

Distribution of 11 β -Hydroxysteroid Dehydrogenase along the Rabbit Nephron

Jean-Pierre Bonvalet, Isabelle Doignon, Marcel Blot-Chaubaud, Philippe Pradelles,* and Nicolette Farman
Institut National de la Santé et de la Recherche Médicale (INSERM) U 246, Service de Biologie Cellulaire, and *Section de Pharmacologie et d'Immunologie, Département de Biologie, CEN Saclay, 91191 Gif-sur-Yvette Cedex, France

Abstract

It has been recently proposed that 11 β -hydroxysteroid dehydrogenase (11 β -OHSD) is responsible for aldosterone tissue specificity. A 11 β -OHSD deficiency has been invoked as a cause of the syndrome of apparent mineralocorticoid excess, and 11 β -OHSD inhibition by liquorice has been invoked to explain the hypertension induced by this drug. Since the renal tubule is composed of aldosterone-sensitive and insensitive segments, we determined the distribution of 11 β -OHSD along the rabbit tubule. Pools of tubular segments isolated by microdissection were incubated for 2 h at 37°C in the presence of [³H]corticosterone (³H-B, 8.10⁻⁹ M). Afterwards, the amounts of ³H-B and of the metabolite 11-dehydrocorticosterone (³H-A) were determined using HPLC analysis. In the proximal tubule, in either its convoluted or straight portion, and in the medullary thick ascending limb, the amount of ³H-A was 19.6 \pm 3.8% ($n = 12$), 17.9 \pm 3.4 ($n = 8$), and 15.0 \pm 2.2 ($n = 4$), respectively, of the sum of ³H-A + ³H-B. In the cortical ascending limb and the collecting tubule in its cortical and medullary parts, it was 74.7 \pm 6.8% ($n = 4$), 74.1 \pm 4.9 ($n = 9$) and 64.6 \pm 14.1 ($n = 3$), respectively. In both proximal and cortical collecting tubule, addition of carbenoxolone 8.10⁻⁴ M, an inhibitor of 11 β -OHSD, almost completely inhibited the conversion of ³H-B to ³H-A. Thus, 11 β -OHSD activity was high in the aldosterone-sensitive segments, and low in the aldosterone-insensitive segments. These results strongly favor the hypothesis that 11 β -OHSD is a key enzyme in mineralocorticoid tissue specificity along the rabbit nephron. They reinforce the notion that a defect in 11 β -OHSD plays a major role in the syndrome of apparent mineralocorticoid excess and liquorice-induced hypertension. (*J. Clin. Invest.* 1990. 86:832-837.)
Key words: aldosterone • corticosterone • isolated tubule

Introduction

The syndrome of apparent mineralocorticoid excess consists of severe hypertension, hypokalemia, sodium retention, and suppression of plasma aldosterone and plasma renin activity (1-4). The hypothesis has been proposed that in this syndrome cortisol acts on the kidney in place of aldosterone (4). This mineralocorticoid effect of cortisol should depend on the lack of conversion of cortisol to cortisone in target cells for aldoste-

rone, due to a deficiency of 11 β -hydroxysteroid dehydrogenase (11 β -OHSD)¹ (4). Indeed, whereas aldosterone and glucocorticoid hormones have the same affinity for the mineralocorticoid receptor in vitro, the in vivo specific binding of aldosterone to mineralocorticoid receptors is much higher than that of glucocorticoids (5). This difference has been ascribed to the intracellular conversion of cortisol or corticosterone to a product with a much lower affinity for mineralocorticoid receptors (5). This allows the mineralocorticoid receptors to remain free for aldosterone binding and action, in face of in vivo concentrations of glucocorticoids much higher than that of aldosterone. On the other hand, it is well known that the ingestion of large amounts of liquorice produces hypertension with characteristics similar to those of the syndrome of apparent mineralocorticoid excess (6-8). Stewart et al. (9) proposed that this effect of liquorice implies an inhibition of 11 β -OHSD activity.

Recently, Funder et al. (5) reported that a high 11 β -OHSD activity is present in tissues with well assessed aldosterone binding and action, such as kidney, parotid, and colon, while it was low or absent in other tissues, such as hippocampus or heart, that specifically bind aldosterone but lack well defined physiological actions of this hormone. Since the renal tubule is composed of several successive epithelia, either aldosterone sensitive (for example the cortical collecting tubule) or aldosterone insensitive (for example the proximal tubule), one can expect that the distribution of 11 β -OHSD along the tubule is restricted to the former type. However, Edwards et al. (10) found no clear difference between proximal- and distal-enriched preparations of rat renal tubules, with no enrichment in 11 β -OHSD in the distal-enriched as compared to the total unpurified fraction. On the other hand, experiments with immunohistochemical methods (10-12) evidenced immunolocalization of 11 β -OHSD in the proximal rather than in the distal nephron, a result hardly compatible with the notion of the role of this enzyme in aldosterone tissue specificity.

To address this point, we examined the conversion of corticosterone to 11-dehydrocorticosterone in discrete isolated tubular segments, using HPLC analysis. The results show that 11 β -OHSD activity is very high in aldosterone-sensitive tubular segments, and low in aldosterone-insensitive segments. These data reinforce the notion that 11 β -OHSD plays a major role in tissue specificity for aldosterone.

Methods

Incubation of cortical kidney slices. Rabbits were killed by a blow behind the neck. Immediately after death, the left kidney was perfused with an ice-cold solution containing (in mM): NaCl, 137; KCl, 5; MgSO₄, 0.8; Na₂HPO₄, 0.33; KH₂PO₄, 0.44; MgCl₂, 1; CaCl₂, 1;

1. *Abbreviations used in this paper:* 11-OHSD, 11-hydroxysteroid dehydrogenase; CBX, carbenoxolone sodium; CCD, cortical collecting tubule; cTAL, cortical ascending limb; OMCT, outer medullary collecting duct; PCT, proximal convoluted tubule; PR, pars recta.

Address reprint requests to Dr. Bonvalet, INSERM U 246, Service de Biologie Cellulaire, Département de Biologie, CEN Saclay, 91191 Gif-sur-Yvette Cedex, France.

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D-glucose, 5; Tris-HCl, 10; pH 7.4 via the renal artery. Then the cortex was sliced and 600 mg of kidney cortex was incubated at 37°C in 4 ml of the same solution added with 1-2[³H]corticosterone (New England Nuclear, Boston, MA) 2.22 TBq/mM). The concentration of [³H]corticosterone was 8.10⁻⁹ M. The incubation solution was bubbled with air. Experiments were done either in the absence of the enzyme inhibitor carbenoxolone sodium, the propionic acid ester of glycyrrhetic acid (3β-hydroxy-11-oxoolean-12-en-30-oic acid 3-hemisuccinate, CBX¹; Sigma Chemical Co., St. Louis, MO) (*n* = 2), or in the presence of 8.10⁻⁵ M (*n* = 2) or 8.10⁻⁴ M (*n* = 2) CBX. 250 μl aliquots of the incubation medium were collected at 30, 60, 90, and 120 min. Samples were immediately frozen at -20°C up to the HPLC analysis.

Isolation of tubular segments. Kidneys were rinsed by intrarenal perfusion as described above. Thereafter, they were prepared for microdissection as previously described (13). A collagenase solution (similar to the perfusion solution, to which 0.1% collagenase [Serva Fine Biochemicals, Inc., Garden City, NY; 0.37 U/mg] was added) was perfused under pressure up to the rupture of the renal capsule. Thin pyramid pieces, including cortex and medulla, were cut and incubated at 30°C for 1 h in the collagenase solution, bubbled with air.

Microdissection of tubular segments was performed at 4°C in 3 ml of microdissection solution (similar to the incubation solution except for the absence of collagenase, 0.25 mM CaCl₂ instead of 1 mM, and the addition of 0.1% bovine serum albumin (Sigma Chemical Co.). The duration of the microdissection period was ≈ 90 min. Fig. 1 illustrates the intrarenal localization of the microdissected tubular segments; the following structures were collected: proximal convoluted tubule (PCT), pars recta (PR), medullary thick ascending limb (mTAL) in its initial portion, cortical ascending limb (cTAL), cortical collecting tubule in its light portion (CCD), and outer medullary collecting duct (OMCT).

Incubation of tubular segments with [³H]corticosterone. At the end of the microdissection, pools of ≈ 50 mm of tubular segments were incubated with [³H]corticosterone. For this purpose, pools of tubules were transferred with 1 μl microdissection solution into Eppendorf tubes containing 4 μl of the same solution plus [³H]corticosterone 8.10⁻⁹ M. In some tubes, CBX was added at the concentration of 8.10⁻⁴ M. Tubes were incubated for 2 h at 37°C. At the end of the incubation, 100 μl of a 19% acetonitrile in 0.08% trifluoroacetic acid (mobile phase) were added, and tubes were frozen at -20°C. On the whole, 12 samples of PCT, 8 of PR, 4 of mTAL, 4 of cTAL, 9 of CCD, and 3 of OMCT, from 10 rabbit kidneys, were incubated in the absence

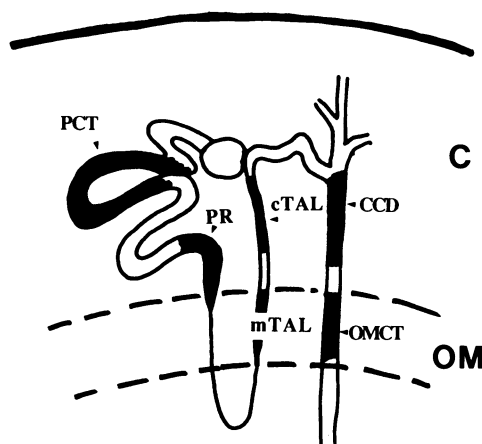


Figure 1. Schematic representation of a rabbit nephron. The dark areas give the localization of the microdissected tubular segments. PCT, proximal convoluted tubule; PR, pars recta, mTAL and cTAL, medullary and cortical parts, respectively, of the thick ascending limb of the loop of Henle; CCD, cortical collecting duct; OMCT, outer medullary collecting tubule. C, cortex; OM, outer medulla.

of carbenoxolone. In addition, five samples of PCT and four of CCD were incubated in the presence of CBX.

We tested the eventual effect of the cofactor NADP/NADPH on the enzyme activity. For this purpose, 26 samples of 10 mm of PCT or CCD were dissected from a rabbit kidney and incubated in the conditions described above, in the presence or absence of 1 μM NADP (Boehringer-Mannheim, Indianapolis, IN), for various times (15, 30, 60, and 120 min)

HPLC analysis. A CN reversed-phase column (μBondapak CN; Waters Associates, Milford, MA) with a precolumn (RP18, 10 μm, Touzart et Matignon, Paris, France) was used. Before injection, samples of incubation medium used for kidney slices were lyophilized (VirTis Co., Gardiner, NY) and taken up in 250 μl mobile phase, which were injected onto the column. The incubation solution containing tubules was centrifuged at 8,000 *g* for 1 min (Microfuge B; Beckman Instruments, Inc., Fullerton, CA). In order to have internal standards, 5 μg unlabeled corticosterone and 5 μg 11-dehydrocorticosterone (both from Sigma Chemical Co.) were added to 100 μl supernatant of tubular samples, which were injected onto the column and eluted isocratically with the mobile phase, 1 ml/min, with the elution profile monitored by absorbance at 240 nm. Fractions of 0.5 ml were collected every 30 s (Retriever III; Isco, Lincoln, NE) for 35 min in counting vials containing 5 ml of scintillation fluid (Instagel; Packard Instruments, Downers Grove, IL). Thus, for each sample, the profile of radioactivity could be compared to the elution profile of unlabeled corticosterone and 11-dehydrocorticosterone. Radioactivity was counted in a liquid scintillation counter (Rackbeta; LKB Instruments, Inc., Gaithersburg, MD).

Results

Table I shows the time-dependent transformation of [³H]corticosterone (³H-B) to [³H]11-dehydrocorticosterone (³H-A) in cortical slices. In the absence of CBX, 40% of ³H-B was metabolized after 30 min incubation time. This percentage increased gradually with time. After 2 h, almost all ³H-B was metabolized into ³H-A. CBX clearly reduced the activity of 11β-OHSD. However we did not observe a complete inhibition of the enzyme, even with high concentrations of CBX. At 2 h, the inhibition was only 30% with 8.10⁻⁵ M CBX. A higher CBX concentration, 8.10⁻⁴ M, was required to obtain a 70% inhibition at this time.

Fig. 2 illustrates the results obtained in PCT and CCD, in the presence or absence of CBX, 8.10⁻⁴ M. After 2 h incubation at 37°C, ~ 20% of ³H-B was converted to ³H-A in PCT; in the same condition, 75% of ³H-B was transformed into ³H-A by CCD. In the presence of CBX, almost no ³H-A was detectable in samples from both PCT and CCD. In Fig. 3, the elution profiles of mTAL and cTAL are represented. Almost

Table I. Effect of CBX on the 11β-OHSD Activity in Kidney Cortical Slices

Time min	Conversion rate (%) of [³ H]corticosterone to [³ H]11-dehydrocorticosterone		
	Without CBX	With CBX 8 × 10 ⁻³ M	With CBX 8 × 10 ⁻⁴ M
30	49.9	10.4	8.6
60	69.6	22.6	10.6
90	83.2	41.1	19.1
120	92.2	65.7	29.8

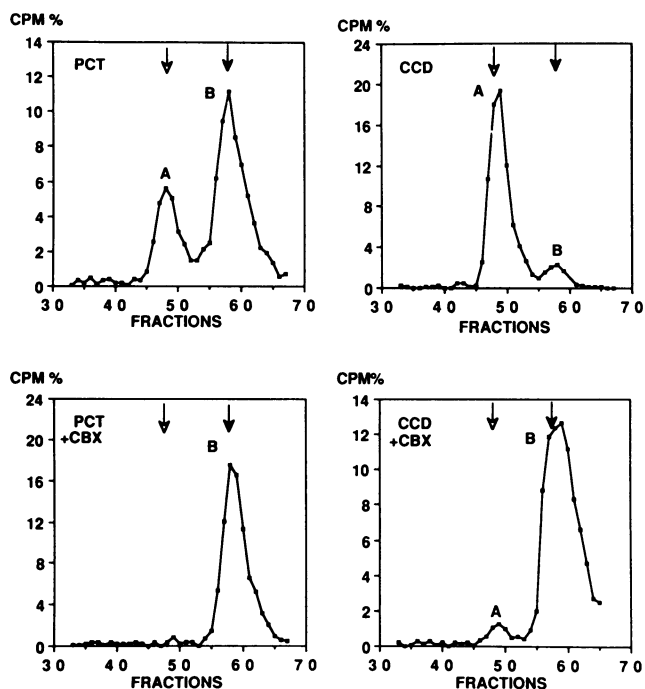


Figure 2. Analysis of in vitro conversion of [^3H]corticosterone (*B*) to [^3H]11-dehydrocorticosterone (*A*) by isolated tubular segments. 50 mm of proximal tubules (PCT, *left*) or cortical collecting tubules (CCD, *right* panels) were incubated for 2 h at 37°C in 5 μl of a solution containing B (8.10^{-9} M) in the absence (*top*) or presence (*bottom*) of carbenoxolone (CBX, 8.10^{-4} M). At the end of incubation, 100 μl mobile phase (19% acetonitrile in 0.08% trifluoro acetic acid) were added and the tubes were centrifuged. Unlabeled corticosterone and 11-dehydrocorticosterone were added to the supernatant. Samples were injected onto CN reversed-phase column and eluted isocratically, 1 ml/min, with the elution profile at 240 nm. Black and white arrows indicate the peak of unlabeled B and A, respectively, as determined by UV detection. Fractions were collected every 30 s. Percentages of counts per minute in fractions are given in ordinate.

no $^3\text{H-A}$ was present in mTAL, whereas, in cTAL, practically all $^3\text{H-B}$ was transformed into $^3\text{H-A}$.

Fig. 4 gives the mean values of the $^3\text{H-A}$ formed by each category of tubular segments, expressed as percentages of the sum $^3\text{H-B} + ^3\text{H-A}$. Clearly, one can distinguish two very dif-

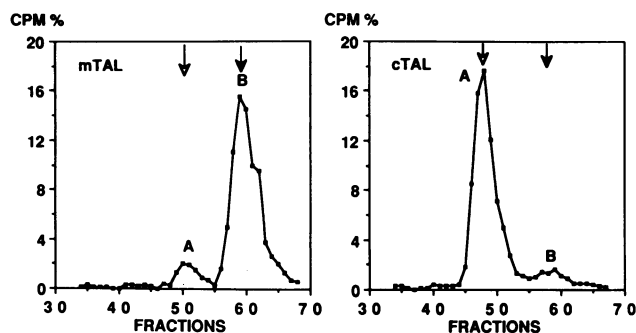


Figure 3. Analysis of in vitro conversion of [^3H]corticosterone (*B*) to [^3H]11-dehydrocorticosterone (*A*) by medullary (*left*, mTAL) or cortical (*right*, cTAL) parts of the thick ascending limb of the loop of Henle. Experimental protocol was similar to that in Fig. 1.

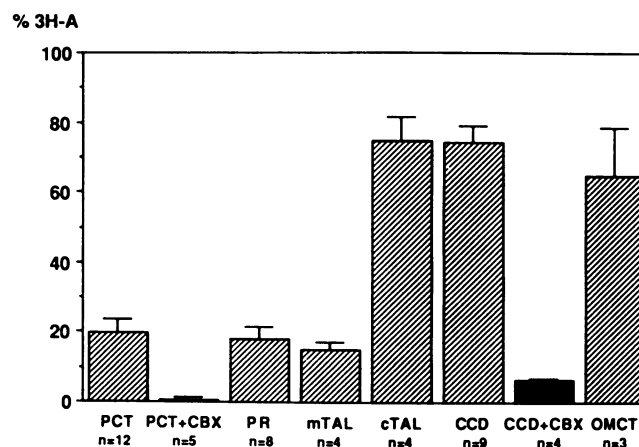


Figure 4. Conversion of [^3H]corticosterone to [^3H]11-dehydrocorticosterone by different types of tubular segments. Radioactivity corresponding to [^3H]11-dehydrocorticosterone is expressed as percentage of the sum of [^3H]corticosterone + [^3H]11-dehydrocorticosterone. Mean values are given with SE. Numbers of tubular samples (50 mm each) are indicated under columns. Black columns correspond to results in the presence of carbenoxolone (CBX, 8.10^{-4} M). PCT, proximal convoluted tubule; PR, straight proximal tubule; mTAL and cTAL, medullary and cortical parts of the thick ascending limb; CCD, cortical collecting tubule; OMCT, medullary collecting tubule from the outer medulla.

ferent patterns. In the proximal tubule (PCT and PR) and the mTAL, only 10–20% $^3\text{H-A}$ was present, attesting to a low activity of 11 β -OHSD. By contrast, cTAL, CCD, and OMCT were the sites of a high 11 β -OHSD activity, attested by the presence of 70–80% $^3\text{H-A}$. CBX strongly inhibited 11 β -OHSD in both PCT (97%) and CCD (91%).

Fig. 5 illustrates the results obtained in the presence or absence of NADP in the incubation medium. Since, in the preceding series, as much as 80% of [^3H]corticosterone was metabolized in 2 h by 50 mm CCD, we reduced the amount of tubular segments to 10 mm, in order to be able to evidence an eventual effect of NADP on enzyme activity after 2 h incubation. It appears clearly that NADP did not significantly influence the 11 β -OHSD activity. In CCD, the percentage of $^3\text{H-A}$

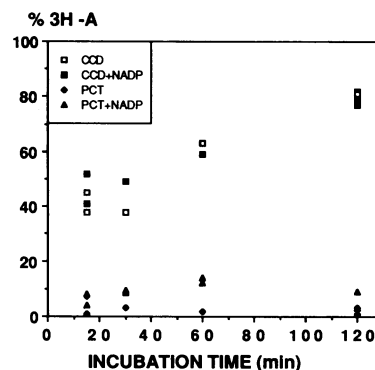


Figure 5. Conversion of [^3H]corticosterone to [^3H]11-dehydrocorticosterone by CCD and PCT. Radioactivity corresponding to [^3H]11-dehydrocorticosterone is expressed as percentage of the sum of [^3H]corticosterone + [^3H]11-dehydrocorticosterone. Each point represents the value corresponding to a single tubular sample. All

samples were dissected from the same kidney. Tubules were incubated at 37°C in 5 μl of a solution containing [^3H]corticosterone (8.10^{-9} M) in the presence or absence of 1 μM NADP. HPLC analysis was performed as described in Fig. 1.

increased from 40% at 15 min to 80% at 120 min, with no detectable difference between samples incubated with or without NADP. The conversion of $^3\text{H-B}$ to $^3\text{H-A}$ was very weak in PCT, even in the presence of NADP.

Discussion

To assess 11β -OHSD as a key enzyme allowing the expression of specific mineralocorticoid effect in tissues that are target for aldosterone, it is necessary to demonstrate that the activity of this enzyme is high in these tissues. Conversely, it must be shown that 11β -OHSD activity is low in tissues devoid of mineralocorticoid specificity. A great deal of evidence along this line has been published. Conversion of cortisol or corticosterone to a 11 -dehydro metabolite has been established in kidney, parotid, and colon, three aldosterone-sensitive tissues, and not in hippocampus or heart, that do not exhibit specific mineralocorticoid effect (5, 10). In addition, whereas, in cytosolic preparations, aldosterone and corticosterone have the same affinity for the type I mineralocorticoid receptor, the *in vivo* specific binding of corticosterone to type I receptor is much lower than that of aldosterone in kidney, colon and parotid (5). Administration of carbenoxolone sodium, a 11β -OHSD inhibitor, results in a similar specific binding of corticosterone and aldosterone in these tissues. Carbenoxolone sodium is the propionic acid ester of glycyrrhetic acid, the active principle of liquorice. It is an effective drug against peptic ulcer, and has been often shown to induce salt and water retention with elevation of blood pressure. Since the product of 11β -OHSD activity, 11 -dehydrocorticosterone, has a much lower *in vitro* affinity for type I receptors than aldosterone or corticosterone (5), it is reasonable to assume that the lower binding of corticosterone as compared to aldosterone results from the local conversion of corticosterone into dehydrocorticosterone. Furthermore, it has been recently shown (14, 15) in toad urinary bladder, an aldosterone sensitive transporting epithelium, that corticosterone exerts a much lower effect on sodium transport than aldosterone does. The addition of carbenoxolone results in a striking increase in the effect of corticosterone, which is then similar to that of aldosterone (14, 15). As early as 1970, Porter (16) reported that CBX enhances the sodium transport induced by submaximal concentrations of aldosterone, whereas it has no effect when administered alone.

Since 11β -OHSD is thought to have a local effect, autocrine and/or paracrine, one can expect that its distribution varies widely within the kidney. Indeed, the renal tubule is composed of several successive epithelia, closely intricated within the kidney. Some of them, such as distal and cortical collecting tubules, are typical target tubular segments for aldosterone (17). Others, such as the proximal tubule, have been repeatedly demonstrated to be devoid of aldosterone receptors (17). Consequently, the demonstration that 11β -OHSD activity is absent or low in the latter and high in the former would constitute a strong argument in favor of a physiological role of this enzyme in tissue specificity for aldosterone.

The present results confirm that 11β -OHSD is present in kidney cortex (5, 10, 18). Its activity is time dependent, resulting, under our conditions, in the conversion of 90% of the $^3\text{H-B}$ to $^3\text{H-A}$ in 2 h. These results are in accordance with those of Funder et al. (5) in rat kidney slices and Monder et al. (19) in suspensions of rat renal tubules. The conversion rate reported by Edwards et al. (10) in either rat cortical slices or

tubular suspensions was somewhat lower (30% in 1 h). 11β -OHSD activity has also been demonstrated in several renal epithelial cell lines derived from mammals (20), and in toad urinary bladder (14). Carbenoxolone clearly inhibited 11β -OHSD in renal cortical slices. This inhibitory effect has been previously reported in rat kidney after *in vivo* administration (5) or *in vitro* addition (19). The high concentration of carbenoxolone (8.10^{-4} M) required to inhibit 11β -OHSD in our experiments is in agreement with the results of Monder et al. (19) in preparations of rat renal cortical tubules. It is also noticeable that high doses of carbenoxolone (10^{-5} – 10^{-4} M) were necessary to increase the effect of corticosterone on Na transport in toad urinary bladder (14, 15).

The present results show that a high rate of conversion of corticosterone into 11 -dehydrocorticosterone was present in the distal cortical parts of the tubule and absent in PCT and PR. This difference cannot be attributed to differences in cofactor availability in the various tubular segments, since the addition of NADP in the incubation medium did not modify these results. Such an absence of effect of NADP on 11β -OHSD activity in tubular suspension has already been reported by Monder et al. (19). This contrasts with the results obtained by Kobayashi et al. (21) on subcellular fractions. This suggests that cofactors might be available in sufficient amounts when intact cells are used, whereas a depletion in cofactors intervenes when subcellular fractions are prepared. On the other hand, it is unlikely that a difference in membrane permeability for steroids could be at the origin of the different rates of [^3H]corticosterone conversion, since it is well assessed that steroids diffuse freely across cell membranes (22).

An attempt to localize 11β -OHSD within the kidney has been performed by Edwards et al. (10). These authors measured the conversion of cortisol to cortisone by proximal- or distal-enriched preparations of rat renal tubules: conversion rate was about 20–30% higher in distal than proximal preparations. Although the difference between proximal and distal tubular preparations is qualitatively in agreement with our own results, it is noteworthy that the differences observed between fractions were small, and that no enrichment of 11β -OHSD was observed in the distal fraction as compared to the unpurified fraction of renal cortical tubules. This might be due to species differences. However, other explanations might be at the origin of this difference, such as a loss of enzyme during the separation procedures or, more likely, an incomplete purification of tubule preparations. Indeed, in the view of the huge enzyme activity in distal cortical segments, 10 mm of CCD converted 80% of the [^3H]corticosterone in 5 μl incubation solution, even a small contamination of proximal suspensions by CCD would result in a significant rate of conversion. Immunohistochemical localization of 11β -OHSD was also examined by these authors (10), by Rundle et al. (11) and by Castello et al. (12). Using a rabbit antibody against purified 11β -OHSD from rat hepatic microsomal preparations, Edwards et al. (10) localized immunostaining in the proximal tubule and along the vasa recta. Immunostaining was absent in the distal nephron. On the other hand, with the same antibody, Rundle et al. (11) found an immunostaining in PCT, medullary rays, and interstitial cells of the papilla. Similar results were reported by Castello et al. (12) with a monoclonal antibody against rat microsomal 11β -OHSD. Thus, in these studies, the immunolocalization of 11β -OHSD was completely different from the distribution of mineralocorticoid receptors along the

nephron (as discussed later). This puzzling dissociation, hardly compatible with the hypothesis of a protective effect of mineralocorticoid receptors by the enzyme, has been recently outlined by Funder (23). This author questioned the specificity of the antibody used, and raised the hypothesis that 11 β -OHSD utilized to obtain the antibody could be one member of a family of related enzymes rather than the renal 11 β -OHSD itself (23).

The distribution of 11 β -OHSD along the tubule, as reported in this paper, strongly favors the view that in the rabbit kidney 11 β -OHSD is a major determinant of the tissue specificity for aldosterone in this species. CCD is a typical aldosterone-sensitive epithelium, as demonstrated by the presence of specific aldosterone binding (13), aldosterone-induced modulation of citrate synthase (24), Na-K-ATPase (25), and electrolyte transport (26). By contrast, neither aldosterone specific binding (13) nor aldosterone-modulated physiological functions has been found (17) along the proximal tubule. Results in other tubular segments also favor the view that the tubular sites of specific aldosterone binding and action are superimposed on those of 11 β -OHSD activity. Regulation of electrolyte transport by both gluco- and mineralocorticoids in the ascending limb of the loop of Henle is well assessed (27). The presence of mineralocorticoid receptors in the cTAL has been directly demonstrated in the rabbit (28), on the basis of a high specific binding of aldosterone in this segment in the presence of the glucocorticoid agonist RU 28362, that occupies glucocorticoid sites (29). The situation is less clear for the mTAL. Although an aldosterone-modulated sodium transport has been reported in this segment (30), Na-K-ATPase activity is stimulated by the glucocorticoid dexamethasone (25, 31, 32) and not by aldosterone (25, 31). On the other hand, aldosterone binding in this segment is low and its mineralocorticoid specificity is not firmly assessed (13). In view of the high 11 β -OHSD activity in the cTAL, contrasting with a low activity in the mTAL, it should be proposed that both gluco- and mineralocorticoids regulate electrolyte transport along the loop: the medullary portion would be predominantly glucocorticoid sensitive, whereas the cortical portion would be mineralocorticoid sensitive. This point warrants further study. Concerning the medullary collecting duct, its mineralocorticoid specificity is very likely. Aldosterone stimulates Na⁺-K⁺-ATPase activity (31) and acid excretion (33, 34) in this segment. The presence of a high 11 β -OHSD activity is thus consistent with this mineralocorticoid sensitivity.

In conclusion, this paper directly demonstrates that the distribution of 11 β -hydroxysteroid dehydrogenase varies widely along the rabbit nephron. A very high activity is present in several parts of the distal nephron, whereas a low activity is observed along the proximal tubule. This distribution is similar to that of mineralocorticoid receptors and is thus compatible with a role of 11 β -hydroxysteroid dehydrogenase in determining tissue specificity for aldosterone.

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