Renal Responses to Actinomycin D and Aldosterone in the Hypophysectomized Rat[1] (35859)

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Aldosterone elicits various renal responses in the hypophysectomized rat (1–3). These responses include: (i) antinatriuresis; (ii) no effect on urinary sodium excretion; and (iii) natriuresis.

In a previous report (3), the authors presented evidence that the level of previous dietary salt and water intake determines the response to exogenous aldosterone. For example, in sodium-deprived rats, aldosterone may not influence sodium excretion, while adequate sodium intake may lead to an antinatriuretic response, as may hydropenia (1, 3). The present experiments were designed to investigate the physiological nature of the changes modifying the renal response to aldosterone in the hypophysectomized rat.

Methods and Materials. Albino rats (100–150 g) were anesthetized with Inaktin (100 mg/kg, ip). Sodium-deprived animals received a sodium-deficient diet (<0.002 mEq of Na/g, Nutritional Biochemicals) in equal quantities for 7 preexperimental days with distilled water *ad libitum*. Sodium-replete rats received Wayne lab blocks (>0.13 mEq of Na/g) and tap water *ad libitum*. Hypophysectomy was accomplished parapharyngeally by Hormone Assay labs. Tracheostomy was followed by jugular vein cannulation and, after a priming dose of 1 ml, mammalian Ringer's solution containing 2 μCi/ml of ^{3}H-methoxy inulin was infused at a rate of 1.3 ml/hr for the duration of the experiment. Both ureters were cannulated, and urine and tail vein blood collections were begun 60 min after the start of infusion. Collections were made over the next 3 hr at 30-min intervals. Rectal temperature was maintained at 38 ± 1°.

Electrolytes were determined by flame photometry. The GFR was calculated from serum and urine radioactivity.

D-Aldosterone was obtained from Mann Research Labs; actinomycin D (Lyovac) came from Merck, Sharp and Dohme.

Experiments were performed on the following preparations, with dosages expressed as weight of compound delivered im/100 g of body weight; the "min" for each group below refer to the number of minutes prior to the start of the first urine collection that injection was made:

I. Unoperated rats, sodium-deprived. 1. Carrier, 90 min.

2. Aldosterone, 4 μg, 90 min.

3. Actinomycin D, 30 μg, 90 min.

II. Hypophysectomized rats, sodium-replete. 1. Carrier, 120 and 90 min.

2. Carrier, 120 min; aldosterone, 4 μg, 90 min.

3. Actinomycin D, 30 μg, 120 min; carrier, 90 min.

4. Actinomycin D, 120 min, 30 μg; aldosterone, 4 μg, 90 min.

III. Hypophysectomized rats, sodium-deprived. 1. Carrier, 120 and 90 min.

2. Carrier, 120 min; aldosterone, 4 μg, 90 min.

3. Actinomycin D, 30 μg, 120 min; carrier, 90 min.

4. Actinomycin D, 30 μg, 120 min; aldosterone 4 μg, 90 min.

5. Aldosterone 4 μg, 120 min; actinomycin D, 30 μg, 90 min.

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TABLE I. Renal Responses to Actinomycin D and Aldosterone in Unoperated and Hypophysectomized Rats.[a]

Line	Type rat, diet, injection	Excretion/kg/min/kidney									GFR ml/min/ kg/kidney
		n	V (μl)	n	Na (μEq)	n	K (μEq)	n	Na/K	n	
i	U, SD, C	150	18.7 ± 1.16	143	0.94 ± 0.19	136	3.51 ± 0.20	136	0.37 ± 0.07	149	3.98 ± 0.16
ii	U, SD, Aldo	107	15.8 ± 0.73[b]	107	0.55 ± 0.12[b]	105	4.09 ± 0.17[b]	105	0.14 ± 0.02[b]	107	4.04 ± 0.18
iii	U, SD, Act D	84	13.9 ± 0.80[b]	74	1.19 ± 0.15	71	2.57 ± 0.16[b]	64	0.53 ± 0.06[b]	84	3.59 ± 0.20
iv	$\overline{H}$, SR, C	95	24.7 ± 1.41	95	1.70 ± 0.25	95	1.60 ± 0.11	95	1.15 ± 0.17	95	2.19 ± 0.15
v	$\overline{H}$, SR, Aldo	96	22.8 ± 1.91	95	1.56 ± 0.26	95	1.50 ± 0.12	95	1.29 ± 0.19	95	2.06 ± 0.18
vi	$\overline{H}$, SR, Act D	24	19.6 ± 1.00[b]	24	2.15 ± 0.21	24	1.17 ± 0.12[b]	24	2.03 ± 0.26[b]	24	2.73 ± 0.20[b]
vii	$\overline{H}$, SR, Act D + Aldo	96	19.1 ± 0.95[b]	95	1.14 ± 0.15[b]	95	1.42 ± 0.07	95	0.93 ± 0.16	96	2.65 ± 0.22[b]
viii	$\overline{H}$, SR, Act D	24	19.6 ± 1.00	24	2.15 ± 0.21	24	1.17 ± 0.12	24	2.03 ± 0.26	24	2.73 ± 0.20
ix	$\overline{H}$, SR, Act D + Aldo	96	19.1 ± 0.95	95	1.14 ± 0.15[b]	95	1.42 ± 0.07	95	0.93 ± 0.16[b]	96	2.65 ± 0.22
x	$\overline{H}$, SR, C	95	24.7 ± 1.41	95	1.70 ± 0.25	95	1.60 ± 0.11	95	1.15 ± 0.17	95	2.19 ± 0.15
xi	$\overline{H}$, SD, C	96	11.2 ± 0.82[b]	92	0.27 ± 0.02[b]	92	1.36 ± 0.13	92	0.29 ± 0.03[b]	96	1.13 ± 0.07[b]
xii	$\overline{H}$, SD, C	96	11.2 ± 0.82	92	0.27 ± 0.02	92	1.36 ± 0.13	92	0.29 ± 0.03	96	1.13 ± 0.07
xiii	$\overline{H}$, SD, Aldo	144	15.7 ± 1.00[b]	141	0.53 ± 0.06[b]	141	1.27 ± 0.07	144	0.57 ± 0.06[b]	144	0.96 ± 0.04[b]
xiv	$\overline{H}$, SD, Act D	66	43.6 ± 3.70[b]	66	3.41 ± 0.30[b]	66	1.88 ± 0.15[b]	66	2.57 ± 0.41[b]	66	1.73 ± 0.10[b]
xv	$\overline{H}$, SD, Act D + Aldo	78	30.6 ± 3.16[b]	77	2.22 ± 0.44[b]	77	1.99 ± 0.17[b]	77	1.14 ± 0.20[b]	78	1.70 ± 0.12[b]
xvi	$\overline{H}$, SD, Aldo + Act D	30	23.4 ± 1.73[b]	30	1.07 ± 0.17[b]	30	2.78 ± 0.20[b]	30	0.44 ± 0.06[b]	30	1.24 ± 0.05
xvii	$\overline{H}$, SD, Act D	66	43.6 ± 3.70	66	3.41 ± 0.30	66	1.88 ± 0.15	66	2.57 ± 0.41	66	1.73 ± 0.10
xviii	$\overline{H}$, SD, Act D + Aldo	78	30.6 ± 3.16[b]	77	2.22 ± 0.44[b]	77	1.99 ± 0.17	77	1.14 ± 0.20[b]	78	1.70 ± 0.12
xix	$\overline{H}$, SD, Act D + Aldo	78	30.6 ± 3.16	77	2.22 ± 0.44	77	1.99 ± 0.17	77	1.14 ± 0.20	78	1.70 ± 0.12
xx	$\overline{H}$, SD, Aldo + Act D	30	23.4 ± 1.73[b]	30	1.07 ± 0.17[b]	30	2.78 ± 0.20[b]	30	0.44 ± 0.06[b]	30	1.24 ± 0.05[b]

[a] Values in the first line of each grouping are the statistical controls for all values in the grouping. U, unoperated; $\overline{H}$, hypophysectomized; SD, sodium deprived; SR, sodium replete; C, carrier; Aldo, D-aldosterone; Act D, actinomycin D; n, number of collection periods. Order written is order of administration where several compounds were injected. Values are means ± SEM.

[b] $p < 0.05$.

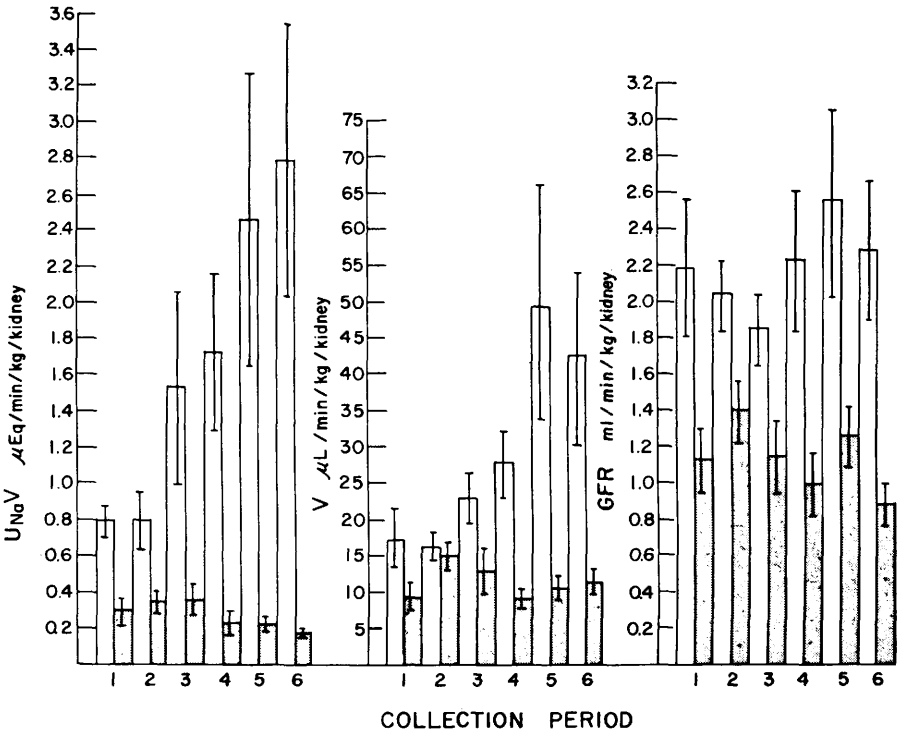

FIG. 1. Effect of sodium deprivation on sodium excretion, urinary volume, and GFR in the hypophysectomized rat. Each collection period was of 30-min duration; (open bars) means of control rats; (stippled bars) means of experimental rats; (lines) standard errors of the means.

Absence of the hypophysis was verified at autopsy. Significance of the differences between the means was determined by Student's *t* test. Significance was assigned to *p* values of less than 0.05.

Results. Unoperated, sodium deprived-rats. The response to aldosterone included antidiuresis, antinatriuresis, kaliuresis, and a lowering of the sodium: potassium excretory ratio. The GFR was unmodified (Table I, ii[3]).

Actinomycin D administration resulted in antidiuresis and antikaliuresis without change in sodium excretion or GFR (iii).

Hypophysectomized, sodium-replete rats. Aldosterone administration did not effect significant change in any of the measured parameters of renal function (v). Actinomycin D, when administered alone, resulted in antidiuresis and antikaliuresis, with concomitant increases in the Na:K excretory ratio, and GFR (vi).

[3] Lower case Roman numerals indicate line on Table I where mean values appear.

By comparison to carrier-receiving controls, the administration of actinomycin D, followed by the administration of aldosterone, resulted in antidiuresis with antinatriuresis and elevated GFR (vii). However, comparison of results from animals which received actinomycin D, then aldosterone, with the results from those receiving only actinomycin D, reveals that aldosterone administration after actinomycin D resulted in antinatriuresis and a lowering of the Na:K excretory ratio (ix).

Hypophysectomized, sodium-deprived rats. Sodium deprivation itself resulted in sharply lowered urinary water and sodium excretion, along with a reduction in the Na:K excretory ratio and GFR (Fig. 1; xi). Interestingly, the natriuresis and diuresis effected by aldosterone, in the sodium-deprived rats, occurred only after a rather lengthy lag period, and are attributable to the last three collection periods. The GFR was slightly depressed by aldosterone in these rats (Fig. 2; xiii). The

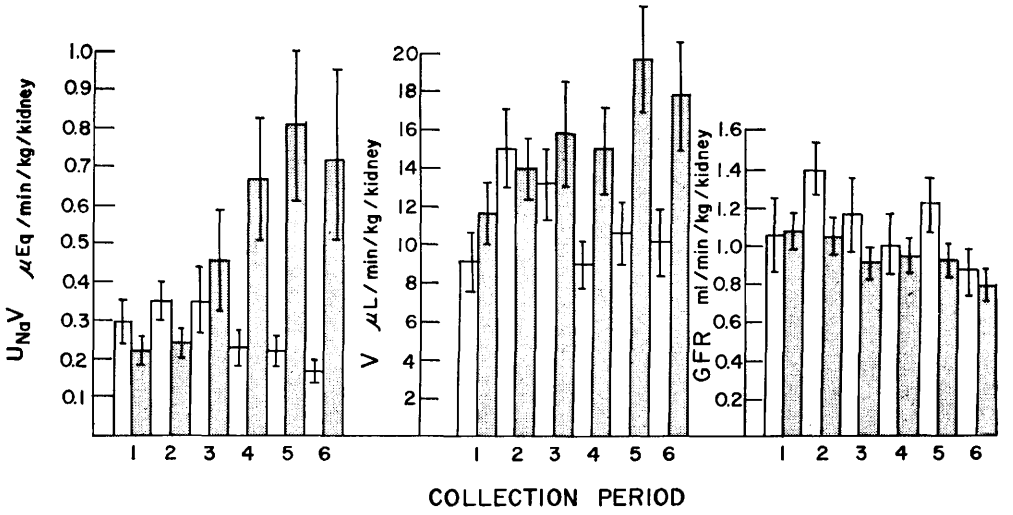

FIG. 2. Effect of aldosterone on sodium excretion, urinary volume, and GFR in the hypophysectomized, sodium-deprived rat.

increasing sodium and water excretion with time mimics the qualitative response to infusion of salt and water observed in the sodium-replete hypophysectomized rat (Fig. 1). Actinomycin D did induce diuresis, natriuresis, and kaliuresis in this group. The Na:K excretory ratio was increased following actinomycin D administration as was the GFR (xiv).

The combination, actinomycin D then aldosterone, elicited relative to controls receiving only carrier, diuresis, natriuresis, and kaliuresis, elevation of the Na:K excretory ratio, and elevation of GFR (xv). However, the addition of aldosterone after actinomycin D resulted in antidiuresis, antinatriuresis and a depression of the Na:K excretory ratio, relative to the values obtained from the animals treated with actinomycin D only (xviii). Reversal of the order of administration, from actinomycin D then aldosterone, to aldosterone, then actinomycin D, resulted in antidiuresis, antinatriuresis, and kaliuresis. The Na:K excretory ratio was lowered, as was the GFR (xx).

Discussion. The results indicate that, though deprived of hypophyseal–adrenal axial regulation, the hypophysectomized rat responds to sodium deprivation with a reduction in sodium excretion that is associated with a reduction in GFR. Micropuncture results from this laboratory (unpublished data) indicate that the reduction in GFR may permit increased fractional proximal reabsorption with consequent reduction of distal load. As it has been reported that the hypophysectomized rat does not increase aldosterone production (4, 5) on sodium deprivation, it appears likely that the enhancement of sodium reabsorption is secondary to a decrease in filtered load.

The refractoriness of the sodium-replete hypophysectomized rat to exogenous aldosterone, as well as the anomalous response of the sodium-depleted rat are more obscure observations. On the face of it, the refractory response suggests an inability on the part of the hypophysectomized rat to respond normally to the administration of aldosterone. That this is, in fact, not the case under all circumstances has been indicated in previous publications which reported the usual sodium-saving action of aldosterone (1, 3). Further, refractoriness *per se* would not explain the observed natriuresis and diuresis of the sodium-deprived hypophysectomized rat. Rather, these observations suggest that, under certain conditions in the hypophysectomized rat, aldosterone may exert an effect other than its effect on the sodium reabsorptive mechanism.

The response to actinomycin D in both the

sodium-depleted and the sodium-replete hypophysectomized rats involved an elevation of GFR. This indicates a particular sensitivity to the antibiotic among the hypophysectomized, as no such response occurred in the unoperated rats. While the means by which actinomycin D increases GFR in the hypophysectomized rat is not clear, it may be a direct consequence of the ability of the antibiotic to blockade aldosterone-stimulated reactions. The evidence suggesting this relationship consists of the observation that, whereas the administration of aldosterone after actinomycin D did not affect the actinomycin D-stimulated increase in GFR, the administration of aldosterone before actinomycin D did largely blunt the increase in GFR. It is also noted with interest that aldosterone alone slightly lowered GFR in the sodium-deprived hypophysectomized rat. These last two observations suggest that in the hypophysectomized rat, the decreased GFR attending sodium deprivation may involve sensitization to a GFR-depressing function of aldosterone which is not observed in the unoperated rat.

The time course of response to aldosterone in the hypophysectomized, sodium-deprived rat indicates that aldosterone promotes "normalization" of the handling of an infused salt and water load. That is, the response of both the unoperated, sodium-deprived rat and the hypophysectomized, sodium-replete rat to the experimental infusion includes a tendency to increase sodium and water excretion through the course of the experiment. If this response is taken as "normal," then the hypophysectomized, sodium-deprived rat may be considered as showing a deficiency in failing to excrete the load in incremental fashion. This deficiency is alleviated by the administration of aldosterone, at least qualitatively, in that the response to aldosterone involves a return to a pattern of increasing rate of excretion of salt and water with time. From this point of view, the response of the sodium-deprived, hypophysectomized rat no longer appears entirely anomalous. It appears that the hormone exerts a function, apart from its sodium-saving function, which is homeostatic in nature in that it serves to facilitate excretion of an infused saline load. This response could possibly reflect a sensitization to aldosterone's glucocorticoid-like activity in the hypophysectomized, and therefore glucocorticoid-deficient, rat.

Summary. Renal excretory patterns of sodium, potassium and water were studied in unoperated and hypophysectomized rats as functions of pretreatment with *d*-aldosterone and actinomycin D. Observations included:

1. A GFR-depressing function of aldosterone in the sodium-depleted hypophysectomized rat.

2. A glucocorticoid-like response to aldosterone in the sodium-depleted hypophysectomized rat.

3. A GFR-stimulating function of actinomycin D, which was reversible by *a priori* but not by *a posteriori* administration of aldosterone.

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