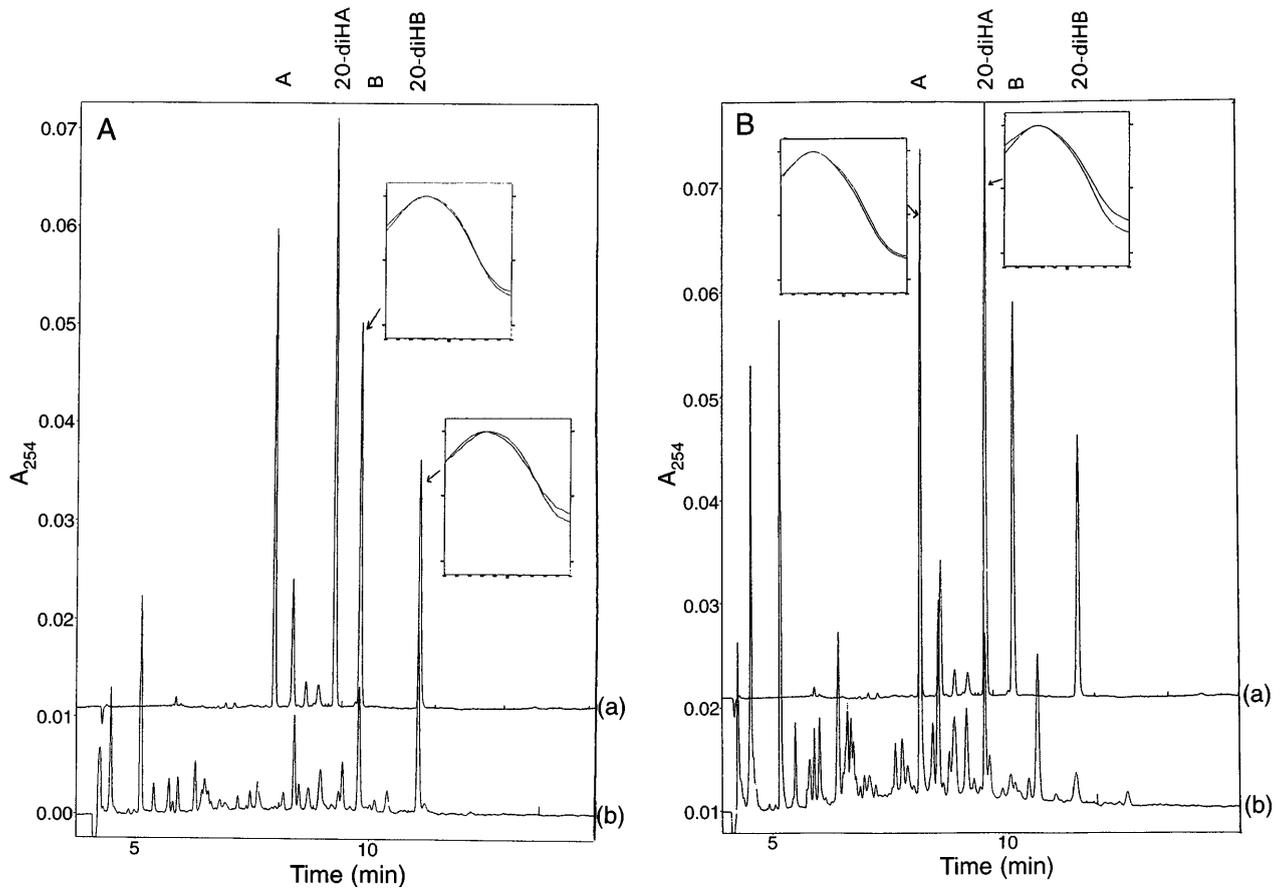


FIG. 4. (A) Metabolism of corticosterone in the hen. Representative MEKC electrophoregrams demonstrate the identification of the corticosterone metabolite. The MEKC electrophoregrams are (a) standards of steroids; abbreviations are the same as in Fig. 3A; additional peaks are contaminants of 11-dehydrocorticosterone; (b) jejunum incubated with unlabeled corticosterone ( $1.45 \mu\text{M}$ ). (B) Metabolism of 11-dehydrocorticosterone by the hen jejunum. The representative electrophoregrams are (a) standards of steroids; abbreviations are the same as in Fig. 3A; (b) jejunum incubated with unlabeled 11-dehydrocorticosterone ( $1.45 \mu\text{M}$ ). (Insets) Comparison of spectra of the steroid metabolites and the corresponding standards (similarity index: A, 0.9997; B, 0.9997; 20-diHA, 0.9978; 20-diHB, 0.9990); the x axis is 230–280 nm.

chicks. The activity was absent in these birds ( $n = 4$  for each segment); this suggests that 20HSD activity is developmentally regulated.

### Intestinal Homogenates

To test the cosubstrate preference of  $11\beta\text{HSD}$  and  $20\text{HSD}$  in the intestinal segments, we used homogenates of ileum and distal colon (colon in hen) and added cosubstrates as indicated in Table 1. In mammalian ileum and distal colon,  $[^3\text{H}]$ corticosterone ( $14.5 \text{ nM}$ ) was converted to 11-dehydrocorticosterone only and the activity of this conversion was higher in the presence of  $\text{NAD}^+$  than  $\text{NADP}^+$  both in guinea pig

distal colon and ileum and in rat distal colon. In the presence of reduced cosubstrates no substrate utilization was apparent in rat, but reduced cosubstrates stimulated conversion of  $[^3\text{H}]$ corticosterone to 11-dehydrocorticosterone in guinea pig. Since no conversion was observed in the absence of reduced or oxidized cosubstrates (data not shown), we suppose that some other system is capable of oxidizing  $\text{NADPH}$  and  $\text{NADH}$  in our assay.

In avian homogenates, the product of transformation of  $[^3\text{H}]$ corticosterone was 11-dehydrocorticosterone in the presence of  $\text{NAD}^+$ ,  $\text{NADP}^+$ , and  $\text{NADH}$  (Table 1). Similar to guinea pigs, the transformation of  $[^3\text{H}]$ corticosterone was zero in the absence of cosub-

TABLE 1

Transformation of [<sup>3</sup>H]Corticosterone in Mammalian and Avian Ileum and Distal Colon (Colon in Hen) in the Presence of Oxidized and Reduced Cosubstrates

	NAD <sup>+</sup>	NADP <sup>+</sup>	NADH	NADPH
	Ileum			
Rat	5.7 ± 1.2*	4.5 ± 1.1*	0	0
Guinea pig	573 ± 105**,**	193 ± 58**	6.2 ± 1.6	14.3 ± 1.2
Hen	13.5 ± 0.9	8.3 ± 0.8	6.9 ± 0.5	16.2 ± 1.5†
	Distal colon (colon in hen)			
Rat	14.5 ± 1.8*‡	7.4 ± 0.9*	0	0
Guinea pig	626 ± 123**,**	237 ± 64**	64.3 ± 13.8	40.4 ± 7.3
Hen	12.0 ± 1.3	6.3 ± 0.8	8.3 ± 0.8	19.1 ± 2.1†

*Note.* Values are means ± SEM,  $n = 6-10$ . Data are given in pmol of corticosterone per mg of protein per hour; concentration of cosubstrates was 0.4 mM and of [<sup>3</sup>H]corticosterone was 14.5 nM. For further details see Materials and Methods. In mammalian intestine, corticosterone was converted only to 11-dehydrocorticosterone. In avian intestine, the product of corticosterone transformation was 11-dehydrocorticosterone in the presence of NAD<sup>+</sup>, NADP<sup>+</sup>, and NADH or 20-dihydrocorticosterone and 11-dehydro-20-dihydrocorticosterone in the presence of NADPH. Analysis of variance demonstrated significant changes among the species and the effect of cosubstrates on the transformation ( $P < 0.001$ ) but no differences among segments. Cosubstrate differences: rat, \*significantly higher than in the presence of reduced cosubstrates ( $P < 0.01$ ), ‡significantly higher than in the presence of NADP<sup>+</sup> ( $P < 0.05$ ); guinea pig, \*\*significantly higher than in the presence of reduced cosubstrates ( $P < 0.05$ ), \*\*\*significantly higher than in the presence of NADP<sup>+</sup> ( $P < 0.01$ ); hen, † significantly higher than in the presence of all other cosubstrates ( $P < 0.01$ ).

strates, and thus we suppose that homogenates converted NADH to NAD<sup>+</sup> and the oxidized cosubstrate was involved in the formation of 11-dehydrocorticosterone. The presence of NADPH activated conversion of [<sup>3</sup>H]corticosterone to 20-dihydrocorticosterone and 11-dehydro-20-dihydrocorticosterone. In colon, 81% of converted corticosterone was recovered as 11-dehydro-20-dihydrocorticosterone and 19% as 20-dihydrocorticosterone; in ileum the respective percentages were 64 and 36%.

## DISCUSSION

The present study was designed to examine the pathways of corticosterone transformation in mammalian and avian intestine and to establish whether the activity of transformation correlates with mineralocorticoid sensitivity of intestinal segments. Our results

clearly indicate that corticosterone is metabolized by the mammalian intestine into 11-dehydrocorticosterone and into three compounds in avian intestine (11-dehydrocorticosterone, 20-dihydrocorticosterone, and 11-dehydro-20-dihydrocorticosterone). Identification of these compounds as the metabolites of corticosterone is based on their comigration with standards when analyzed by two different analytical methods (HPLC, MEKC). Supporting indirect evidence is the very high correlation between spectra of known standards and unknown metabolites and the fact that isolated enterocytes of duck colon extensively metabolize corticosterone to 20-dihydrocorticosterone (DiBattista *et al.*, 1989). These three independent methods seem to be sufficient for our conclusion about the nature of the metabolites.

As documented by the results obtained with slices, mammalian intestine displays only oxidative activity of 11 $\beta$ HSD, whereas avian intestine possesses reductive activity of 20HSD only. If intestinal slices reflect the endogenous pattern of dehydrogenases, it can be concluded that there are differences of corticosterone metabolism in mammalian and avian intestine. The experiments with homogenates demonstrated, however, the presence of 11 $\beta$ HSD in avian intestine. In the presence of oxidized cosubstrates (NAD<sup>+</sup>, NADP<sup>+</sup>) apparent oxidase activity of 11 $\beta$ HSD was displayed. When avian homogenates were incubated with labeled corticosterone and NADPH, 11-dehydro-20-dihydrocorticosterone and 20-dihydrocorticosterone were identified. This means that in the presence of NADPH, but not NADH, both 20HSD and 11 $\beta$ HSD operate. The pathway of 11-dehydro-20-dihydrocorticosterone formation (via 11-dehydrocorticosterone or 20-dihydrocorticosterone) remains to be evaluated. The basis of the discrepancy between absence of 11 $\beta$ HSD activity in intact cells and presence in homogenate is unclear but not unique to the avian intestine. Thus, transfection of COS 7 cells with a plasmid encoding 11 $\beta$ HSD1 produces exclusively 11 $\beta$ -reduction activity in intact cells, but potent dehydrogenation is revealed when the transfected cells are homogenized (Low *et al.*, 1994). Also the cultures of primary hippocampal and hepatic cells display only 11 $\beta$ -reduction activity, whereas homogenates have significant 11 $\beta$ -dehydrogenase activity (Jamieson *et al.*, 1995; Rajan *et al.*, 1996). Any variation in the ratio of oxidized and reduced cosubstrates is unlikely to be sufficient to account for the

dramatic changes in reaction direction (Jamieson *et al.*, 1995) and, consequently, it seems more likely that the subcellular structure, which is disrupted during homogenization, determines the enzyme activity.

One possible explanation for the present findings is that the reduction of corticosterone at the C-20 keto group might be an alternative system for facilitating aldosterone binding to mineralocorticoid receptors in the avian intestine. Various lines of evidence support such a conclusion. First, isolated duck colonocytes convert corticosterone to 20 $\beta$ -dihydrocorticosterone and have much lower cytosolic binding affinity for this compound than for corticosterone (DiBattista *et al.*, 1989). Second, the effect of corticosterone on mineralocorticoid transport function can be stimulated by carbenoxolone, which is not only the competitive inhibitor of 11 $\beta$ HSD but also of other dehydrogenases including 20HSD (Baker and Fanestil, 1991) even if glycyrrhetic acid and its derivative carbenoxolone are extremely potent inhibitors of 11 $\beta$ HSD ( $K_i$  in nanomolar range) but not 20HSD ( $K_i$  in micromolar range) (Monder *et al.*, 1989; Ghosh *et al.*, 1992). Grubb and Bentley (1992) have recently demonstrated that corticosterone stimulates *in vitro* electrogenic amiloride-sensitive Na<sup>+</sup> transport more in the presence than in the absence of 0.1 mM carbenoxolone. As the incubation system was very similar to our "slice experiments," we cannot exclude the possibility that the inhibition of corticosterone metabolism via 20HSD is responsible for the increased effect of corticosterone.

In contrast to avian intestine metabolizing corticosterone into three compounds, mammalian intestine appears to metabolize corticosterone only to 11-dehydrocorticosterone. Our experiments demonstrate that a considerable level of 11 $\beta$ HSD activity in the large intestine is a common feature of both the mammalian species studied, and the distribution of 11 $\beta$ HSD confirms earlier reports about mineralocorticoid sensitivity of intestinal segments. Some of the segments, such as the distal colon and cecum, are typical target segments for aldosterone (Loeschke and Müller, 1975; Sellin and DeSoignie, 1993; Binder and Sandle, 1994), whereas the others are mineralocorticoid insensitive or mineralocorticoids exert only a modest effect on these segments, such as in the ileum (Will *et al.*, 1985). Even if the rats are not under the influence of aldosterone and do not exhibit aldosterone-induced Na<sup>+</sup> transport

mechanisms under normal conditions (Pácha and Pohlová, 1995), the activity of 11 $\beta$ HSD is relatively high in mineralocorticoid target epithelia. The results are similar to guinea pig, in which Clauss *et al.* (1985) demonstrated a typical electrogenic Na<sup>+</sup> pathway. The finding of very high 11 $\beta$ HSD activity in guinea pig is consistent with a very similar affinity of aldosterone and cortisol to mineralocorticoid receptors in guinea pig colon and extraordinarily high circulating cortisol level in this species (Myles and Funder, 1994). At the moment it is not possible to conclude whether the 11 $\beta$ HSD system is operationally sufficient for discriminating between mineralocorticoids and glucocorticoids. Although some segments of guinea pig intestine have very high activity of 11 $\beta$ HSD (cecum, distal colon), the others have activity similar to rat (ileum, proximal colon). In addition, no correlation between plasma cortisol and 11 $\beta$ HSD activity in cardiac and vascular tissue of cortisol-dominant species was found, even if 11 $\beta$ HSD activity was much higher in corticosterone- than in cortisol-dominant species (Slight *et al.*, 1994). As we used a 14.5 nM substrate concentration in the experiments with intestinal homogenates and observed "mixed" cofactor preference, our data seem to reflect the presence of both isoforms of 11 $\beta$ HSD in mammalian and avian intestine.

In conclusion, this study demonstrates that the intestinal 11 $\beta$ HSD is expressed in both mammalian and avian intestine but that avian intestine metabolizes corticosterone in a manner different from that of the mammals. It is tempting to speculate that the mechanism conferring aldosterone selectivity on mineralocorticoid receptors in epithelial target tissue is different in the mammalian and avian intestine.

## ACKNOWLEDGMENT

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## REFERENCES

- Agarwal, A. K., Monder, C., Eckstein, B., and White, P. C. (1989). Cloning and expression of rat cDNA encoding corticosteroid 11 $\beta$ dehydrogenase. *J. Biol. Chem.* **264**, 18939–18943.

- Agarwal, A. K., Mune, T., Monder, C., and White, P. C. (1994). NAD<sup>+</sup>-dependent isoform of 11 $\beta$ -hydroxysteroid dehydrogenase. *J. Biol. Chem.* **269**, 25959–25962.
- Albiston, A. L., Obeyesekere, V. R., Smith, R. E., and Krozowski, Z. S. (1994). Cloning and tissue distribution of the human 11 $\beta$ -hydroxysteroid dehydrogenase type 2 enzyme. *Mol. Cell. Endocrinol.* **105**, R11–R17.
- Baker, M. E., and Fanestil, D. D. (1991). Licorice, computer-based analyses of dehydrogenase sequences, and the regulation of steroid and prostaglandin action. *Mol. Cell. Endocrinol.* **105**, C99–C105.
- Binder, H. J., and Sandle, G. I. (1994). Electrolyte transport in the mammalian colon. In "Physiology of the Gastrointestinal Tract" (L. R. Johnson, Ed.), pp. 2133–2171. Raven, New York.
- Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **72**, 248–254.
- Brem, A. S., Matheson, K. L., Latif, S., and Morris, D. J. (1993). Activity of 11 $\beta$ -hydroxysteroid dehydrogenase in toad bladder: Effects of 11 $\beta$ -dehydrocorticosterone. *Am. J. Physiol.* **264**, F854–F858.
- Brown, R. W., Chapman, K. E., Edwards, C. R. W., and Seckl, J. R. (1993). Human placental 11 $\beta$ -hydroxysteroid dehydrogenase: Evidence for and partial purification of a distinct NAD-dependent isoform. *Endocrinology* **132**, 2614–2621.
- Clauss, W., and Skadhauge, E. (1988). Modulation of Na and Cl transport by mineralocorticoids. *Comp. Biochem. Physiol.* **90A**, 583–589.
- Clauss, W., Dürr, J., and Rechkemmer, G. (1985). Characterization of conductive pathways in guinea pig distal colon in vitro. *Am. J. Physiol.* **248**, G176–G183.
- DiBattista, J. A., Mehdi, A. Z., and Sandor, T. (1989). Steroid C-20 oxidoreductase activity of duck intestinal mucosa: The interrelations of the enzymatic activity with steroid binding. *Gen. Comp. Endocrinol.* **74**, 136–147.
- Dixon, W. J. (1988). "BMDP Statistical Software Manual." Univ. of California Press, Berkeley.
- Edwards, C. R. W., and Stewart, P. M. (1991). The cortisol-cortisone shuttle and the apparent specificity of glucocorticoid and mineralocorticoid receptors. *J. Steroid Biochem. Mol. Biol.* **39**, 859–865.
- Funder, J. W., Pearce, P. T., Smith, R., and Smith, A. I. (1988). Mineralocorticoid action: Target tissue specificity is enzyme, not receptor, mediated. *Science* **242**, 583–585.
- Ghosh, D., Erman, M., Pangborn, W., Duax, W. L., and Baker, M. E. (1992). Inhibition of *Streptomyces hydrogenans* 3 $\alpha$ ,20 $\beta$ -hydroxysteroid dehydrogenase by licorice-derived compounds and crystallization of an enzyme-cofactor-inhibitor-complex. *J. Steroid Biochem. Mol. Biol.* **42**, 849–853.
- Grubb, B. R., and Bentley, P. J. (1992). Effects of corticosteroids on short-circuit current across the cecum of the domestic fowl, *Gallus domesticus*. *J. Comp. Physiol. B* **162**, 690–695.
- Jamieson, P. M., Chapman, K. E., Edwards, C. R. W., and Seckl, J. R. (1995). 11 $\beta$ -hydroxysteroid dehydrogenase is an exclusive 11 $\beta$ -reductase in primary cultures of rat hepatocytes: Effect of physicochemical and hormonal manipulations. *Endocrinology* **136**, 4754–4761.
- Krozowski, Z., Albiston, A. L., Obeyesekere, V. R., Andrews, R. K., and Smith, R. E. (1995). The human 11 $\beta$ -hydroxysteroid dehydrogenase type 2 enzyme: Comparisons with other species and localization to the distal nephron. *J. Steroid Biochem. Mol. Biol.* **55**, 457–464.
- Kyossef, Z., Walker, P. D., and Reeves, W. B. (1996). Immunolocalization of NAD-dependent 11 $\beta$ -hydroxysteroid dehydrogenase in human kidney and colon. *Kidney Int.* **49**, 271–281.
- Lakshmi, V., and Monder, C. (1988). Purification and characterization of the corticosteroid 11 $\beta$ -dehydrogenase component of the rat liver 11 $\beta$ -hydroxysteroid dehydrogenase complex. *Endocrinology* **123**, 2390–2398.
- Leckie, C., Chapman, K. E., Edwards, C. R. W., and Seckl, J. R. (1995). LLC-PK<sub>1</sub> cells model 11 $\beta$ -hydroxysteroid dehydrogenase type 2 regulation of glucocorticoid access to renal mineralocorticoid receptors. *Endocrinology* **136**, 5561–5569.
- Loeschke, K., and Müller, O. A. (1975). Hormones and the stimulated sodium transport in cecum hypertrophy. *Pflügers Arch.* **355**, 273–280.
- Low, S. C., Chapman, K. E., Edwards, C. R. W., and Seckl, J. R. (1994). Liver-type 11 $\beta$ -hydroxysteroid dehydrogenase cDNA encodes reductase but not dehydrogenase activity in intact mammalian COS-7 cells. *J. Mol. Endocrinol.* **13**, 167–174.
- Monder, C. (1991). Corticosteroids, receptors, and the organ-specific functions of 11 $\beta$ -hydroxysteroid dehydrogenase. *FASEB J.* **5**, 3047–3054.
- Monder, C., Stewart, P. M., Lakshmi, V., Valentino, R., Burt, D., and Edwards, C. R. W. (1989). Licorice inhibits corticosteroid 11 $\beta$ -dehydrogenase of rat kidney and liver: In vivo and in vitro studies. *Endocrinology* **125**, 1046–1053.
- Myles, K., and Funder, J. W. (1994). Type I (mineralocorticoid) receptors in the guinea pig. *Am. J. Physiol.* **267**, E268–E272.
- Pácha, J., and Mikšík, I. (1994). Distribution of 11 $\beta$ -hydroxysteroid dehydrogenase along the rat intestine. *Life Sci.* **54**, 745–749.
- Pácha, J., and Mikšík, I. (1996). 11 $\beta$ -hydroxysteroid dehydrogenase in developing rat intestine. *J. Endocrinol.* **148**, 561–566.
- Pácha, J., and Pohlová, I. (1995). Relationship between dietary Na<sup>+</sup> intake, aldosterone and colonic amiloride-sensitive Na<sup>+</sup> transport. *Br. J. Nutr.* **73**, 633–640.
- Rajan, V., Edwards, C. R. W., and Seckl, J. R. (1996). 11 $\beta$ -hydroxysteroid dehydrogenase in cultured hippocampal cells reactivates inert 11-dehydrocorticosterone, potentiating neurotoxicity. *J. Neurosci.* **16**, 65–70.
- Rusvai, E., and Naray-Fejes-Toth, A. (1993). A new isoform of 11 $\beta$ -hydroxysteroid dehydrogenase in aldosterone target cells. *J. Biol. Chem.* **268**, 10717–10720.
- Sellin, J. H., and DeSoignie, R. (1993). Steroids alter ion transport and absorptive capacity in proximal distal colon. *Am. J. Physiol.* **249**, G113–G119.
- Souness, G. W., and Morris, D. J. (1989). The antinatriuretic and kaliuretic effect of the glucocorticoids corticosterone and cortisol following pretreatment with carbenoxolone sodium (a liquorice derivative) in the adrenalectomized rat. *Endocrinology* **124**, 1588–1590.
- Slight, S., Ganjam, V. K., and Weber, K. T. (1994). Species diversity of

- 11 $\beta$ -hydroxysteroid dehydrogenase in the cardiovascular system. *J. Lab. Clin. Med.* **124**, 821–826.
- Walker, B. R., Campbell, J. C., Williams, B. C., and Edwards, C. R. W. (1992). Tissue-specific distribution of the NAD<sup>+</sup>-dependent isoform of 11 $\beta$ -hydroxysteroid dehydrogenase. *Endocrinology* **131**, 970–972.
- Whorwood, C. B., Ricketts, M. L., and Stewart, P. M. (1994). Epithelial cell localization of type 2 11 $\beta$ -hydroxysteroid dehydrogenase in rat and human colon. *Endocrinology* **135**, 2533–2541.
- Will, P. C., Cortright, R. N., Groseclose, R. G., and Hopfer, U. (1985). Amiloride-sensitive salt and fluid absorption in small intestine of sodium-depleted rats. *Am. J. Physiol.* **248**, G133–G141.