J. steroid Biochem. Vol. 23, No. 4, pp. 503–509, 1985
Printed in Great Britain.

0022-4731/85 $3.00 + 0.00

THE SYNTHESIS OF REDUCED METABOLITES OF ALDOSTERONE BY SUBCELLULAR FRACTIONS OF RAT KIDNEY: EFFECTS OF ANTIMINERALOCORTICOIDS

MARTIN J. MCDERMOTT*, MARY FREIBERGER, SYED A. LATIF and DAVID J. MORRIS†
Department of Pathology and Laboratory Medicine, The Miriam Hospital, Division of Biology and Medicine, Brown University, Providence, RI 02906, U.S.A.

(*Received* 10 *December* 1984)

Summary—Subcellular fractionation of male rat kidney revealed that the nuclear and plasma membrane fractions isolated from the 1,000 *g* pellet retained a significant proportion of the aldosterone ring-A reducing activity. Improved HPLC solvent systems separated all six possible ring-A reduced metabolites of aldosterone and revealed that 80–90% of the reduced metabolites synthesized by purified nuclei and plasma membranes were 5α-reduced compounds consisting of 5α-DHA and 3α,5α-THA in ratios of 1:2 (nuclei) and 1:1 (membranes). The 105,000 *g* cytosol also synthesized significant quantities of reduced, hydroxylated, and conjugated metabolites of aldosterone. In contrast, the majority of the reduced metabolites of aldosterone synthesized by kidney cytosol were 5β-products, consisting principally of 5β-DHA and smaller quantities of 3α,5β-THA and 3β,5β-THA. The synthesis of reduced aldosterone metabolites in the cytosol, nuclear, and plasma membrane fraction was inhibited by both 5 and 50 μM concentrations of the antimineralocorticoids, progesterone, K^+-canrenoate, and corticosterone. Progesterone was the strongest inhibitor of the synthesis of 5α-DHA and 3α,5α-THA in both nuclei and plasma membranes. The overall order of inhibition of the synthesis of ring-A reduced metabolites in the kidney subcellular fractions was progesterone > K^+-canrenoate > corticosterone; both progesterone and K^+-canrenoate inhibited 5α-reduction more than 5β-reduction.

INTRODUCTION

The synthesis of 5α-reduced metabolites of both testosterone and progesterone has been shown to be an important step in the mechanism of action of these hormones in several of their target tissues [1–3]. Our studies have suggested that the metabolism of aldosterone is important in the expression and/or regulation of its physiological actions in the kidney [4].

Experiments in our laboratories have shown that rat kidney slices synthesize significant quantities of 5α- and 5β-reduced, as well as several polar metabolites of aldosterone [5]; several of these metabolites possess significant mineralocorticoid activity [4, 6, 7]. The mineralocorticoid antagonists, progesterone, corticosterone, and spironolactone (90–200 μM) markedly inhibited aldosterone metabolism in kidney slices and in particular the synthesis of the ring-A reduced products [5]. The present study was therefore undertaken to further investigate the subcellular location of the renal transformations of aldosterone, to identify which specific individual ring-A reduced metabolites of aldosterone are synthesized at these sites using improved HPLC techniques, and to determine the effects of lower concentrations of antimineralocorticoids on the synthesis of these metabolites.

*Work submitted as part of Ph.D. Thesis.
†To whom correspondence should be addressed.
‡3α,5β-THA: 3α,11β,21-trihydroxy-20-oxo-5β-pregnan-18-al; 3β,5β-THA: 3β,11β-21-trihydroxy-20-oxo-5β-pregnan-18-al; 3α,5α-THA: 3α,11β,21-trihydroxy-20-oxo-5α-pregnan-18-al; 3β,5α-THA: 3β,11β,21-trihydroxy-20-oxo-5α-pregnan-18-al; 5β-DHA: 11β,21-dihydroxy-3,20-dioxo-5β-pregnan-18-al; 5α-DHA: 11β,21-dihydroxy-3,20-dioxo-5α-pregnan-18-al; K^+-canrenoate: Potassium 17-hydroxy-3-oxo-17α-pregna-4,6-diene-21-carboxylate.

EXPERIMENTAL

Chemicals

1,2-[^{3}H]aldosterone and 1,2-[^{3}H]3α,5β-tetrahydroaldosterone (3α,5β-THA)‡; with sp. act. of 46.2 Ci/mmol were obtained from New England Nuclear Corporation. The ^{3}H-steroids were purified by high pressure liquid chromatography (HPLC) before use. Nonradioactive aldosterone, 3α,5β-THA, 3β,5α-tetrahydroaldosterone (3β,5α-THA); 3β,5β-tetrahydroaldosterone (3β,5β-THA); progesterone, corticosterone, and NADPH were obtained from Sigma Chemical Company. [^{3}H]5α-DHA was prepared by incubating [^{3}H]-aldosterone with microsomes from female rat liver and [^{3}H]3α,5α-THA was synthesized by incubating [^{3}H]5α-DHA with 3α-hydroxysteroid dehydrogenase [8]. 5β-Dihydroaldosterone (5β-DHA) was received as a gift from Professor D. N. Kirk (MRC, London) and 5α-DHA and 3α,5α-THA from Professor M. Harnik, University of Tel Aviv, Israel. Potassium canrenoate

(spironolactone, SC 14266) was a gift from G. D. Searle Company.

Animals

Adult male Sprague–Dawley rats (Charles River, CD), 50–70 days old, and maintained on Purina Laboratory Chow and tap water *ad libitum*, were used in all experiments. Rats were kept in a temperature and light controlled room, and were bilaterally adrenalectomized 48 h prior to sacrifice. Adrenalectomized rats were maintained on 0.9% saline to drink.

In vitro *incubations of subcellular fractions*

Preparation of mitochondria, microsomes, and cytosolic subcellular fractions. Decapsulated kidneys (4 g) were homogenized (10% w/v) with a Teflon homogenizer in ice cold *Buffer I*, 0.25 M sucrose containing 100 mM potassium phosphate buffer (pH 7.2), 5 mM dithiothreitol (DTT), and 50 μg/ml soybean trypsin inhibitor (Sigma Chemical Company). The 1,000 g pellet and mitochondrial, microsomal, and cytosolic fractions were isolated by differential centrifugation and incubated for 120 min at 37°C with 21.5 nM [^{3}H]aldosterone as previously described [5]. Reactions were stopped by the addition of 3 ml acetone–ethanol (1:1, v:v), and extracted overnight at 37°C. Cytosol, purified nuclei, and plasma membrane fractions were also co-incubated with 5 or 50 μM progesterone, corticosterone, and K^+-canrenoate.

Preparation of nuclei and plasma membrane subcellular fractions. Purified nuclei and plasma membranes were prepared using a modification of the procedures of Cheng and Karavolas[9], and Fitzpatrick *et al.*[10]. Decapsulated kidneys (46 g) were rinsed in the homogenization buffer and the minced material was homogenized with a Teflon pestle in *Buffer II*, 0.88 M sucrose (20% w/v) containing 100 mM potassium phosphate (pH 7.2), 1.5 mM $CaCl_2$, 1 mM $MgSO_4$, 5 mM DTT, and 50 μg/ml trypsin inhibitor, and filtered through 4 layers of cotton gauze. The filtrate was re-homogenized using a glass pestle and filtered through 3 layers of nylon mesh (200 μm). The filtered homogenate was then centrifuged at 800 g and the pellet was washed twice and resuspended in 200 ml *Buffer II*, homogenized once more using a glass pestle, and filtered again through 2 layers of nylon mesh.

After a final 800 g spin, the pellet was layered on 2 vol of 2.2 M sucrose containing 100 mM potassium phosphate (pH 7.2), 1.5 mM $CaCl_2$, 5 mM DTT, and 50 μg/ml trypsin inhibitor, and centrifuged at 50,000 g for 70 min using a SW 50.1 (Beckman) swinging bucket rotor. The resultant pellet, consisting of purified nuclei, was suspended in 25 ml *Buffer III*, 100 mM potassium phosphate, pH 7.2, containing 1 mM $MgCl_2$, 5 mM DTT, and 50 μg/ml trypsin inhibitor, centrifuged at 800 g for 10 min, and resuspended in 10 ml of the same buffer.

The top layer from the 50,000 g spin, consisting of crude plasma membranes, was diluted to isotonicity with 7 vol of distilled water and centrifuged for 15 min at 35,000 g [10]. The pellet was washed with 25 ml of *Buffer I* and recentrifuged at 35,000 g. The final pellet of crude plasma membranes was suspended in 15 ml *Buffer III*.

Aliquots (250 μl) of both the nuclei and plasma membrane fractions were incubated separately with 21.5 nM [^{3}H]aldosterone in 0.5 ml *Buffer III* containing 2.76 mM NADPH and 2% ethanol.

HPLC analysis of aldosterone metabolites

The acetone–ethanol extracts of the subcellular fraction incubations were centrifuged at 1000 g for 10 min, and aliquots of the supernatant were evaporated to dryness under N_2, dissolved in 15% aqueous methanol, and chromatographed on a Dupont Zorbax C_8 reverse phase column by stepwise elution with 15, 33, and 50% aqueous methanol (Solvent System A) as previously described [5]. Separate aliquots of the acetone–ethanol extract were chromatographed and eluted with 40% aqueous methanol containing 0.5% acetonitrile (Solvent System M). The HPLC peak containing 5α-DHA and 3α,5α-THA (which could not be separated and eluted as a single peak) was re-chromatographed using 37% aqueous methanol containing 2% tetrahydrofuran, 0.5% acetonitrile, and 0.2% acetic acid (Solvent System Q). Non-radioactive and radioactive standards were also chromatographed using Solvent Systems M and Q and monitored at 190 nm with a Spectroflow SF 770 u.v. detector (Schoeffel Instrument Corporation) as well as for ^{3}H-radioactivity.

RESULTS

Subcellular localization of aldosterone metabolism

Subcellular fractionation of male rat kidney revealed that several fractions are capable of synthesizing aldosterone metabolites (Table 1). The kidney homogenate synthesized significant quantities of both polar and reduced metabolites (Fig. 1). Approximately 21% of the steroid ring-A reducing activity (pmol/g tissue weight) was located in the 1000 g pellet. Further purification of this pellet showed that significant aldosterone reductase activity is located in both the purified nuclei and plasma membranes. In these preparations, neither the nuclei nor plasma membrane fractions synthesized polar metabolites of aldosterone. The cytosol fraction on the other hand was active in synthesizing both polar (data not shown) as well as reduced metabolites of aldosterone. Thus, expressing the enzymatic activity per g kidney, the cytosol contained 68% of the overall reductase activity. The remaining reductase activity was present in both microsomes and mitochondria. When the reductase activity is expressed as femtomoles of product per mg protein (Table 1), the

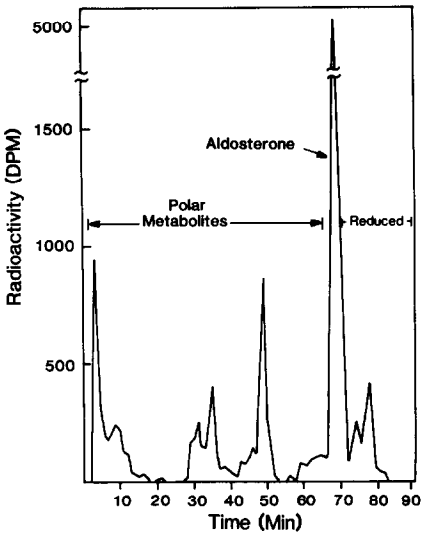

Fig. 1. HPLC analysis of aldosterone metabolites synthesized by kidney homogenate using Solvent System A. Kidney homogenates were incubated with 21.5 nM [^{3}H]aldosterone as described above for 120 min at 37°C. Extracts were chromatographed on a Zorbax C_8 reverse phase column with stepwise elution with 15, 33 and 50% aqueous methanol for 20, 30 and 35 min respectively at a flow rate of 1 ml/min.

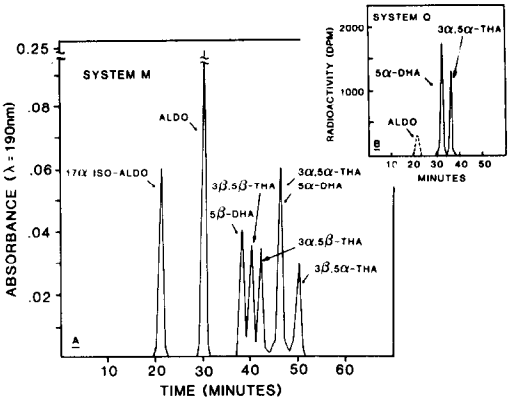

Fig. 2. HPLC analysis of reduced aldosterone metabolite standards. Ten μg (or 500–1000 dpm) of each standard were injected onto a Zorbax C_8 reverse phase column and eluted with Solvent System *M*; 40% aqueous methanol containing 0.5% acetonitrile or Solvent System *Q*; 37% aqueous methanol containing 2% tetrahydrofuran, 0.5% acetonitrile, and 0.2% acetic acid at a flow rate of 1 ml/min. The standards were monitored at 190 nm using a flow-through u.v. detector, or 1 ml aliquots of each fraction were counted as described above.

1000 *g* pellet and cytosol contained 42 and 38% respectively of the aldosterone reducing activity. Upon further purification of the 1000 *g* pellet, the nuclear and plasma membrane fractions retained 62 and 38% respectively of this activity. Light and electron microscopic examination of these subcellular fractions revealed minimal cross-contamination.

HPLC separation of reduced aldosterone metabolites

Solvent System A resolves metabolites of aldosterone into several polar products and two peaks, eluting at 73–75 and 76–78 min (Fig. 1) which contained the ring-A reduced metabolites of aldosterone. This solvent system was used to measure overall metabolism of aldosterone by the kidney subcellular fractions. Solvent System M (Fig. 2A) resolves the six ring-A reduced metabolite standards of aldosterone into 5 distinct peaks. 5β-DHA, 3β,5β-THA, 3α,5β-THA, and 3β,5α-THA eluted at 38, 40, 42 and 50 min, respectively and 5α-DHA and 3α,5α-THA eluted as a single peak at 46 min. The 46 min peak containing 5α-DHA and 3α,5α-THA was isolated and separated by a second Solvent System (System Q), into distinct peaks eluting at 31 and 35 min respectively (Fig. 2B). In these experiments, only the pooled reduced metabolites (separated by Solvent System A) synthesized by cytosol, nuclear, and plasma membrane fractions contained sufficient radioactivity for further analysis using Solvent System M and only nuclei and plasma membranes were further analyzed using Solvent System Q.

Analysis of reduced aldosterone metabolites synthesized by kidney subcellular fractions

The pattern of ring-A reduced products synthesized by the cytosol was markedly different from that observed with the nuclear and plasma membrane fractions (Table 2). The cytosol synthesized mainly 5β-products. In contrast 80–90% of the reduced metabolites synthesized by the nuclei and plasma membrane fractions were 5α-reduced products. The nuclear fraction synthesized 2-fold more 5α-reduced products than the plasma membrane fractions

Table 1. Reduced aldosterone metabolites synthesised by subcellular fractions of kidney

Subcellular fraction	Reduced aldosterone metabolites synthesized (pmol/g tissue/2 h)	(fmol/mg protein/2 h)
1000 *g* pellet	63 ± 2	147 ± 3
Nuclei	21 ± 1	91 ± 5
Crude plasma membranes	11 ± 2	54 ± 8
Cytosol	202 ± 18	134 ± 12
Microsomes	13 ± 5	35 ± 12
Mitochondria	19 ± 4	32 ± 6

Subcellular fractions were incubated with 21.5 nM [^{3}H]aldosterone at 37°C/120 min, and products quantitated by HPLC using Solvent System A as described above. Values represent mean ± SE, $n = 6$.

Table 2. HPLC analysis of reduced metabolites of aldosterone synthesized by kidney subcellular fractions

	Aldosterone metabolites synthesized (fmol/mg protein/2 h)		
	Nuclei	Crude plasma membranes	Cytosol
5α-DHA	82 ± 8	33 ± 6	3 ± 2
3α,5α-THA (5α-DHA and 3α,5α-THA)*	(34 and 66%)*	(47 and 53%)*	
3β,5α-THA	1 ± 1	10 ± 4	21 ± 5
Total 5α-reduced	83 ± 10	43 ± 10	24 ± 6
5β-DHA	4 ± 2	0	101 ± 7
3α,5β-THA	0	6 ± 3	7 ± 4
3β,5β-THA	5 ± 3	5 ± 3	2 ± 2
Total 5β-reduced	9 ± 5	11 ± 6	110 ± 10

The reduced metabolites following incubation of subcellular fractions with 21.5 nM [^{3}H]aldosterone at 37°C/120 min were analyzed by HPLC using Solvent Systems M and Q as described above. Values represent mean ± SE; $n = 6$. *As shown by Solvent System Q.

($P < 0.05$). The majority of the 5α-products synthesized by these fractions (>99% and >75% respectively) were shown to be 5α-DHA and 3α,5α-THA. The ratios of 5α-DHA and 3α,5α-THA synthesized by nuclear and plasma membrane fractions were significantly different, being 1:2 and 1:1, respectively. The cytosol synthesized only trace quantities of these metabolites, but did product significant amounts of 3β,5α-THA.

HPLC analysis (Table 2) revealed that the majority (>90%) of the 5β-reduced metabolites of aldosterone synthesized in the cytosol was co-chromatographed with 5β-DHA; small quantities of both 3α,5β-THA, 3β,5β-THA were also present. The nuclear and plasma membrane fractions also synthesized small quantities of 5β-reduced metabolites.

Effects of aldosterone antagonists on the synthesis of reduced aldosterone metabolites

The synthesis of reduced aldosterone metabolites by kidney cytosol, nuclear and plasma membrane fractions was inhibited by both 5 and 10 μM concentrations of the three antimineralocorticoids tested (Fig. 3A–C). However, the inhibition by progesterone was significantly greater ($P < 0.01$–0.001) than that of K^+-canrenoate and corticosterone in both the nuclei and plasma membranes even at 5 μM. In the cytosol, this effect of progesterone was only significantly greater at 50 μM ($P < 0.05$). In the nuclear and plasma membrane fractions, where 5α-reduced metabolites were principally synthesized, progesterone inhibited aldosterone metabolism by 85–100% at both 5 and 50 μM concentrations. However, in the cytosol, where 5β-reduced metabolites were principally synthesized, progesterone inhibited aldosterone metabolism by 58 and 85% at 5 and 50 μM concentrations, respectively.

In the nuclei K^+-canrenoate was a more potent inhibitor of aldosterone reduction (42 and 80% at 5 and 50 μM concentrations, respectively) than corticosterone. In contrast, this soluble spironolactone inhibited the synthesis of reduced aldosterone metabolites to the same extent as corticosterone in both the plasma membranes and cytosol fractions. It is important to note that in the nuclear experiments, corticosterone is a relatively weak inhibitor (18% at 5 μM and 47% at 50 μM ($P < 0.01$)), whereas, this

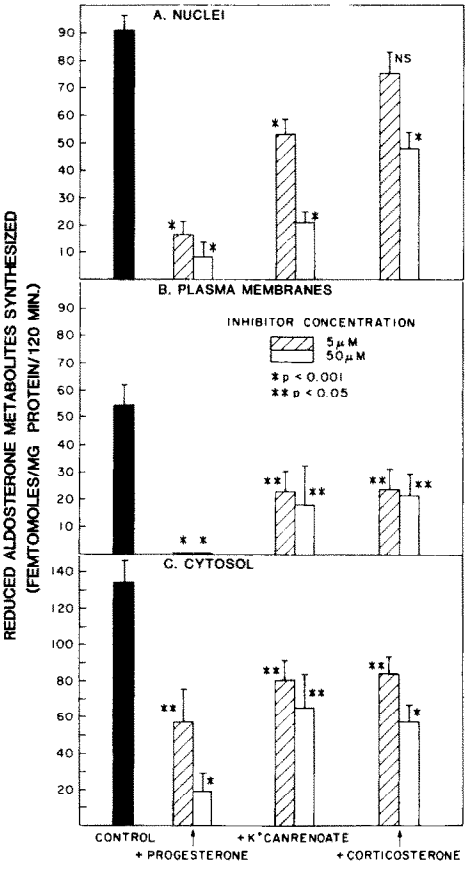

Fig. 3. The effects of progesterone, K^+-canrenoate and corticosterone on the synthesis of reduced metabolites of aldosterone in kidney subcellular fractions. Purified nuclei (Fig. 3A), plasma membranes (Fig. 3B), and cytosol (Fig. 3C) were incubated with 21.5 nM [^{3}H]aldosterone with and without 5 μM and 50 μM of each of these steroids for 120 min at 37°C. The reduced products synthesized were analyzed by HPLC as described above. Values represent mean ± SE, $n = 6$ (*$P < 0.001$, **$P < 0.05$ vs controls).

steroid inhibited aldosterone metabolism in the plasma membranes by 50–60% ($P < 0.01$) at both 5 μM and 50 μM concentrations.

DISCUSSION

Previous investigators have reported that rat kidney possesses several steroid metabolizing enzymes including microsomal 5α-reductase [11–13]; cytosolic 5β-reductase [11]; microsomal, cytosolic, and nuclear 3α-hydroxysteroid dehydrogenase (3α-HSD) [12–16] and cytosolic 3β-hydroxysteroid dehydrogenase (3β-HSD) [11]. Steroid hydroxylating enzymes, as well as glucuronyl transferase, sulfotransferase, sulfatase and beta-glucuronidase are also known to be present in kidney [11]. We recently reported that slices of kidney cortex and medulla from adrenalectomized male rats metabolized aldosterone to several 5α- and 5β-reduced metabolites in addition to at least 4 weeks of polar metabolites [5].

The present experiments confirmed our earlier reports that a major proportion of the reduced metabolites synthesized in kidney of male adrenalectomized rats takes place in the 1000 ***g*** pellet. Most strikingly, improved HPLC systems demonstrated that purified nuclei and plasma membrane fractions obtained from this pellet metabolize aldosterone principally to 5α-DHA and 3α,5α-THA in ratios of 2:1 and 1:1, respectively. Interestingly, the cytosol which also possessed a large capacity to enzymatically reduce aldosterone, synthesized almost exclusively 5β-reduced products, consisting principally of 5β-DHA and smaller quantities of 3α,5β-THA and 3β,5β-THA. Whereas the nuclei and plasma membranes synthesized only reduced metabolite products, the cytosol fraction metabolized aldosterone to both polar and reduced metabolites. In addition, small quantities of reduced metabolites were synthesized from aldosterone in the microsomes and mitochondria. The polar metabolites synthesized consist of a variety of neutral and conjugated products which are under current investigation.

Other studies in our laboratory have shown that 5α-reduced metabolites are more potent mineralocorticoids than the 5β-reduced isomers. 5α-DHA and 3α,5α-THA possess $\frac{1}{10}$ and $\frac{1}{30}$ respectively, of the antinatriuretic activity of aldosterone. 3α,5β-THA was shown to possess $\frac{1}{80}$ to $\frac{1}{100}$ the antinatriuretic activity of aldosterone [6]. Thus, at least three of the reduced metabolites of aldosterone synthesized at specific subcellular sites in the target tissue, kidney, do possess significant mineralocorticoid properties. Localization of the synthesis of 5α-DHA and 3α,5α-THA in the nuclei indicates that transformation of aldosterone to biologically active products takes place near the site of action of the hormone–receptor complex with chromatin, an important step in the accepted mechanism of action of the hormone [17–19]. In other related experiments, incubation of [^{3}H]aldosterone with the isolated toad bladder has shown that this target tissue also synthesized 5α- and 5β-reduced products similar to those observed in the kidney [20].

It is important to note that both testosterone and progesterone are also converted to 5α-dihydro and 3α,5α-tetrahydro-reduced metabolites in their target tissues [1–3, 21, 22]. These metabolites are synthesized by both the nuclear and plasma membrane subcellular fractions, and are considered to be important in the mechanism of these hormones. Rat kidney nuclei also synthesize similar 5α-reduced metabolites of testosterone which may have physiological importance [11]. Corticosterone [23] and cortisol [24] have been shown to be metabolized in rat kidney to several 5α-reduced and 20-reduced products.

We realize that the subcellular fractions we have examined at this time are derived from the whole kidney and therefore, represent a variety of cell types. Additional experiments are necessary to locate those portions of the kidney tubules which do possess enzymes responsible for the metabolic transformations of aldosterone described above. It is also possible that the proportions of the individual metabolites of aldosterone synthesized *in vitro* may be different from those generated in whole kidney cells or slices. However, these experiments do indicate that several subcellular locations in kidney are capable of generating specific metabolites of aldosterone which possess physiological activity.

In our earlier studies the antimineralocorticoids, progesterone, and K^+-canrenoate (a soluble spironolactone) both markedly inhibited the metabolism of aldosterone to both reduced and polar metabolites in rat kidney slices [5]. Corticosterone, which has been shown to antagonize the antinatriuretic response to aldosterone [25, 26] strongly inhibited the synthesis of polar metabolites, but only slightly inhibited the synthesis of ring-A reduced products [5]. In the present studies, progesterone was the strongest inhibitor of aldosterone metabolism in all of the subcellular fractions tested. Interestingly, the synthesis of 5α-DHA and 3α,5α-THA in both the nuclear and plasma membrane fractions was inhibited by 85–100%, even at 5 μM concentrations. Other investigators have reported that progesterone inhibits 5α-reduction of testosterone in rat kidney nuclei [12]. Progesterone also inhibited the synthesis of 5α-reduced products in the cytosol by 85% at 50 μM; however, at lower concentrations, (5 μM), their synthesis was only inhibited by 58%. K^+-canrenoate inhibited the synthesis of 5α-DHA and 3α,5α-THA in nuclei more than corticosterone but their inhibitory effects were similar in the plasma membrane and cytosol fractions.

Thus, the order of inhibition of the synthesis of reduced metabolites of aldosterone in kidney subcellular fractions was shown to be: progesterone > K^+-canrenoate > corticosterone. Although all three antimineralocorticoids inhibited

both 5α- and 5β-reduction of aldosterone, progesterone and K^+-canrenoate inhibited 5α-reduction more than 5β-reduction. Further experiments are needed to determine the specificity and mode of action of these inhibitors.

Both progesterone, [27–29] and K^+-canrenoate [30, 31] have been suggested to exert their antimineralocorticoid effects by binding directly to cytosolic mineralocorticoid receptors in the kidney and preventing translocation of the hormone–receptor complex to the nucleus. The present investigations demonstrate clearly that these two antimineralocorticoids inhibit specific metabolism of aldosterone in kidney nuclei and plasma membrane fractions. Although our experiments have shown that corticosterone strongly inhibits the synthesis of polar metabolites of aldosterone in rat kidney slices this inhibitor of the antinatriuretic response to aldosterone [25, 26] less markedly inhibited the synthesis of ring-A reduced products in kidney subcellular fractions. This observation suggests that its mechanism of inhibition may be different from that of progesterone and K^+-canrenoate.

Although the importance of renal metabolism of aldosterone remains unknown at this time, it is very possible that nuclear synthesis of these metabolities may be a post-receptor event and also be involved in the biochemical events leading to the physiological responses to aldosterone. It has previously been shown that aldosterone induces renal flavokinase in adrenalectomized male rats and spironolactone inhibits this effect [32]. In addition, the 5α-reduced metabolites, 5α-DHA and 3α,5α-THA have also been shown recently to induce renal flavokinase, and likewise this effect is inhibited by spironolactone [33], again suggesting their possible role at the nuclear level for the expression of this hormonal effect. The importance of the synthesis of 5α-reduced metabolites in plasma membranes and 5β-reduced metabolites in the cytosol fraction remains unknown. The recent finding that 3α,5β-THA does not bind to cytosolic mineralocorticoid receptors but does possess significant mineralocorticoid activity which can be inhibited by spironolactone is likewise interesting [34]. Further studies are necessary to determine more about the uptake and translocation of both aldosterone and its metabolites to the nucleus. These and other experiments will help clarify the role of aldosterone metabolites in target tissues, and specifically at what point in the sequence of events they are required for the expression or regulation of the physiological actions of aldosterone.

Acknowledgements—This work was supported in part by a grant AM-21404 from the NIH. The authors wish to thank Kathleen King for excellent secretarial assistance.

REFERENCES

1. Bruchovsky N. and Wilson J. D.: The conversion of testosterone to 5α-androstan-17β-ol-3-one by rat prostate *in vivo* and *in vitro*. *J. biol. Chem.* **243** (1967) 2012–2021.
2. Martini L.: The 5α-reduction of testosterone in the neuroendocrine structures. Biochemical and physiological implications. *Endocr. Rev.* **3** (1982) 1–25.
3. Karavolas J. H. and Nuti K. M.: Progesterone metabolism by neuroendocrine tissues. In *Subcellullar Mechanisms in Reproductive Neuroendocrinology*. (Edited by F. Naftolin). Elsevier, Amsterdam (1976) pp. 305–326.
4. Morris D. J.: The metabolism and mechanism of action of aldosterone. *Endocr. Rev.* **2** (1981) 234–247.
5. McDermott M. J., Latif S. A. and Morris D. J.: The metabolism of aldosterone in kidney. *J. steroid Biochem.* **19** (1983) 1205–1211.
6. Kenyon C. J., McDermott M. J., DeConti G. A., Latif S. A., Brem A. S. and Morris D. J.: Antinatriuretic and kaliuretic activities of reduced derivatives of aldosterone. *Endocrinology* **112** (1983) 1852–1856.
7. Morris D. J., Kenyon C. J., Latif S. A., McDermott M. J. and Goodfriend T.: The possible biological significance of aldosterone metabolites. *Hypertension* (Suppl. I) **5** (1983) I35–I40.
8. Latif S. A. and Morris D. J.: Enzymatic synthesis of 3α,5α-tetrahydroaldosterone. Unpublished data.
9. Cheng Y. J. and Karavolas H. J.: Subcellular distribution and properties of progesterone (Δ4-steroid) 5α-reductase in rat medial basal hypothalamus. *J. biol. Chem.* **250** (1975) 7997–8003.
10. Fitzpatrick D. F., Davenport G. R., Forte L. and Lanslon E.: Characterization of plasma membrane proteins in mammalian kidney. *J. biol. Chem.* **244** (1969) 3561–3569.
11. Uotila P. J., Hartiala W. and Neinstedt W.: The metabolism of testosterone by human, rabbit, and rat kidney. *Int. J. Biochem.* **12** (1980) 345–351.
12. Verhoeven G. and DeMoor P.: Kinetic data on the formation of 17β-hydroxy-5α-androstene-3-one in renal nuclei from male and female rats. *Endocrinology* **91** (1972) 54–64.
13. Gustafsson J. Å. and Pousette A.: Different mechanisms of regulation of nuclear reduced nicotinamide-adenine dinucleotide phosphate dependent 3-oxo steroid 5α-reductase activity in rat liver, kidney, and prostate. *Biochem. J.* **142** (1974) 273–277.
14. Ghraf R., Lax E. R., Hoff H. G. and Schriefers A. H.: The role of the gonads and hypophesis in the regulation of hydroxysteroid dehydrogenase activities in rat kidney. *Hoppe-Seyler's Z. physiol. Chem.* **356** (1975) 135–147.
15. Verhoeven G., Heyns W. and DeMoor P.: Interconversion between 17β-hydroxy-5α-androstan-3-one (5α-dihydrotestosterone) and 5α-androstan-3α,17β-diol in rat kidney: Heterogeneity of 3α-hydroxysteroid oxidoreductases. *Eur. J. Biochem.* **65** (1976) 565–576.
16. Ghraf R., Lax E. R. and Schriefers H.: Regulation of 3α-hydroxysteroid dehydrogenase activities in rat cytosol: Dependence of estrogenic induction on the endocrine state. *Hoppe-Seyler's Z. physiol. Chem.* **358** (1977) 699–702.
17. Edelman I. S. and Fimognari G. M.: On the mechanism of action of aldosterone. *Recent Prog. Horm. Res.* **24** (1968) 1–34.
18. Crabbé J.: The mechanism of action of aldosterone. In *Receptors and Mechanism of Action of Steroid Hormones* (Edited by J. R. Pasqualini). Marcel Dekker, New York (1977) p. 513.
19. Pasqualini J. R. and Sumida C.: Mineralocorticoid receptors and target tissues. In *Receptors and Mechanism of Action of Steroid Hormones* (Edited by J. R. Pasqualini). Marcel Dekker, New York (1977) p. 399.
20. Brem A., Pacholski M. and Morris D. J.: The metabolism of aldosterone in the isolated toad bladder. Unpublished data.

21. Schmidt H., Giba-Tziampiri O., Rotteck G. and Voigt K. D.: Metabolism and mode of action of androgens in target tissue of male rats. IV—Mode of action of 5α-androstene-3α,17β-diol and 5α-androstene-3, 17-dione and 5α-androstene-3β,17β-diol at a cellular level on seminal vesicles and prostates of rats. *Acta endocr., Copenh.* **73** (1973) 599–611.
22. Krause J. E. and Karavolas H. J.: Properties of the hypothalamic 5α-dihydroprogesterone NADH- and NADPH-linked 3α-hydroxysteroid oxidoreductase activities. *J. steroid Biochem.* **14** (1981) 63–69.
23. Hierholzer K., Schoneshofer M., Siebe H., Tsiakiras D., Weskamp P. and Lichtenstein I.: Corticosterone metabolism in isolated rat kidney *in vitro*. I. Formation of lipid soluble metabolites from corticosterone (B) in renal tissue from male rats. *Pflügers Arch.* **400** (1984) 363–371.
24. Reach G., Nakane H., Nakane Y., Auzan C. and Corvol P.: Cortisol metabolism and excretion in the isolated perfused kidney. *Steroids* **30** (1977) 621–635.
25. Uete T. and Venning E. H.: The effect of cortisone, hydrocortisone and 9α-fluoro,16β-hydroxy-cortisol on the action of desoxycorticosterone and aldosterone with respect to electrolyte excretion. *Endocrinology* **67** (1960) 62–69.
26. Kenyon C. J., Saccoccio N. A. and Morris D. J.: Glucocorticoid inhibition of mineralocorticoid action in the rat. *Clin. Sci.* **67** (1984) 329–335.
27. Landau R. and Lugibihl K.: Inhibition of the sodium-retaining influence of aldosterone by progesterone. *J. clin. Invest.* **18** (1958) 1237–1245.
28. Wambach G. J. and Higgins R.: Antimineralocorticoid action of progesterone in the rat: Correlation of the effect on electrolyte excretion and interaction with renal mineralocorticoid receptors. *Endocrinology* **102** (1978) 1686–1693.
29. Lan N. C., Graham B., Bartter F. C. and Baxter J. D.: Binding of steroids to mineralocorticoid receptors: Implications for *in vivo* occupancy by glucocorticoids. *J. clin. Endocr. Metab.* **54** (1982) 332–341.
30. Funder J., Feldman D., Highland E. and Edelman I.: Molecular modifications of anti-aldosterone compounds: Effects on affinity of spironolactone for renal aldosterone receptors. *Biochem. Pharmac.* **23** (1974) 1493–1501.
31. Marver D., Stewart J., Funder J. W., Feldman D. and Edelman I. S.: Renal aldosterone receptors: Studies with [^{3}H]aldosterone and the antimineralocorticoid [^{3}H]spironolactone. *Proc. natn. Acad. Sci. U.S.A.* **71** (1974) 1431–1435.
32. Tan E. L. and Trachewsky D.: Effect of aldosterone on flavin coenzyme biosynthesis in the kidney. *J. steroid Bicohem.* **6** (1975) 1471–1475.
33. Trachewsky D., Oakes M. L. and Morris D. J.: Induction of flavokinase by aldosterone in the rat kidney. *Endocrinology* **116** (1985) 879–888.
34. Gomez-Sanchez C. E., Smith J. S., Ferris M. W. and Gomez-Sanchez E. P.: Renal receptor-binding activity of reduced metabolites of aldosterone: Evidence for a mineralocorticoid effect outside of the classic aldosterone receptor system. *Endocrinology* **115** (1984) 712–715.