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Corticosterone metabolism in chicken tissues: Evidence for tissue-specific distribution of steroid dehydrogenases

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Abstract

Glucocorticoids influence the function of numerous tissues. Although there are a very large number of studies that have investigated the local metabolism of glucocorticoids in mammals, the knowledge of this metabolism in birds is limited. The local concentration of corticosterone is critical for both glucocorticoid- and mineralocorticoid-dependent activity, and we have therefore carried out studies of corticosterone metabolism in various chicken organs. It was found that corticosterone was metabolized to 20-dihydrocorticosterone, and in some tissues also to 11-dehydrocorticosterone and 11-dehydro-20-dihydrocorticosterone. The activity of 20-hydroxysteroid dehydrogenase (20HSD), responsible for the transformation of corticosterone to 20-hydroxy derivatives, was abundant in the kidney and intestine, with lower levels in the liver and testis. Low levels of 20HSD were detected in the brain and ovaries. In contrast, 11-hydroxysteroid dehydrogenase (11HSD) activity was only found in the kidney and intestine. No activity was observed in the brain, testis, or ovaries. The treatment of chickens with estrogens stimulated 20HSD activity in the kidney, intestine, and oviduct and 11HSD activity in the liver and oviduct. Kinetic studies for corticosterone yielded an apparent K_m for 11HSD in the nanomolar ($K_m = 21 \pm 5\,\text{nmol} \cdot \text{l}^{-1}$) and for 20HSD in the micromolar range ($K_m = 3.7 \pm 0.3\,\mu\text{mol} \cdot \text{l}^{-1}$). When progesterone or 5α-dihydrotestosterone were used instead of corticosterone, the tissues reduced the former to 20β-dihydroprogesterone and the latter to both 5α,3α- and 5α,3β-dihydrotestosterone. The data presents the first evidence for corticosterone metabolism via 11β-, 3α/3β-, and 20β-hydroxysteroid dehydrogenases in various chicken organs and provide support for the theory of prereceptor modulation of glucocorticoid signals in avian tissues.

Keywords: Chicken 11β-hydroxysteroid dehydrogenase; Chicken 20β-hydroxysteroid dehydrogenase; Corticosterone metabolism

1. Introduction

Glucocorticoids are hormones that influence a number of functions. Until recently, it was thought that their activity depends purely on the circulating hormone concentrations, their binding by plasma proteins, and the density of receptors in target tissues. However, the circulating levels of glucocorticoids reach every organ and the glucocorticoid and mineralocorticoid receptors bind endogenous glucocorticoids with a high affinity. It is therefore apparent that organ- or cell-specific reactions must be determined by additional mechanisms. Studies in the last 10 years have demonstrated that an important level of hormone control is exerted by prereceptor modulation of the glucocorticoid signal due to the cell- and tissue-specific metabolism of steroids (Stewart and Krozowski, 1999; Seckl and Walker, 2001). The intracellular availability of glucocorticoids is predominantly modulated by 11β-hydroxysteroid dehydrogenase (11HSD) that converts the 11-hydroxysteroids cortisol and corticosterone into the hormonally inactive 11-oxosteroids cortisone and 11-dehydrocorticosterone and vice versa. Extensive mammalian studies have identified two isoforms of 11HSD-type 1 (11HSD1) and type 2 (11HSD2). Whereas 11HSD1 is a $NADP^+$(H)-dependent dehydrogenase which is active in both the oxidative and reductive direction and has a Michaelis-Menten constant (K_m) in the micromolar range, 11HSD2 exclusively exhibits NAD^+-dependent oxidase activity and its K_m is in the

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nanomolar range. 11HSD1 is principally found in glucocorticoid target tissues such as the liver, gonads, and adipose tissue, where its reductase activity predominates. In contrast, 11HSD2 is located in the placenta and mineralocorticoid target tissues such as the kidney and colon, where it is believed to protect these tissues against an excess of active glucocorticoids and their binding to the mineralocorticoid receptors (Stewart and Krozowski, 1999; Seckl and Walker, 2001).

As far as the glucocorticoid metabolism of non-mammalian vertebrates is concerned, only a few studies have been performed. It has been suggested that there is an 11HSD2-dependent mineralocorticoid selectivity mechanism similar to mammals in the toad bladder (Brem et al., 1989; Gaeggeler et al., 1989) and avian intestine (Mazancová et al., 2005). In addition, the essential role of 11HSD has been shown for the biosynthesis of a potent androgen, 11-ketotestosterone, in the testes of male teleost fish (Kusakabe et al., 2003). However, in contrast to mammalian tissues, in which glucocorticoids such as cortisol and corticosterone are oxidized to 11-oxoderivatives, the avian intestine (DiBattista et al., 1989; Mazancová et al., 2005; Vylitová et al., 1998) and toad bladder (Sabatini et al., 1993) also convert glucocorticoids to 20-derivatives via 20-hydroxysteroid dehydrogenase (20HSD).

The purpose of the study presented here was to extend our knowledge of the metabolism of corticosteroids in avian tissues and to relate this metabolism to its putative physiological role. As far as we know, this is the first systematic comparison of corticosterone metabolism in avian glucocorticoid and mineralocorticoid target tissues with respect to 11HSD and 20HSD.

Materials and methods

1.1. Animals and chemicals

Experiments were performed on Brown Leghorn chickens obtained from the hatchery of the Institute of Molecular Genetics (Czech Acad. Sci. 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 the age of 5–7 weeks, the chickens were killed by decapitation and exsanguination and various tissues (kidney, liver, intestine, brain, various regions of the oviduct, and gonads) were quickly removed. Two groups of animals were used: untreated controls and chickens that were treated with diethylstilbesterol (DES) daily with an s.c. injection of 2 mg DES kg^{-1} in polypropylene glycol for 7 days starting from day 22 (primary stimulation). Some chickens were treated again between days 42 and 46 (secondary stimulation). The chickens were treated with DES to determine the effect of estrogens on corticosterone metabolism and to induce the development of the oviduct in immature birds. It is well known that the oviduct is extremely sensitive to estrogens that induce the differentiation and proliferation of oviduct cells and the synthesis of egg white proteins. When the administration of estrogens is halted, the oviduct regresses and production of egg white proteins ceases; however, this decrease can be stopped and reversed by secondary stimulation with both estrogens and non-estrogenic steroid hormones (Dougherty and Sanders, 2005; Muramatsu and Sanders, 1995). Therefore, some animals were subjected to secondary stimulation and corticosterone metabolism was studied not only after primary stimulation but also after secondary. 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), deoxycorticosterone (4-pregnen-21-ol-3,20-dione), 5α-dihydrotestosterone (5α-androstan-17β-ol-3-on), 5α-androstan-3α,17β-diol, and 5α-androstan-3β,17β-diol were purchased from Steraloids (Newport, RI). All other chemicals were obtained from Sigma (St. Louis, MO).

1.2. Measurement of corticosteroid metabolism

Corticosterone metabolism was studied in tissue fragments as described previously (Pácha and Mikšík, 1996; Vylitová et al., 1998). Briefly, tissue fragments (approximately 300 mg wet weight) were placed in flasks containing 10 ml of bicarbonate buffer solution and corticosterone or other steroids dissolved in methanol were added to each sample to a final concentration of 1.45 μmol L^{-1}, which was close to the K_m value for 20HSD (DiBattista et al., 1989; McNatt et al., 1992; Nakajin et al., 1988a). At the end of incubation, an internal standard of deoxycorticosterone was added. The incubation times were selected in such a way that the velocities of the individual reactions were linear.

As some 20HSD's also show 3α/3β-hydroxysteroid dehydrogenase activity (Edwards and Orr, 1978; Ohno et al., 1991), we studied the conversion of 5α-dihydrotestosterone under the same conditions as mentioned above. To identify the stereospecificity of 20HSD, progesterone was incubated with intestinal fragments and the products of stereospecific reduction of the 20-keto group were investigated. Progesterone was used in these experiments instead of corticosterone, because 20α-dihydrocorticosterone was not available in the market. Enzyme activity was calculated as the sum of the reaction products of 11HSD (11-dehydrocorticosterone and 11-dehydro-20-dihydrocorticosterone) and 20HSD (20-dihydrocorticosterone and 11-dehydro-20-dihydrocorticosterone) and expressed in picomoles per hour per mg dry weight (DW).

The kinetic parameters of 11HSD and 20HSD were measured in microsomal and cytosolic fractions of the renal and intestinal homogenates, respectively. The subcellular fractions were prepared from homogenates (1:9 w/v; sucrose 200 mmol L^{-1}, Tris/HCl 10 mmol L^{-1}, pH 8.5) after sequential centrifugation of the supernatant at 400g for 10 min, 9000g for 10 min, and 105,000g for 60 min. The conversion of corticosterone was assayed, as described previously (Vylitová et al., 1998), in tubes containing the buffer (KCl, 100 mmol L^{-1}; Tris/HCl, 50 mmol L^{-1}, pH 8.5), cosubstrate (NAD^+ or NADPH; 0.4 mmol L^{-1}), [^{3}H]corticosterone (12.5 or 25 nmol L^{-1}), and non-radioactive corticosterone as the variable substrate. K_m and V_{max} were derived by plotting the enzyme activity against the corticosterone concentration.

1.3. Steroid analysis

After incubation, the samples were placed on ice and immediately extracted from the incubation buffer by Sep-Pak cartridges (Waters, Milford, MA) and separated by liquid chromatography–mass spectrometry (Pácha et al., 2004). Non-radioactive steroids were detected on-line using ultraviolet absorbance detection, whereas a solid and flow cell detector (Radiomatic 150TR, Canberra Packard) was used for the detection of radioactive steroids. The metabolites formed from corticosterone were further verified by HPLC/mass spectrometry (mass detector LC/MSD, Hewlett-Packard, Palo Alto, CA) with positive mode atmospheric pressure ionization-electrospray ionization (API-ESI). The separation was performed in a C18 column using a water-methanol gradient (for details see Pácha et al., 2004).

1.4. Statistical analysis

Values were expressed as means ± SEM and were analyzed by Student's t test. Significance was set at $P < 0.05$. The kinetic determination of

K_m and V_{max} were performed by fitting the apparent enzyme activity against the concentration of substrate to the Michaelis–Menten equation using the iterative least squares curve-fitting technique.

2. Results

The metabolism of corticosterone was investigated in various organs possessing either mineralocorticoid or glucocorticoid sensitivity, in particular, the kidney, liver, intestine, brain, testis, ovary, and oviduct. Incubation with corticosterone yielded three metabolites that cochromatographed with 11-dehydrocorticosterone, 20-dihydrocorticosterone, and 11-dehydro-20-dihydrocorticosterone, and were further confirmed by mass spectrometry. These findings show that corticosterone is oxidized at position C_{11} by 11HSD and reduced at position C_{20} by 20HSD. However, the ability to oxidize and reduce corticosterone differs among the various organs (Fig. 1). The oxidation of corticosterone by 11HSD was most powerful in the kidney and intestine, whereas it was not detected in significant amounts in the liver, brain, testis, and ovary (Fig. 1A). A relatively high 11HSD activity was also found in the magnum and uterus regions of the oviduct of DES-primed immature chicks after primary stimulation. The data in Table 1 shows that stimulations of immature chickens with DES were associated with upregulation of 11HSD in the liver and magnum but not in the kidney, intestine, or uterus.

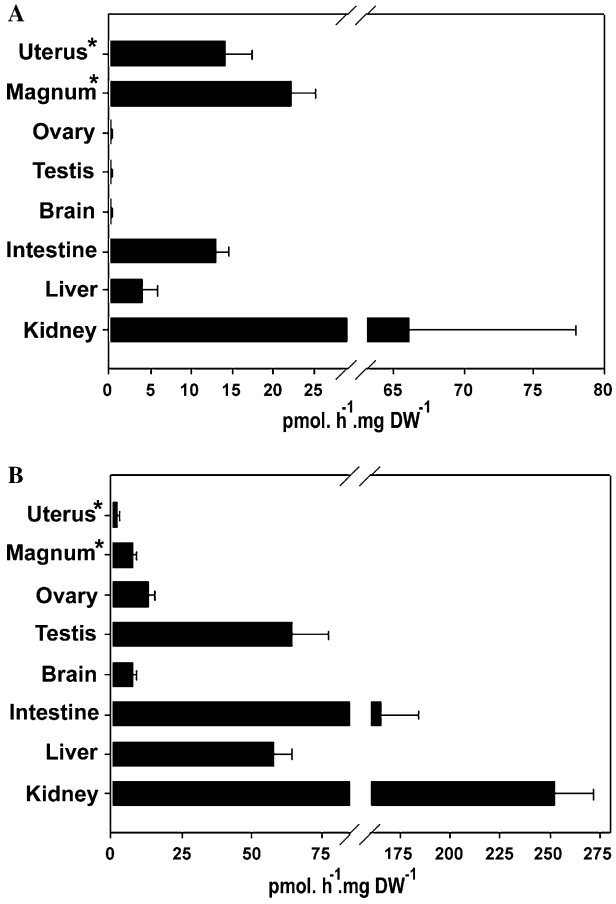

Fig. 1. 11HSD (A) and 20HSD activity (B) in tissue fragments of chicken organs. Each tissue incubated with corticosterone and then subjected to HPLC–MS analysis. Each column represents means ± SEM of 7–10 animals. *Treatment with DES for 7 days prior to the experiment. Values of 11HSD activity in ovary, testis, and brain were not significantly different from zero.

In contrast to C_{11} oxidation, the reduction at position C_{20} was detected in all tissues investigated, with the exception of the uterus. The 20HSD activity was largest in the kidney and intestine, medium in the liver and testis and low in the brain, ovary, and oviduct of chickens treated with DES (Fig. 1). The stimulation of 20HSD activity was found in the kidney and intestine after primary stimulation with estrogens and in the magnum region of the oviduct after secondary restimulation (Table 1). To identify the stereospecificity of 20HSD, progesterone was incubated with tissue fragments that exhibit the highest activity of this enzyme. Under these conditions, progesterone was converted to the β-epimer of 20-dihydroprogesterone (Fig. 2A). As can be seen in Fig. 2B, both renal and intestinal 20HSD reduced progesterone, but the catalytic efficiency/substrate specificity for progesterone was in the range of 5–15% of that observed with corticosterone as the substrate.

The apparent K_m values for both 11HSD and 20HSD were determined using various concentrations of corticosterone at a fixed concentration of NAD^+ or NADPH. As 11HSD is an enzyme of the endoplasmic reticulum (Stewart and Krozowski, 1999) and 20HSD is a cytosolic enzyme (DiBattista et al., 1989), 11HSD was measured in the microsomes and 20HSD in the cytosol fraction. The kinetic parameters for both enzymes are given in Table 2.

Table 1
11HSD and 20HSD activities in various organs of chickens treated with diethylstilbesterol

	Control		Primary stimulation		Secondary stimulation	
	11HSD	20HSD	11HSD	20HSD	11HSD	20HSD
Kidney	66 ± 12 (10)	252 ± 20	55 ± 7 (5)	326 ± 16[a]	n.d.	n.d.
Liver	2.5 ± 1.8 (10)[c]	58 ± 6	9.3 ± 2.3 (13)[a]	46 ± 4	n.d.	n.d.
Intestine	13 ± 2 (7)	158 ± 21	12 ± 2 (7)	222 ± 21[a]	n.d.	n.d.
Magnum	n.d.	n.d.	22 ± 9 (8)	8.0 ± 1.0	55 ± 6 (5)[b]	45 ± 4[b]
Uterus	n.d.	n.d.	14 ± 4 (8)	2.2 ± 0.7[c]	23 ± 3 (5)	0.9 ± 0.5[c]

Activities of enzymes expressed in picomoles of products per hour per mg dry weight. Values given as means ± SEM. Numbers of animals given in parentheses.

[a] Significantly different from control animals.

[b] Significant difference between primary and secondary stimulation.

[c] Not significantly different from zero; ($P < 0.05$).

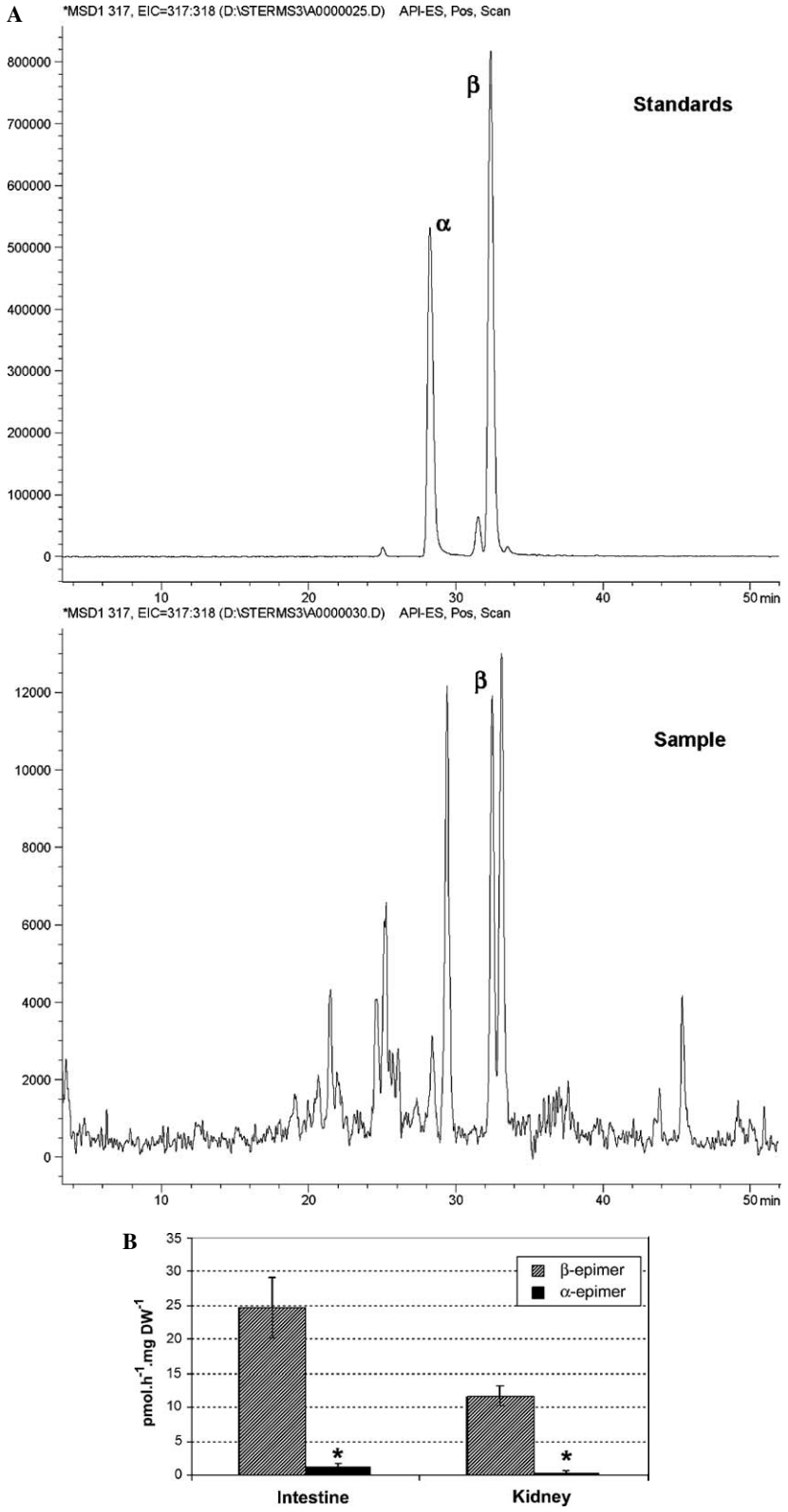

Fig. 2. (A) Chromatographic analysis (HPLC/MS) of 20α- and 20β-dihydroprogesterone metabolites. Comparison of standards (above) and actual incubation of intestinal slices with 1.45 μmol L^{-1} progesterone (below). Chromatograms expressed as records of extracted ions specific to dihydroprogesterones (m/z 317); *y*-axis: ion current, *x*-axis: retention time in minutes. (B) Reduction of progesterone to 20-dihydroprogesterone in kidney and intestine. Each column represents means ± SEM of 12–15 animals.

Table 2
Kinetic parameters of chicken 11HSD and 20HSD

Enzyme	Substrate	K_m (μmol L^{-1})	V_{max} (nmol h^{-1} mg $prot^{-1}$)
11HSD	Corticosterone, NAD^+	0.021 ± 0.005	0.36 ± 0.04
20HSD	Corticosterone, NADPH	3.742 ± 0.331	4.39 ± 0.24

Assay method as described in Materials and methods, corticosterone concentration varied whereas cosubstrate concentrations were fixed (0.4 mmol L^{-1}). Michaelis constant K_m and maximum velocity V_{max} obtained by least squares curve-fitting technique.

Table 3
Conversion of 5α-dihydrotestosterone in tissue fragments of chicken intestine, brain, and magnum region of oviduct

	5α,3α-DH-T	5α,3β-DH-T
Intestine	210 ± 27 (14)[b]	324 ± 37
Brain	40 ± 5 (8)	40 ± 4
Magnum[a]	293 ± 46 (7)[b]	121 ± 13

Conversion of 5α-dihydrotestosterone (DH-T) expressed in picomoles of products per hour per mg dry weight. Values given as mean ± SEM.

[a] Treatment with DES for 7 days prior to the experiment. Numbers of animals given in parentheses; 5α,3α-DH-T, 5α-androstan-3α,17β-diol; 5α,3β-DH-T, 5α-androstan-3β,17β-diol.

[b] Significantly different from 5α,3β-DH-T.

As there have been several reports on bifunctional or polyfunctional hydroxysteroid dehydrogenases having 20HSD activity such as 3α,20βHSD or 3α/β,20βHSD (Edwards and Orr, 1978; Ohno et al., 1991), we further investigated the catalytic activity of various tissues for the reduction of the hydroxyl group at position C_3 of the steroid nucleus. The incubation of tissue fragments revealed the effective conversion of 5α-dihydrotestosterone to both 3α- and 3β-reduced metabolites (Table 3), but this reduction differed among the various tissues, with the lowest level in the brain.

3. Discussion

The results of this study support the idea that corticosterone inactivation is a general attribute of many avian tissues. Based on our steroid analysis, we could detect 20HSD activity in the majority of organs used in this study and thus, the reduction at position C_{20} seems to be a common feature of avian tissues. On the other hand, we have demonstrated that the oxidase activity of 11HSD is only expressed in some tissues, predominantly in mineralocorticoid target tissues such as the kidney and intestine, and in the oviduct. With regard to the properties of 11HSD isoforms (Stewart and Krozowski, 1999; Seckl and Walker, 2001), it is obvious that the inactivation of corticosterone at position C_{11} reflects the activity of 11HSD isoform 2. However, the results described here provide evidence of a second enzyme, 20HSD, which is able to metabolize corticosterone both in tissues with low and high 11HSD oxidase activities. Considering that 20HSD can not only metabolize corticosterone but also steroids that do not have an oxygen-containing functional group at C_{11} to serve as a substrate for 11HSD, it is reasonable to consider the role of 20HSD in the metabolism of other steroids such as progesterone.

The data presented in this paper demonstrates that the majority of avian organs possess stereospecific 20HSD that exhibits 20β- but not 20α-reductase activity. To the best of our knowledge, this is the first report showing the nearly ubiquitous expression of this enzyme in avian tissues. Earlier studies only detected 20HSD in duck and chicken intestine (DiBattista et al., 1989; Vylitová et al., 1998; Mazancová et al., 2005). Mammalian 20βHSD was first identified in the pig testis and was shown to possess 3α/3β-dehydrogenase activity (Nakajin et al., 1988a; Ohno et al., 1991). Later studies revealed that, 3α/3β,20βHSD is expressed in many other tissues especially in the kidney, liver, and testis (Kobayashi et al., 1996) and that K_m for the 20β-reduction of various steroid substrates lies in the micromolar range (Nakajin et al., 1988a). Our data indicates that avian 20βHSD might be similar to mammalian 3α/3β,20βHSD. First, avian 20βHSD catalyzes the stereospecific reduction of a ketone group at position C_{20}, it is localized in the cytosol and its K_m for corticosterone reduction is in the micromolar range. Second, it is abundantly expressed in the kidney, intestine, liver, and testis and third, 20β-reduction is colocalized with a reduction at C_3 that is not stereospecific.

The physiological role of 20HSD in avian tissues is not yet entirely clear. First, in mineralocorticoid target tissues such as the kidney and intestine, 20βHSD together with 11HSD might play a role in preventing corticosterone from binding to the mineralocorticoid receptors. In chickens, the circulating corticosterone levels are much higher than the aldosterone levels (Rosenberg and Hurwitz, 1987). The reduction of corticosterone to 20β-dihydrocorticosterone might therefore be an alternative system to 11β-oxidation to facilitate aldosterone binding because this metabolite has been shown to possess no affinity for aldosterone receptors (DiBattista et al., 1989). Furthermore, our recent data suggests that neither 20-dihydrocorticosterone nor 11-dehydrocorticosterone was able to induce electrogenic Na^+ transport in chicken intestine (Mazancová et al., 2005). Second, 20HSD might be involved in progesterone metabolism similar to mammals (Quinkler et al., 1999) and third, several findings suggest that 20HSD has a role in the androgen synthesis regulatory pathway. 20β-Hydroxy-C_{21} steroids have been shown to strongly inhibit cytochrome $P450_{C\,17}$ and thus to modulate androgen synthesis in the testis (Nakajin et al., 1998b).

Surprisingly, 20HSD is not only abundantly expressed in the testis but also in tissues such as the kidney and intestine that coexpress 11HSD. Why two corticosterone inactivation enzymes operate in one tissue is not entirely clear. However, there is some evidence that C_{20}-reduction may further decrease the biological activity of 11-oxo derivatives (Sabatini et al., 1993). In addition, the functional coupling of 11β-oxidation followed by reduction of the ketone group at position C_{20} via cosubstrates might amplify the

effect of steroid dehydrogenases (Mercer and Krozowski, 1992). Finally, it is well known that steroid receptors have similar binding properties for a variety of steroid ligands operating as agonists or antagonists (Sandor et al., 1989; Myles and Funder, 1996). The upregulation of chicken 20HSD in mineralocorticoid target tissues such as the kidney and colon, but not in non-mineralocorticoid target tissue such as the liver (Mazancová et al., 2005; Rafestin-Oblin et al., 1989; Sandor et al., 1989) suggests that the estrogen stimulation of 20HSD might play a role in the protection of mineralocorticoid receptors against progesterone. Such situations arise during the onset of egg laying when both estrogen and progesterone plasma levels are increased (Su et al., 1996).

When considering that the C_{11}-oxidation of corticosterone is associated in intact cells with 11HSD2 and the reduction of 11-dehydrocorticosterone with 11HSD1 (Stewart and Krozowski, 1999), it is obvious that 11HSD2 operates in chicken mineralocorticoid target tissues similarly to mammals, where 11HSD2 prevents mineralocorticoid receptors from binding with glucocorticoids (Farman and Rafestin-Oblin, 2001). This conclusion agrees with several findings. Our previous study in the chicken intestine demonstrated that 11HSD protects the mineralocorticoid regulatory pathway against stimulation by corticosterone (Mazancová et al., 2005) and the findings of Sandor et al. (1989) and Rafestin-Oblin et al. (1989) show that avian mineralocorticoid receptors bind aldosterone and corticosterone with nearly equal affinities. The demonstration of relatively high 11β-oxidation in the oviduct suggests that 11HSD2 might protect these organs against an excess of glucocorticoids. In the magnum segment of the chicken oviduct, several egg white proteins are secreted in large amounts, regulated by estrogens, progestins, androgens, and glucocorticoids. However, most steroid-specific genes are specific to estrogens during primary stimulation and can only be induced by progestins, androgens, and glucocorticoids after a secondary restimulation with estrogens (Muramatsu and Sanders, 1995). We therefore cannot exclude the possibility that 11HSD protects oviduct steroid-specific genes against premature induction by glucocorticoids. The exact role of 11HSD in the oviduct will have to be elucidated by further experiments.

In conclusion, the data presented here suggests that the metabolism of corticosterone in avian organs depends on the activity of two steroid dehydrogenases–11HSD and 20HSD, which operate in opposite directions and are not distributed homogeneously in all organs. The tissue-specific heterogeneity of these enzymes strongly indicates differences in the local accessibility of corticosterone.

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