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FECAL AND URINARY EXCRETION OF [^{3}H]-ALDOSTERONE AND ITS SEX DEPENDENCE IN RATS

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SUMMARY

Following subcutaneous injection of [^{3}H]-aldosterone, the excretion of [^{3}H]-radioactivity in the urine was demonstrated to be sex-dependent in both adrenalectomized and intact rats. More radiometabolites of aldosterone were excreted in the urine of the male rats than the female rats. The excretion of [^{3}H]-radioactivity in the feces of these rats was also sex-dependent. In contrast, more radiometabolites of aldosterone were excreted in the feces of the females than the males. In both male and female rats, adrenalectomized and intact, the principal route of excretion of aldosterone and its metabolites was *via* the feces.

In all rats, the urinary excretion of the radiometabolites of aldosterone consisted of an initial major peak within the first few hours and a subsequent smaller peak, 6–18 h post-injection of the [^{3}H]-aldosterone. The enterohepatic circulation of aldosterone and its metabolites appears to be responsible for this second peak of radiometabolites excreted in the urine, but its relationship to the initial [^{3}H]-radioactivity excreted in the urine remains unclear. The majority of the radiometabolites excreted in both the urine and feces of these rats are polar non-extractable metabolites of aldosterone. The quantities of free aldosterone and tetrahydroaldosterone extractable from both the urine and feces of these rats represent very small percentages of the administered [^{3}H]-aldosterone.

INTRODUCTION

From previous studies[1, 2] it has been shown that following administration of physiological quantities of [^{3}H]-aldosterone in both adrenalectomized and intact rats, the rates of clearance of total plasma radioactivity were rapid and sex-dependent.

The biliary route of excretion of aldosterone[2–5], cortisol[6], corticosterone and other steroids[7–11] has been shown to be the major pathway of excretion of these steroids in rats. The biliary excretion of aldosterone and its metabolites has been demonstrated to be considerably greater in female than in male rats[2, 5]. Similar differences in the rates of clearance of corticosterone from plasma of male and female rats[11–14] has been demonstrated to be related to differences in the routes of hepatic metabolism of this hormone. The pathways of metabolism and excretion, and the pharmacological response of many drugs are also sex-dependent in rats[15–17].

The fecal and urinary excretion of corticosterone and other steroids have been previously demonstrated[18, 19] to be sex-dependent in rats. This paper describes that both the fecal and urinary routes of excretion of aldosterone and its metabolites in rats are also sex-dependent.

MATERIALS AND METHODS

Adrenalectomized and intact male and female Sprague–Dawley (Charles River, CD) rats, weighing between 160 and 180 g, were used in all experiments. The adrenalectomized rats were used 5–8 days post-adrenalectomy and were maintained on 0.9% NaCl *ad lib.* as drinking water. The intact rats were maintained on tap water *ad lib.* as drinking water. Diet consisted of Purina rat chow fed *ad lib.*

Four groups of rats: intact male and female rats, and adrenalectomized male and female rats, were housed in Hoeltge metabolic cages. Each group consisted of 6 rats; 2 rats per metabolic cage unit.

Chromatographically purified D-[1,2,-^{3}H]-aldosterone was obtained under nitrogen from the New England Nuclear Corporation. The specific radioactivity was 30–50 Ci/mmol. Labeled aldosterone was dissolved in 0.14 M NaCl under nitrogen just prior to use to give a dosage of 0.081 μg/rat in 0.2 ml (17.7×10^6 d.p.m. per rat).

The rats were injected subcutaneously with [^{3}H]-aldosterone and spontaneously voided urine, free of feces was collected for successive hourly intervals throughout the first 6 h. The residual bladder urine at the end of each time interval was obtained by gentle suprapubic pressure. Continuous collection of urine from these animals was then carried out for successive 6 h intervals over a total period of 48 h. Feces were also continuously collected from these animals for successive 6 h intervals over a total period of 48 h. The urine and feces were stored at 4°C.

The volume of each urine collection was recorded and the total radioactitivity of aliquots (0.1 ml) of each urine collection were counted directly using Beckman Biosolv as a solubilizer in 10 ml of toluene scintillation solution, as previously described[20].

The collections of feces for each time period of each group of rats were pooled and homogenized in a Waring Blender in 0.14 M NaCl at 0°C. Total vol. of homogenates of feces were recorded and the total radioactivity of aliquots (0.2 ml) of each homogenate were counted directly as above.

Aliquots (2 ml) of each urine collection and of each fecal homogenate were extracted twice with equal vol. of CH_2Cl_2 at 0°C. Radioactivity in aliquots (0.1 ml) of the aqueous phase after extraction was counted directly to determine the percentage of extractable radioactivity in each urine and fecal sample.

After extraction with CH_2Cl_2, samples of urine (1 ml) were hydrolysed either at pH 1 at room temperature for 48 h or with 14,000 Fishman units of *Helix pomatia* β-glucuronidase containing sulphatase activity (Sigma, Type H-1) in 0.1 M acetate buffer, at pH 5.0 for 48 h at 37°C. Following hydrolysis, each aliquot of urine was washed twice with dichloromethane to determine the percentage of extractable label.

The dichloromethane extracts from both urine and feces were concentrated under nitrogen, and as previously described[20] chromatographed with non-radioactive aldosterone and 3α,5β-tetrahydroaldosterone as markers on paper using the Bush B_5 solvent system.

To minimize error in the total quantity of [^{3}H]-aldosterone administered subcutaneously, all rats were injected on a given day with the same fresh preparation of [^{3}H]-aldosterone.

Statistical analysis of the resultant data was performed utilizing the Student's *t* test.

RESULTS

Excretion studies of [^{3}H]-aldosterone in male and female rats

1. *Comparison of urine excretion in adrenalectomized rats.* The total radioactivity excreted in the urine of adrenalectomized male and female rats was markedly different (Table 1). Larger quantities of [^{3}H]-radioactivity were excreted in the urine of the males. From 4 to 48 h post-injection of the [^{3}H]-aldosterone, the percentage of administered radioactivity excreted was almost 3 times greater in the males than in the females ($P < 0.005$). By 48 h, 18.2% of the administered [^{3}H]-radioactivity was excreted in the urine of males, whereas only 6.9% was excreted in the urine of the females (Table 1).

The majority of the radioactivity was excreted within the first 5 h in the males and within the first 3 h in the females (Table 1). In both the males and females, additional radioactivity was excreted between 6–18 h post-injection of the hormone. Both the initial and the 6–18 h urinary excretion of [^{3}H]-radioactivity were significantly greater ($P < 0.05$) for the males than for the females. Subsequent excretion of radiometabolites in the urine was very small.

The percentages of CH_2Cl_2-extractable label from the urine, 6–11%, were similar for the males at all time intervals studied up to 18 h post-injection. The female values, 13–21%, at these time intervals were also similar. All the female values, however, were significantly greater ($P < 0.05$) than the male values. Paper chromatography showed that the CH_2Cl_2 extracts of the urine from both the males and females contained only aldosterone and tetrahydroaldosterone in a ratio of 4:1.

2. *Comparison of urine excretion in intact rats.* Similar to the findings for adrenalectomized rats, the urinary excretion of [^{3}H]-radioactivity was markedly greater in male than in female intact rats (Table 1). By 48 h, 14.6% of the administered [^{3}H]-radioactivity was excreted in the urine of males, whereas only 4.9% was excreted ($P < 0.005$) in the urine of the females (Table 1). As found in the adrenalectomized rats, both the initial and the 6–18 h urinary excretion of [^{3}H]-radioactivity were significantly greater in males than in females ($P < 0.05$).

Table 1. Cumulative excretion of [^{3}H]-radioactivity in urine

Post injection time (h)	Adrenalectomized rats				Intact rats			
	Male		Female		Male		Female	
	c.p.m. rat × 10^5	%†	c.p.m rat × 10^5	%†	c.p.m. rat × 10^5	%†	c.p.m. rat × 10^5	%†
1	2.03	2.6	1.49	1.9	3.13	4.1	1.18	1.5
2	4.85	6.3	2.36	3.1	5.77	7.5	1.44	1.9
3	5.72	7.4	2.50	3.3	6.79	8.8	1.49	1.9
4	6.37	8.3	2.50	3.3	7.07	9.2	1.54	2.0
5	8.72	11.3	2.53	3.3	7.36	9.6	1.59	2.1
6	9.03	11.7	2.58	3.4	7.54	9.8	1.67	2.2
12	11.00	14.3	3.06	4.0	9.16	11.9	2.26	2.9
18	12.43	16.1	4.04	5.3	10.34	13.4	2.98	3.9
24	13.00	17.0	4.39	5.7	10.64	13.8	3.22	4.2
48	14.00	18.2	5.28	6.9	11.25	14.6	3.79	4.9

Urine was collected from rats following subcutaneous injection of 0.081 μg [^{3}H]-aldosterone. All values are represented as mean values ($n = 6$).
† Percentage of administered [^{3}H]-aldosterone.

Table 2. Percentage of administered [^{3}H]-radioactivity excreted in feces

Post injection time (h)	Adrenalectomized rats		Intact rats	
	Male	Female	Male	Female
6	<0.1	<0.1	<0.1	<0.1
12	1.3	0.3	25.3	0.3
24	27.5	22.7	44.7	41.0
48	41.7	63.6	46.9	58.4

Feces were collected from rats following subcutaneous injection of 0.081 μg [^{3}H]-aldosterone.

All per cent values are represented as mean values ($n = 6$).

The percentages of CH_2Cl_2-extractable label from the urine, 6–14%, were similar for the males at all time intervals studied up to 18 h post-injection. The female values, 30–49%, at these time intervals were also similar. All the values for the females were significantly greater ($P < 0.005$) than the male values. Paper chromatography showed that the CH_2Cl_2 extracts from the urine of the males contained only aldosterone and tetrahydroaldosterone in a ratio of 3:1. However, the extracts from the urine of the females contained principally aldosterone (>97%).

pH 1 or β-glucuronidase hydrolysis experiments showed that only a further 9% of the [^{3}H]-radioactivity in the urine of adrenalectomized and intact male rats could be hydrolysed to yield CH_2Cl_2-extractable steroids. In similar hydrolysis experiments with the urine of adrenalectomized and intact female rats, a further 18% of the urine [^{3}H]-radioactivity was hydrolysed. Paper chromatography of the CH_2Cl_2-extractable label produced in all these hydrolysis experiments revealed the presence of at least five peaks of label, aldosterone and tetrahydroaldosterone being minor components. The structures of these polar metabolites of aldosterone, found in both the urine and feces, are under current investigation.

3. *Comparison of fecal excretion in adrenalectomized rats.* Very little radioactivity was excreted in the feces of adrenalectomized male and female rats, during the first 12 h (Table 2). The excretion of radiometabolites in the feces of males increased from 27.5% at 24 h to 41.7% of the administered [^{3}H]-radioactivity by 48 h post-injection of the hormone. The corresponding increase in the excretion of radioactivity in the feces of the females, from 22.7% at 24 h to 63.6% by 48 h, was significantly greater than that in the males.

4. *Comparison of fecal excretion in intact rats.* Very little radioactivity was excreted in the feces of intact male and female rats during the first 6 h (Table 2). By 12 h post-injection of the hormone, 25.3% of the administered [^{3}H]-radioactivity was excreted in the feces of the males, compared to only a trace in the female rats. The excretion of radiometabolites in the feces of the males then increased to 44.7% at 24 h, reaching 46.9% by 48 h. In contrast, the females excreted 41% by 24 h, reaching 58.4% by 48 h.

The percentage of CH_2Cl_2-extractable label from the feces of both the adrenalectomized male and female rats at 24 and 48 h were similar, 9–13%. These values were less than 4% for both the intact male and female rats. Paper chromatography of the dichloromethane extracts of the feces from both the adrenalectomized and intact rats indicated the presence of at least 4 peaks of label, including aldosterone (25%) and tetrahydroaldosterone (25%).

DISCUSSION

Substantial quantities of radiometabolites of both cortisol[6] and aldosterone[3] have been previously demonstrated to be excreted in the urine of male rats. From bile duct cannulation experiments the majority of the radiometabolites of cortisol and aldosterone have been shown to be rapidly excreted into the bile of these animals[2, 3, 5, 6]. The presence of the radiometabolites of these two steroids in the urine has been suggested[3, 6] to be due to their reabsorption from the gut into the enterohepatic circulation. The presence of metabolites of corticosterone and aldosterone in the cloacal fluid of ducks has been likewise demonstrated to be due to the enterohepatic circulation of these compounds[21].

The excretion of corticosterone and other steroids in the feces and urine have been previously demonstrated[18, 19] to be sex-dependent in rats. These differences appear to be due to the different routes of hepatic metabolism of these steroids[18, 19, 22]. The biliary excretion of aldosterone and its metabolites has been demonstrated in these laboratories to be considerably greater in female than in male rats[2, 5]. We were therefore prompted to investigate whether sex differences in the urinary and fecal excretion of aldosterone and its metabolites exist in rats.

The studies described in this paper indicate that the considerable percentages of radiometabolites of aldosterone excreted in the urine in both adrenalectomized and intact male rats were significantly greater than in the corresponding females. In all rats the majority of the [^{3}H]-radioactivity was excreted in the urine within the first few hours and also during a later period, 6–18 h post-injection of the hormone. The urinary excretion of [^{3}H]-radioactivity which occurred between 6 and 18 h post-injection in all rats may be due to the enterohepatic circulation, as has been previously suggested[3]. However, it is not yet clear whether the initial major peak of urinary excretion of aldosterone and its metabolites is also due to the enterohepatic circulation.

The aldosterone and tetrahydroaldosterone, extractable from the urine of male and female rats, represent very small percentages of the administered [^{3}H]-aldosterone. The finding that the extractable label from the urine of intact female rats was (chromatographically) essentially aldosterone is interesting, since the extractable label from the urine of the other groups of rats was shown to contain not only aldo-

sterone but also considerable proportions of tetrahydroaldosterone. Assuming that the injected [^{3}H]-aldosterone is metabolized and cleared from the plasma of intact rats in a similar fashion to the endogenous aldosterone, this finding could be related to the higher secretion rates[23] and peripheral plasma levels[24] of aldosterone in female rats, and sex differences in the routes of hepatic metabolism of this hormone[20]. The percentages of CH_2Cl_2-extractable radioactivity in the urine, both before and after hydrolysis, were significantly greater for the females than for the males. These findings also suggest that the sex differences in the urinary excretion of [^{3}H]-aldosterone are due to differences in the metabolism of aldosterone in the liver of male and female rats.

The fecal excretion of radiometabolites was initially somewhat slower in female than in male rats. However, over a 48 h period, significantly greater quantities of [^{3}H]-radioactivity were excreted in the feces of the female rats (both adrenalectomized and intact). Interestingly, the rates of excretion of the radiometabolites of aldosterone in the feces of both the males and particularly the females, were significantly slower than those indicated from bile duct cannulation experiments[2, 3, 5]. These findings also suggest that a considerable enterohepatic circulation of the radiometabolites of aldosterone exists in both males and females. Preliminary studies in these laboratories[25], injecting bile containing radiometabolites of aldosterone intraduodenally into both male and female rats, respectively, have confirmed the existence of this enterohepatic circulation[3] in both sexes. Within 3 h, 10–15% of the injected radioactivity reappeared in the bile of both male and female rats. Further experimentation is required to determine the role of the enterohepatic circulation of aldosterone and its metabolites and its possible relationship to the mechanism of action of aldosterone in rats.

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