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Glucocorticoid Metabolism and Na^+ Transport in Chicken Intestine

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ABSTRACT The role of aldosterone in regulation of electrogenic Na^+ transport is well established, though mineralocorticoid receptors bind glucocorticoids with similar binding affinity as aldosterone and plasma concentration of aldosterone is much lower than glucocorticoids. In mammals, the aldosterone specificity is conferred on the low-selective mineralocorticoid receptors by glucocorticoid inactivating enzyme 11β-hydroxysteroid dehydrogenase (11HSD) that converts cortisol or corticosterone into metabolites (cortisone, 11–dehydrocorticosterone) with lower affinity for these receptors. The present study examined the chicken intestine, whether changes in 11HSD activity are able to modulate the effect of corticosterone on Na^+ transport, and how the metabolism of this hormone is distributed within the intestinal wall. This study shows that not only aldosterone, but also corticosterone (B), was able to increase the electrogenic Na^+ transport in chicken caecum in vitro. The effect of corticosterone was higher in the presence of carbenoxolone, an inhibitor of steroid dehydrogenases, and was comparable to the effect of aldosterone. The metabolism of B in the intestine was studied; results showed oxidation of this steroid to 11–dehydrocorticosterone (A) and reduction to 11–dehydro–20β–dihydrocorticosterone (20diA) as the main metabolic products at low nanomolar concentration of the substrate. In contrast, 20β–dihydrocorticosterone and 20diA were the major products at micromolar concentration of B. Progesterone was converted to 20β-dihydroprogesterone. The metabolism of corticosterone was localized predominantly in the intestinal mucosa (enterocytes). In conclusion, the oxidation at position C_{11} and reduction at position C_{20} suggest that both 11HSD and 20β-hydroxysteroid dehydrogenase (20HSD) operate in the chicken intestine and that the mucosa of avian intestine possesses a partly different system of modulation of corticosteroid signals than mammals. This system seems to protect the aldosterone target tissue against excessive concentration of corticosterone and progesterone. *J. Exp. Zool. 303A:113–122, 2005.*

INTRODUCTION

It is well established in mammals that glucocorticoids, cortisol, or corticosterone are able to serve as ligands of not only glucocorticoid, but also mineralocorticoid receptors. The extent of binding of these hormones to the receptors is determined by their concentrations. These in turn depend on local glucocorticoid metabolism. The enzyme 11β-hydroxysteroid dehydrogenase (11HSD) plays the key role in this steroid metabolism. This enzyme catalyzes the reversible conversion of physiologically active cortisol and corticosterone into their 11–oxo derivatives cortisone and 11–dehydrocorticosterone, that have lower affinity to the corticosteroid receptors. To date, two distinct forms of 11HSD have been identified: 11HSD type 1 shows both oxidative and reductive activities and has a relatively low affinity for glucocorticoid substrates, whereas 11HSD type 2 is primarily oxidative with a high affinity for glucocorticoids (Stewart and Krozowski, '99). The co-localization of 11HSD type 2 and the mineralocorticoid receptors has been shown to be responsible for assignment of the mineralocorticoid sensitivity of target tissues, such as renal and colonic epithelium (Stewart and Krozowski, '99). A mechanism similar to mineralocorticoid selectivity in mammals is assumed to operate in non-mammalian vertebrates (Brem et al., '89; Gaeggeler et al., '89), even though some data indicate that glucocorticoid metabolism is not identical in mammals (DiBattista et al., '89; Sabatini et al., '93; Vylitová et al., '98).

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In birds, due to the relatively weak concentration ability of the avian kidney and lack of a urinary bladder, urine is modified by retrograde movement into segments of the lower intestine, coprodeum, colon, and caeca, where water and NaCl are reabsorbed (Skadhauge, '93). This postrenal modification of urine is regulated in large part by aldosterone. In the domestic fowl aldosterone/low salt diet eliminate the colonic Na^+-linked cotransport (Garriga et al., 2001; Laverty et al., 2001), while concurrently inducing the expression of electrogenic sodium transport via amiloride-sensitive Na^+ channels (Thomas and Skadhauge, '82, '89; Grubb and Bentley, '87; Arnason and Skadhauge, '91) and apical Na^+/H^+-exchanger (De La Horra et al., 2001) in the ileum and lower intestine. The avian mineralocorticoid receptors in the gut bind aldosterone and corticosterone with nearly equal affinity (Rafestin-Oblin et al., '89; Sandor et al., '89), however, the plasma concentration of aldosterone is much lower than that of corticosterone, the dominant glucocorticoid in birds (Skadhauge et al., '83). One mechanism for avoiding excessive corticosterone binding to the mineralocorticoid receptors might be the competition of intestinal glucocorticoid and mineralocorticoid receptors for binding corticosterone. However, the binding affinity of this hormone to both glucocorticoid and mineralocorticoid receptors is similar. Another mechanism for reducing corticosterone binding to mineralocorticoid receptors is the metabolic conversion of corticosterone to derivatives with lower binding affinity. It has been shown that carbenoxolone, a non-specific inhibitor of steroid dehydrogenases (Baker and Fanestil, '91), increases the stimulatory effect of corticosterone on Na^+ transport in chicken intestine (Grubb and Bentley, '92). However, the studies performed on avian tissues have demonstrated that corticosterone can be transformed by several dehydrogenases (DiBattista et al., '89; Vylitová et al., '98). These studies have led to investigation of 1) whether inhibition of 11HSD modulates the effect of corticosterone on avian Na^+ transport and 2) how the metabolism of corticosterone is distributed within the intestinal wall.

MATERIALS AND METHODS

Animals and chemicals

Experiments were performed on Brown Leghorn chickens obtained from the hatchery of the Institute of Molecular Genetics (Czech Academy of Sciences, Prague). They were raised under controlled conditions (12–h light: 12–h dark photoperiod), fed a commercial poultry diet, and had free access to water. At 5–7 weeks the chickens were killed by decapitation and exsanguination, and the intestine was taken up. All experiments were approved by the Institutional Review Board.

The corticosteroids 11–dehydrocorticosterone (4–pregnen–21–ol–3,11,20–trione); corticosterone (4–pregnen–11β,21–diol–3,20–dione), 20β-dihydrocorticosterone (4–pregnen–11β,20β,21–triol–3–one), 11–dehydro–20–dihydrocorticosterone (4–pregnen–20β,21–diol–3,11–dione), progesterone (4–pregnen–3,20–dione), 20α-dihydroprogesterone (4–pregnen–20α-ol–3–one), 20β-dihydroprogesterone (4–pregnen–20β –ol–3–one) and deoxycorticosterone (4–pregnen–21–ol–3,20–dione) were purchased from Steraloids (Newport, RI). All other chemicals were from Sigma (St. Louis, MO).

Measurement of electrogenic Na^+ transport

The effect of corticosterone on the induction of electrogenic Na^+ transport was measured according to Grubb and Bentley ('92), with some modifications. Briefly, the caecum was mounted between two Ussing chambers and both the serosal and mucosal surfaces of the tissue were bathed in a bicarbonate buffer solution containing (in $mmol.l^{-1}$): NaCl, 119; $NaHCO_3$, 21; K_2HPO_4, 2.4; KH_2PO_4, 0.6; $CaCl_2$, 1.2; $MgCl_2$, 1.1; glucose, 10; glutamine, 2.5; β-hydroxybutyrate, 0.5; mannitol, 10.0, warmed to 37°C and aerated with 95% O_2/5% CO_2. The antibiotic azlocillin (50 $mg.l^{-1}$) was added to the incubation medium to prevent microbial degradation. Epithelial potential was measured continuously, except for brief periods when the tissue was short-circuited. The tissue was incubated in the presence or absence of aldosterone or corticosterone (both 15 $nmol.l^{-1}$) for 6 hours. In some experiments carbenoxolone (0.2 $mmol.l^{-1}$), a non-specific inhibitor of steroid dehydrogenases (Baker and Fanestill, '91), was added 1 h prior to adding corticosterone. The concentration of corticosterone was chosen because it has been found previously to induce submaximal stimulation of the short-circuit current in caeca, whereas the concentration of aldosterone was shown to induce maximal effect (Grubb and Bentley, '92). Seven hours after incubation of the tissue, amiloride (final concentration 10 $\mu mol.l^{-1}$) was added to the mucosal side

and the drop of the short-circuit current was recognized as electrogenic Na^+ transport.

Measurement of corticosteroid metabolism

The metabolism of corticosterone was studied in intestinal fragments, homogenates of the whole intestine or intestinal mucosa, and in isolated enterocytes.

Intestinal fragments

The intestinal fragments were prepared and their metabolism analyzed as described previously (Pácha and Mikšík, '96; Vylitová et al., '98). Briefly, the tissue slices (less than 1 mm thick) were cut from the blocks of the intestine vertically to the mucosal surface and collected in the ice-cold bicarbonate buffer mentioned above. Tissue fragments (300 mg) were placed in flasks containing 10 ml of bicarbonate buffer solution. Corticosterone dissolved in methanol was added to each sample at a final concentration of 15 $nmol.l^{-1}$ ([^{3}H]corticosterone; Amersham, UK). In some experiments corticosterone or other steroids yielded a final concentration 1.45 $\mu mol.l^{-1}$ close to K_m values of 20–hydroxysteroid dehydrogenases for various steroids (Nakajin et al., '88; DiBattista et al., '89; McNatt et al., '92) and allowing the analysis of the steroid products by mass spectrometry. The flasks were gassed with 95% O_2/5% CO_2, stoppered, and incubated at 37°C in a shaking water bath. The incubation times were selected to ensure that the velocities of the individual reactions would be linear. The reaction was stopped by cooling, tissue slices were removed, and the incubation buffer was extracted. To identify the stereospecificity of 20HSD, progesterone (final concentration 1.45 $\mu mol.l^{-1}$) was incubated with the intestinal fragments and the products of the stereospecific reduction of the 20–keto group was investigated. Progesterone was used instead of corticosterone in these experiments, because 20α-dihydrocorticosterone was not available on the market.

Intestinal homogenates

Intestinal tissue cut into small pieces or mucosal scrapings were homogenized (1:9 w/v) in ice-cold buffer containing sucrose 200 $mmol.l^{-1}$ and TRIS/HCl 10 $mmol.l^{-1}$ (pH 8.5) by a Polytron or Teflon homogenizer, respectively. The homogenate was centrifuged at 1000 × *g* for 10 min and the supernatant was assayed for protein concentration using the Bradford Coomassie blue method. Conversion of corticosterone was assayed as described previously (Vylitová et al., '98) in tubes containing buffer (100 $mmol.l^{-1}$ KCl; 50 $mmol.l^{-1}$ TRIS/HCl; pH 8.5), cosubstrate (NAD^+, $NADP^+$, NADH, or NADPH; 0.4 $mmol.l^{-1}$) and 1 mg (whole intestine) or 0.25 mg of protein (mucosa). After 10 min preincubation at 37°C [^{3}H]corticosterone (final concentration 20 $nmol.l^{-1}$) was added to the assay and incubation continued for 45 min (whole intestine) or 30 min (mucosa). The reaction was stopped by cooling and the steroids were extracted. To eliminate the possibility that $NADP^+$ produced by oxidation of NADPH was converted into NAD^+ by pyrophosphatases, some experiments were performed in the presence of 50 $mmol.l^{-1}$ sodium pyrophosphate (Gomez-Sanchez et al., '97).

Enterocytes

Enterocytes were isolated by hyaluronidase isolation procedure (Kimmich, '90) from the small intestine. The isolated enterocytes were added to pre-gassed (95% O_2/5% CO_2) incubation solution identical to the buffer used for incubation of intestinal slices. Corticosterone (1.45 $\mu mol.l^{-1}$) was added after 25 min of pre-incubation and the reaction was stopped 80 min later by cooling. The cells were centrifuged. The pellet was used for protein determination and the supernatant for steroid extraction.

Steroid extraction, identification, and quantification

The steroids were extracted from the incubation buffer by Sep-Pak cartridges (Waters, Milford, MA) and separated by HPLC. Non-radioactive steroids were detected on-line using ultraviolet absorbance detection, whereas the solid cell detector (Beckman Type 171, Fullerton, CA) was used (Vylitová et al., '98) for detection of radioactive steroids. The metabolites formed from corticosterone were further verified by HPLC/mass spectrometry (atmospheric pressure ionization-electrospray ionization, API-ESI, operating in positive mode, 1100 MSD from Hewlett Packard), (Mikšík et al., '99). Operating conditions were optimized by flow-injection analysis and were determined as follows: drying gas (N_2), 10 l/min; drying gas temperature, 350°C; nebulizator pressure, 20 psi (138 kPa); capillary voltage, 4 500 V; ions were observed at mass range *m*/*z* 200–500; fragmentor was set at 80 V. Reconstructed ion chromatogram for selected ion was set considering

the whole mass range of the considered ion, e.g. for *m/z* 347, the mass range *m/z* was 347–348.

NAD^+(H) and $NADP^+$(H) extraction and quantification

The cosubstrates NAD^+, $NADP^+$, NADH, and NADPH were quantified according to the enzymatic cycling method (Blomquist and Hakanson, '91). For NAD^+ and $NADP^+$, the mucosal scrapings were homogenized in 500 mmol.l^{-1} ice-cold perchloric acid, sonicated, left on ice for 15 min, and then centrifuged (1,500 × g, 10 min). For reduced cofactors (NADH, NADPH), the mucosa was homogenized in 250 mmol.l^{-1} NaOH, heated to 60°C for 5 min and centrifuged. Bicine (1 mol.l^{-1}; pH 8.0) was added to the supernatant, pH was titrated to 8.0, and the samples stored at −20°C. The extracted NAD^+/NADH and $NADP^+$/NADPH were quantitated by the cycling method using alcohol dehydrogenase (Sigma A–3263) and isocitrate dehydrogenase (Sigma I–1877), respectively. The efficacy of extraction was estimated in preliminary experiments and the data corrected accordingly.

Statistics

Experimental points are the mean ± S.E.M. Data were compared by Student's *t*-test or by analysis of variance and Neuman-Keuls post-hoc test, as appropriate. Probabilities less than 0.05 were considered significant.

RESULTS

Membrane transport studies

Incubation of chicken caeca with aldosterone and corticosterone resulted in a time-dependent increase of the short-circuit current. The hormone effect on the short-circuit current was distinguishable after a 2–3 h incubation period and reached a maximum 5 h later. Adding amiloride after 7 h of incubation led to an abrupt fall of the short-circuit current. It can be seen from Fig. 1 that aldosterone had greater potency to stimulate amiloride-sensitive short-circuit current than the same concentration of corticosterone. However, corticosterone stimulated amiloride-sensitive short-circuit current significantly more in the presence of carbenoxolone than with corticosterone alone, and its potency was similar to aldosterone. Carbenoxolone alone had no effect on short-circuit current. Similarly, the derivatives of corticosterone, 11–dehydrocorticosterone, 20–dihydrocorticosterone, or 11–dehydro–20–dihydrocorticosterone (100 nmol.l^{-1}) did not affect stimulation of

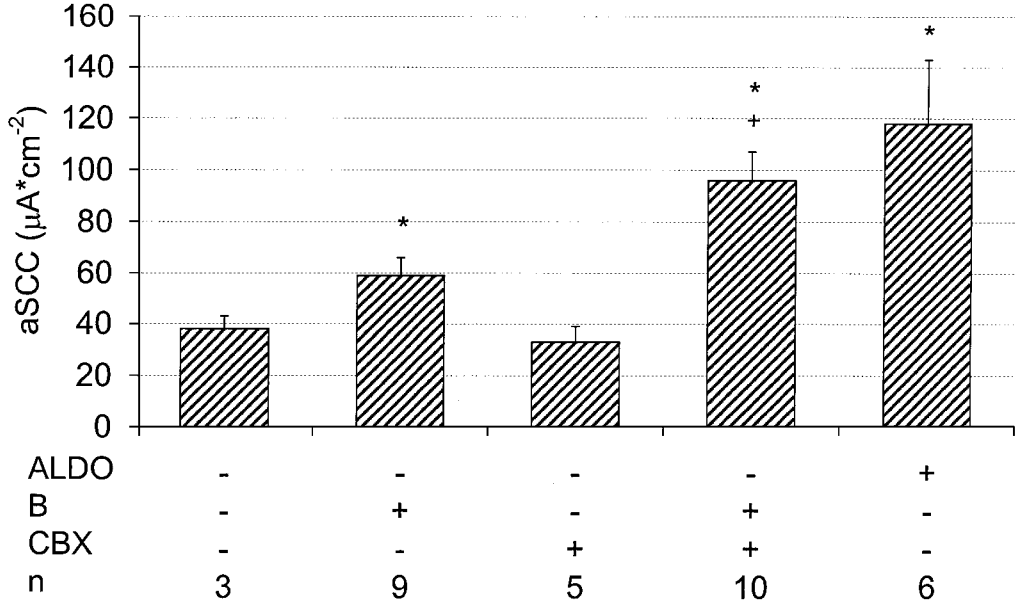

Fig. 1. Effect of corticosterone (B; 15 nmol.l^{-1}), aldosterone (ALDO, 15 nmol.l^{-1}) and carbenoxolone (CBX; 0.2 mmol.l^{-1}) on stimulation of amiloride-sensitive short-circuit current (aSCC) in chicken caecum. Amiloride (10 μmol.l^{-1}) was added to the mucosal side to obtain amiloride-sensitive short-circuit current as a measure of electrogenic Na^+ transport after 6 h incubation in the presence of corticosteroids. Total short-circuit current at the end of incubation was 75 ± 20 μA.cm^{-2} in control tissues in the absence of corticosteroids and carbenoxolone. *Significantly different from controls ($P < 0.05$). *Significantly different from corticosterone treated tissue in the absence of carbenoxolone ($P < 0.05$); n, numbers of animals.

amiloride-sensitive short-circuit current (not shown).

Corticosterone and progesterone transformation in chicken intestine

The conversion of corticosterone in intestinal fragments was investigated to determine whether the inhibitory effect of carbenoxolone on the induction of the short-circuit current was related to corticosterone metabolism. The intestine converted [^{3}H]corticosterone (15 nmol.l^{-1}) into two metabolites that cochromatographed with 11–dehydrocorticosterone and 11–dehydro–20–dihydrocorticosterone; i.e. the hormone was oxidized at position C_{11} by 11HSD and reduced at position C_{20} by 20–hydroxysteroid dehydrogenase (20HSD). A minor metabolite, 20–dihydrocorticosterone, was detected only in some samples. Figure 2 summarizes the percentage yield of steroid products in the absence and presence of carbenoxolone.

To characterize further the reductase activity of 20HSD, additional experiments were done at corticosterone concentration (1.45 μmol.l^{-1}) close to the K_m values of 20HSD. In this set of experiments corticosterone metabolism showed a different pattern than in experiments (Fig. 2) using corticosterone concentration that was close to the K_m value for 11HSD type 2 (Stewart and Krozowski, '99). The structure of the isolated products was identified by mass spectroscopy (Fig. 3). As shown in Table 1, corticosterone was converted predominantly into 20–dihydrocorticosterone and much less into 11–dehydro–20–dihydrocorticosterone and 11–dehydrocorticosterone, whereas 11–dehydrocorticosterone was metabolized only into 11–dehydro–20–dihydrocorticosterone. There was no detectable reduction of 11–dehydrocorticosterone to corticosterone. Similarly, 20–dihydrocorticosterone was oxidized only to 11–dehydro–20–dihydrocorticosterone (6.6 ± 0.8 pmol.mg DW^{-1}.h^{-1}) that was not changed further. Carbenoxolone decreased the conversion of corticosterone and 11–dehydrocorticosterone by $78 \pm 5\%$ and $72 \pm 11\%$, respectively.

To identify the stereospecificity of 20HSD the 20α- and 20β-dihydroderivatives of progesterone were analyzed under identical conditions, as in the experiments with corticosterone. Under these conditions progesterone was converted in both epimers of 20–dihydroprogesterone (α-epimer: 2.0 ± 0.8 and β-epimer 13.2 ± 1.0 pmol.mg DW^{-1}.h^{-1}). However only 20β-dihydroprogesterone seems to represent the biological activity of the tissue. The production of α-epimer reflects the spontaneous epimerisation, because the incubation of 20β-dihydroprogesterone resulted *per* hour

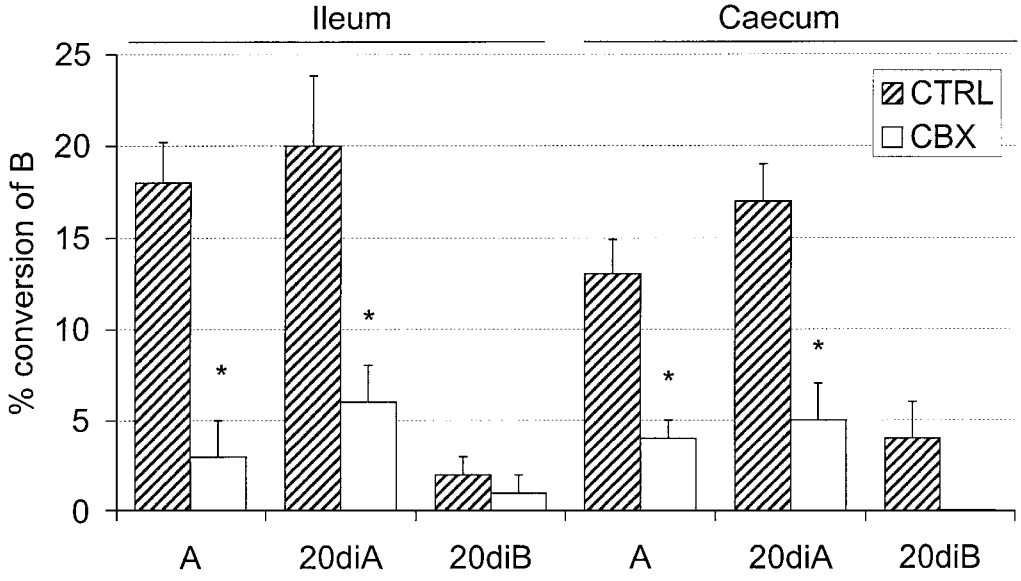

Fig. 2. Percentage conversion of [^{3}H]corticosterone (15 nmol.l^{-1}) in ileal and caecal fragments incubated in the presence or absence of carbenoxolone (CBX, 0.2 mmol.l^{-1}). Values are shown as percent conversion of [^{3}H]corticosterone to [^{3}H]11–dehydrocorticosterone in the incubation medium. Mean ± SEM of 9 (ileum) or 12 (caecum) animals. A, 11–dehydrocorticosterone; 20diB, 20–dihydrocorticosterone; 20diA, 11–dehydro–20–dihydrocorticosterone. *Significantly different from the values in the absence of carbenoxolone ($P < 0.05$).

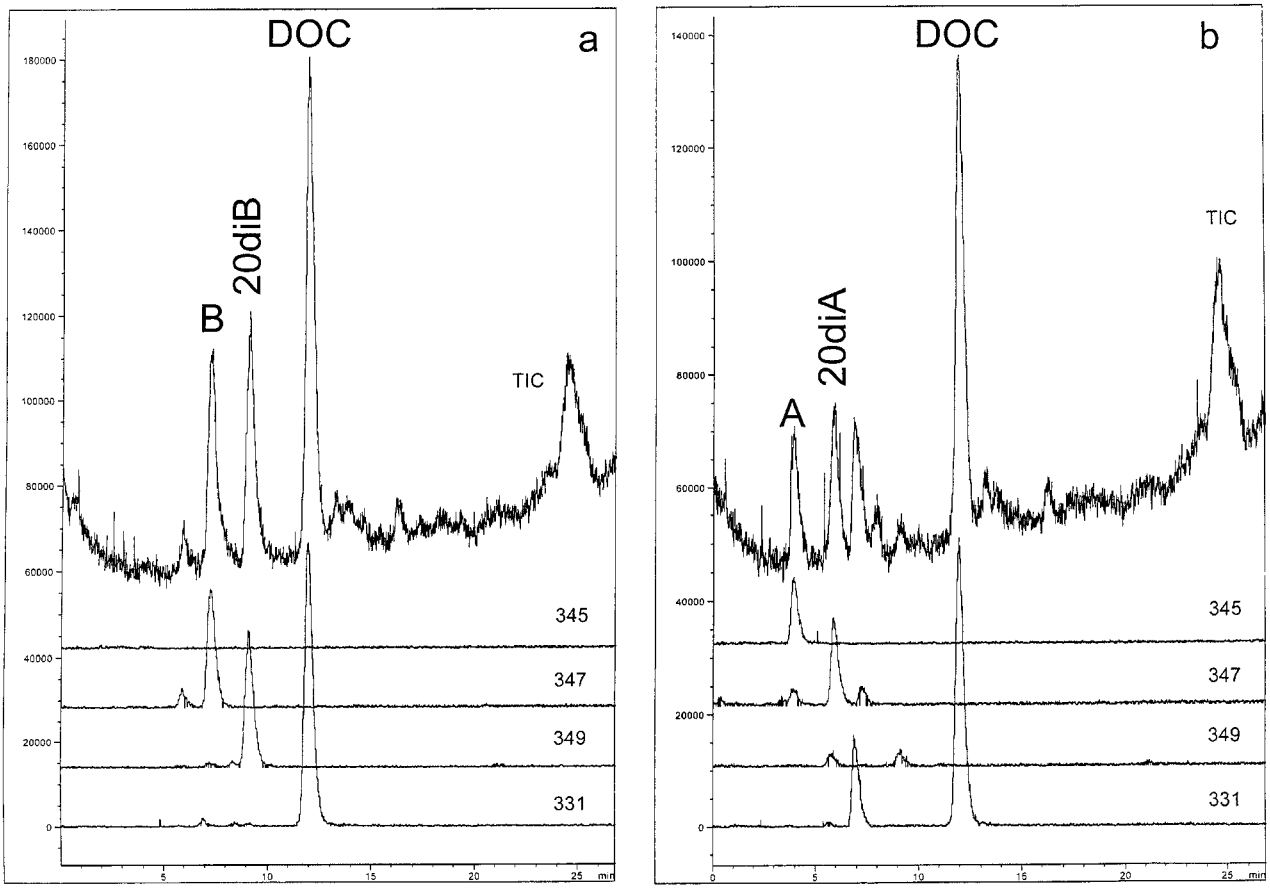

Fig. 3. Chromatographic (HPLC/MS) analysis of incubation of intestinal slices with corticosterone (a) and 11–dehydrocorticosterone (b) in the concentration 1.45 μmol.l^{-1}. *y-axis*: ion current, *x-axis*: retention time in minutes. First line: total ion current (***TIC***) for scan of 200–500 m/z, another lines are currents for extracted ions characteristic for individual steroids (m/z): ***345***, 11–dehydrocorticosterone (A); ***347***, corticosterone (B) and 11–dehydro–20–dihydrocorticosterone (20diA); ***349***, 20β–dihydrocorticosterone (20diB); and ***331***, deoxycorticosterone (DOC). Ranges at the y-axis are stretched for maximum response.

TABLE 1. Conversion of corticosterone and 11-dehydrocorticosterone in intestinal fragments

Product	B			A		
	20diB	A	20diA	20diA	B	20diB
Ileum	65±7	12±3	7±3	56±8	0	0
Caecum	58±8	7±4	6±3	51±7	0	0

Conversion of corticosterone (B; 1.45 μmol.l^{-1}) or 11-dehydrocorticosterone (A; 1.45 μmol.l^{-1}) is expressed as the synthesis of 20-dihydrocorticosterone (20diB), 11-dehydro-20-dihydrocorticosterone (20diA), and A, respectively, in picomoles of products per mg of dry weight per hour. Numbers of animals 10–12.

in spontaneous epimerization of 15% of 20β-dihydroprogesterone to 20α-dihydroprogesterone, whereas the opposite process was less than 1%. Similarly, in experiments with tissue, the transformation of progesterone into 20α-dihydroprogesterone represented only 15% of transformation into 20β-dihydroprogesterone.

Localization of steroid dehydrogenases within the intestinal wall

To test where the conversion of corticosterone is localized, its metabolism in three locations, the intestinal wall, enterocytes, and intestinal mucosa, was compared. Similar to intestinal fragments,

TABLE 2. Conversion of corticosterone in the whole intestine and intestinal mucosa and its inhibition by carbenoxolone in the presence of various cosubstrates

		n		A	20diA	20diB
Ileum	Gut wall	8	NAD^+	12±2	0	0
		10	NADPH	0	11±2	5±1
	Mucosa	19	NAD^+	134±11	5±4	0
		6	NAD^+CBX	5±1*	0	0
		21	NADPH	2±2	97±12	13±4
		8	NADPH+CBX	0	9±1*	0*
		11	Cosubstrate mixture	74±11*	40±7*	0*
Caecum	Gut wall	7	NAD^+	25±2	0	0
		7	NADPH	0	14±2	3±1
	Mucosa	8	NAD^+	150±14	0	0
		8	NADPH	7±4	82±15	31±4

Corticosterone (15 $nmol.l^{-1}$) was converted into 3 products: 11-dehydrocorticosterone (A), 20-dihydrocorticosterone (20diB), and 11-dehydro-20-dihydrocorticosterone (20diA), CBX, carbenoxolone (0.1 $mmol.l^{-1}$). Cosubstrate mixture means the mix of NAD^+, NADH, $NADP^+$ and NDPH in the ratio 20:6:1:9 that reflects the ratio of these cosubstrates in intestinal mucosa. The conversion is expressed as picomoles of product per mg protein and hour. Values are mean ± SEM; n, numbers of animals. *Significantly different from the synthesis in the presence of NAD+ or NADPH ($P < 0.05$).

TABLE 3. Levels of cosubstrates in intestinal mucosa

	NAD^+	NADH	$NADP^+$	NADPH
$nmol.mg\ prot^{-1}$	10.9±1.6	3.3±0.4	0.5±0.1	4.6±0.5

Analysis of variance demonstrated significant changes among cosubstrate concentration ($P < 0.001$). Numbers of animals 5–9.

isolated enterocytes converted corticosterone into 20–dihydrocorticosterone. However, this conversion was much higher than that of the intestinal wall without intestinal mucosa (342±51 (8) vs. 8.2±5.0 (5) $pmol.mg\ prot^{-1}.h^{-1}$). No reduction of 11–dehydrocorticosterone to corticosterone was observed in enterocytes. Similar distribution of corticosterone metabolizing enzymes was found when the activities of homogenates prepared from the whole intestinal wall and intestinal mucosa were compared. As shown in Table 2, 11–oxidation of corticosterone in mucosa significantly exceeded the conversion revealed in the whole intestine both in ileum and caecum.

Corticosterone was converted into 11–dehydrocorticosterone in the presence of NAD^+, whereas two products, 20–dihydrocorticosterone and 11–dehydro–20–dihydrocorticosterone, were found if NADPH was used as a cosubstrate (Table 2). Carbenoxolone blocked the activity of both enzymes. Additional experiments have been performed in the buffer containing sodium pyrophosphate, to determine whether the activity of 11HSD reflects, in the presence of NADPH, the activity of pyrophosphatases that split $NADP^+$ into NAD^+ and phosphate. Incubation in the presence of pyrophosphate significantly decreased the synthesis of 11–dehydro–20–dihydrocorticosterone (by 81±7%).

As C_{11}–oxidation and C_{20}–reduction have reciprocal demand on NAD^+/NADH and $NADP^+$/NADPH, intracellular cosubstrate concentration in intestinal mucosa (Table 3) was measured, and the intracellular cosubstrate ratio calculated. The ratio NAD^+:NADH:$NADP^+$:NADPH was 20:6:1:9. Therefore, the radiometric assay of corticosterone in the presence of all cosubstrates was repeated. In comparison with NADPH, the ''cocktail'' of cosubstrates stimulated the synthesis of 11–dehydrocorticosterone, whereas the synthesis of 11–dehydro–20–dihydrocoreticosterone was decreased. In comparison with NAD^+, the synthesis of 11–dehydrocorticosterone was suppressed and the formation of 11–dehydro–20–dihydrocorticosterone was increased (Table 2).

DISCUSSION

The studies performed both in vivo and in vitro clearly showed that aldosterone is a major regulator of Na^+ transport in hen intestine during changes in dietary NaCl intake. Whereas aldosterone changes from nearly zero values (NaCl surplus) to around 300–400 $pg.ml^{-1}$ at NaCl depletion in non-growing adult birds, corticosterone at a value 1–2 $ng.ml^{-1}$ undergoes no major changes (Arnason and Skadhauge, '91; Skadhauge, '93). In the young, growing chicks used in

this study, the regulation of plasma aldosterone is identical, but seems to occur at an even higher Na^+ intake, as additional Na^+ is needed due to the growth of bones and extracellular volume (Rosenberg and Hurwitz, '87). As the mineralocorticoid receptor displays the same or even higher affinity for corticosterone than for aldosterone (Sandor et al., '89), and the former hormone is more abundant in the plasma than aldosterone (Rosenberg and Hurwitz, '87; Arnason and Skadhauge, '91; Skadhauge, '93), it is obvious that some mechanism(s) are necessary to protect the mineralocorticoid receptors against permanent occupancy by corticosterone. Otherwise, corticosterone would be expected to fully occupy these receptors under most conditions. It is now well established in mammals that specificity of receptor occupancy is achieved by the juxtaposition of 11HSD with the mineralocorticoid receptors in epithelial cells and that this enzyme plays a pivotal role in their protection (Farman and Rafestin-Oblin, 2001).

The present study supports the hypothesis that peripheral metabolism of glucocorticoids has a physiological role in the avian intestine. Data are consistent with the idea that two enzymes, 11HSD and 20HSD, are involved in conversion of corticosterone into biologically inactive metabolites when the hormone diffuses from the intestinal capillaries to the receptors of effector epithelial cells; this metabolism might prevent the stimulation of Na^+ transport by glucocorticoids. First, the rapid conversion of corticosterone catalyzed by 11HSD and 20HSD was found in the ileum and caecum, i.e. in intestinal segments whose transport properties are regulated by aldosterone (Grubb and Bentley, '87, '92; Thomas and Skadhauge, '89; De La Horra et al., 2001; Garriga et al., 2001). Second, corticosterone stimulated electrogenic Na^+ transport more in the presence of the steroid dehydrogenase inhibitor than in its absence. Third, the steroid dehydrogenase activities were much higher in the intestinal mucosa and isolated enterocytes than in the whole intestine, i.e. 11HSD and 20HSD are localized in specific cell types and morphologically distinct regions of the intestine whose transport functions are regulated by aldosterone. Fourth, binding of corticosterone to enterocyte mineralocorticoid receptors is increased in the presence of carbenoxolone when the activity of 11HSD is significantly decreased (Sheppard et al., '99).

The ability of chicken intestinal mucosa to transform corticosterone sequentially into a number of metabolites has been demonstrated. It is oxidized at position C_{11} and also reduced at position C_{20}. The substrate of 11HSD is not only corticosterone, but also 20–dihydrocorticosterone. The substrate of 20HSD is both corticosterone and 11–dehydrocorticosterone. Even if the activity of 20HSD is absent in mammalian intestine (Vylitová et al., '98), it is present in the chicken (Fig. 2, Tables 1 and 2) and duck intestine (DiBattista et al., '89), and in the chorioallantoic membrane of the chick embryo, that transports Na^+ via electrogenic transport (Pácha et al., '85) and inactivates corticosterone via 20HSD (McNatt et al., '92).

It is well known that mineralocorticoid, glucocorticoid, androgen, and progesterone receptors have similar properties of binding a variety of steroids. In terms of potential competing steroids, corticosterone, deoxycorticosterone, and progesterone have considerable affinity to mineralocorticoid receptors and were found to operate as agonists (corticosterone, deoxycorticosterone) or antagonists (progesterone) of mineralocorticoid receptors and progesterone, and also as an inhibitor of 11HSD (Sandor et al., '89; Myles and Funder, '96; Latif et al., '97). The steroid oxidoreductase systems such as 11HSD and 20HSD might filter, therefore, the physiologically inappropriate signals relevant not only to the regulation of Na^+ transport but also to other regulatory pathways. For example, elevated dietary Na^+ intake suppresses plasma aldosterone concentration, but neither corticosterone, deoxycorticosterone nor progesterone, and plasma progesterone but not aldosterone level is subjected to considerable changes during onset of egg laying (Skadhauge et al., '83; Su et al., '96). Considering that neither deoxycorticosterone nor progesterone have an oxygen function at C_{11} to serve as a substrate for 11HSD, but both of them are reduced by 20HSD, co-expression of both dehydrogenases in mineralocrticoid target tissue might expulse various incorrect steroid signals from the mineralocorticoid receptors. This role of 20HSD has been recently demonstrated in the progesterone metabolism of mammals (Quinkler et al., '99); the 20–hydroxyderivatives of progesterone have been shown to have negligible binding to mineralocorticoid receptors (Butkus et al., '82).

These data about 11HSD and 20HSD may be reconciled by considering the interconnection between these dehydrogenases in such a way that C_{11}–oxidation and C_{20}–reduction are linked in intestinal mucosa. 11HSD and 20HSD have

reciprocal demands on NAD^+/NADH and $NADP^+$/NADPH ratio (Nakajin et al., '88; Noda et al., '91; Stewart and Krozowski, '99) and thus their functional coupling via cosubstrates might serve to amplify their effect. This conclusion is supported by Mercer and Krozowski ('92), who demonstrated in a histochemical study of rat kidney that the NAD^+-dependent oxidation of 11β-hydroxyandrostenedione led to staining that was abolished by carbenoxolone or deoxycorticosterone. In contrast, no reaction product was observed if cortisol or corticosterone were used in the presence of NAD^+ or $NADP^+$. These findings can be interpreted as evidence that C_{20}–reductase activity exploits the reduced cosubstrate equivalents at the expense of the color histochemical reaction.

These results show that low physiological concentrations of corticosterone are converted into two compounds in the avian intestine, 11–dehydrocorticosterone and 11–dehydro–20–dihydrocorticosterone. However, the question is whether these metabolites possess any biological activity and whether both 11HSD and 20HSD contribute to the inactivation system. When tested, 11–dehydrocorticosterone or 11–dehydro–20–dihydrocorticosterone have not shown any effect on the short-circuit current in the chicken intestine. However, Brem et al. ('91) have shown that 11–dehydrocorticosterone might be an active mineralocorticoid inhibitor and/or regulator that could modify the response to circulating aldosterone. Even if the actual physiological mechanisms and the relationship among C_{11} and C_{20} derivatives of corticosterone require additional investigation, it seems likely that C_{20}–-reduction may further decrease the biological activity of 11–oxo derivatives as was shown in case of 11–dehydrocorticosterone and 20–dihydrocorticosterone vs. 11–dehydro–20–dihydrocorticosterone (Sabatini et al., '93).

In summary, evidence is provided in this study that peripheral metabolism of glucocorticoids is involved in the endocrine regulation of intestinal transport and that this metabolism depends on the activity of two steroid dehydrogenases – 11HSD and 20 HSD –which operate in opposite directions. Whereas 11HSD catalyzes oxidation at the position C_{11}, 20HSD is responsible for reduction at the position C_{20}. The result of these reactions is that steroids of lower biologic activity are synthesized. As both dehydrogenases are expressed predominantly in intestinal mucosa, they seem to play a role in modulation of the potency of glucocorticoids to stimulate Na^+ transport.

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