Effect of Spironolactone on Spontaneous NaCl Intake of Adrenalectomized Rats.* (31650)

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Nearly 30 years ago, Richter(11) first showed that adrenalectomized rats ingested more NaCl solution than water if given a choice between the two. Under similar experimental conditions others have shown that administration of graded doses of the mineralocorticoid hormones, aldosterone, desoxycorticosterone acetate (DOCA) and 9-α-fluorocortisol, to adrenalectomized rats progressively reduced their elevated salt intake until a certain dose was reached(14,4). Administration of doses of hormone greater than this progressively returned intake of sale solution toward that of untreated, adrenalectomized rats. Thus, a U-shaped dose-response relationship was observed between NaCl intake and dose of mineralocorticoid administered. The specificity of the effect of mineralocorticoids on NaCl intake was tested by administration of cortisone acetate, testosterone propionate, estrone and thyroxine to adrenalectomized rats. None of these affected NaCl intake significantly(4,5). The present study is a further test of specificity of the mineralocorticoid effect on the NaCl intake of adrenalectomized rats. The mineralocorticoid inhibitor, spironolactone, which is known to inhibit the effect of DOCA on renal tubular reabsorption of sodium(6,13), is used and its efficacy in reversing the effect of DOCA on NaCl intake of adrenalectomized rats is tested.

Methods. Twenty-five male rats of the Carworth CFN strain weighing initially from 360 to 400 g were bilaterally adrenalectomized using pentobarbital for anesthesia (50 mg/kg intraperitoneally). After operation, all rats were given 0.15 M NaCl solution to drink and Rockland pellets to eat. Three weeks after operation the rats were placed in individual, round metabolism cages and given choice between distilled water and 0.15 M NaCl solution to drink. Fluid containers were infant nursing bottles with cast aluminum fountains as described by Lazarow(8). The positions of the 2 bottles on each cage were interchanged daily to avoid habit formation in selection of drinking fluid. Finely powdered Rockland Rat Diet was given in spillproof feeders described elsewhere in detail (2). Intakes were measured for 5 days prior to initiation of the control period to be certain that a spontaneous salt appetite was present. Only animals with an increased total fluid intake of which 75% or more was NaCl solution were used in this experiment.

Upon completion of the initial screening procedure, which was designed to eliminate partially adrenalectomized rats, the animals were divided randomly into 5 equal groups. The first group (5 rats) received 0.2 ml peanut oil subcutaneously daily. The second to fifth groups inclusive (5 rats each) received subcutaneously daily 50, 100, 200 and 400 μg desoxycorticosterone acetate (DOCA) per 100 g body weight dissolved in 0.2 ml peanut oil. Three days after initiation of the hormone treatment, a 4-day control period began. During this time body weight and individual daily intakes of water, 0.15 M NaCl solution and food (Rockland Rat Diet) were measured for 4 days. DOCA was administered daily throughout this control period as well as throughout all periods mentioned below.

At the completion of this control period, all rats were given Rockland Diet into which was thoroughly mixed 400 mg spironolactone per kg food. Measurement of intakes and body weight continued for another 4-day period at the end of which all rats were given Rockland Diet without spironolactone.

During the third 4-day period, (second control period) intakes and body weight were measured daily as described. At the end of this control period, all rats were given Rockland Diet into which was thoroughly mixed 800 mg spironolactone per kg food. Measurement of intakes and body weight continued

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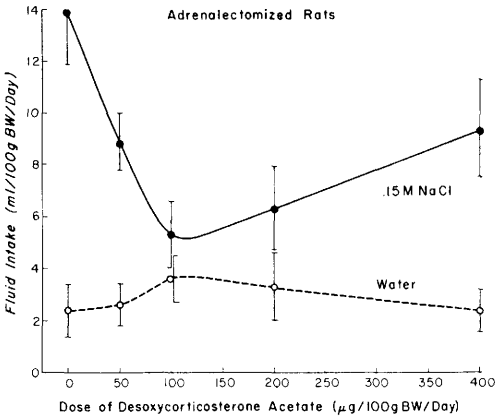

FIG. 1. Effect of administration of desoxycorticosterone acetate on intakes of water (0) and 0.15M NaCl solution (●) by adrenalectomized rats. ± one standard error is set off at each mean.

for the final 4-day period of the experiment.

These experiments were set up according to a randomized statistical design so that an analysis of variance could be used to aid in interpretation of the data(12).

Results. The effect of administration of DOCA at graded dose levels on the spontaneous intakes of 0.15 M NaCl solution and water by adrenalectomized rats is shown in Fig. 1. The reduction in intake of NaCl solution, reaching a minimum when approximately 100 μg DOCA/100 g body weight/day was administered, has been described(4). Also described earlier is the tendency for NaCl intake to return toward the level of untreated controls when doses of DOCA greater than 100 μg/100 g body weight/day are administered. Water intake tends to be the mirror image of NaCl intake and is of less magnitude because the total fluid intake of adrenalectomized rats under these conditions consists mainly of NaCl solution.

A compound which successfully blocks the effects of all the dose levels of DOCA shown in Fig. 1 would be expected to yield intakes of NaCl solution similar to that of the untreated adrenalectomized controls. Such a compound would thus be expected to reduce the U-shaped dose-response curve of Fig. 1 to a straight line. Hence, high doses of spironolactone were administred simultaneously with DOCA in an attempt to achieve this effect.

We attempted to reduce experimental variability in our design of the experiment by comparing intake of each rat during a control period with its intake during the following treatment period. The difference is expressed as percent change from pre-spironolactone control period. This procedure is also consistent with the method of expression of change used in our earlier studies and lends itself well to analysis of variance.

The relationship between percentage change in intake of 0.15 M NaCl solution and dose of DOCA administered during simultaneous treatment with spironolactone may be represented by a curve with a maximum somewhere between 100 and 200 μg DOCA/100 g body weight/day (Fig. 2A). The shape of the curve obtained during simultaneous administration of graded doses of DOCA and

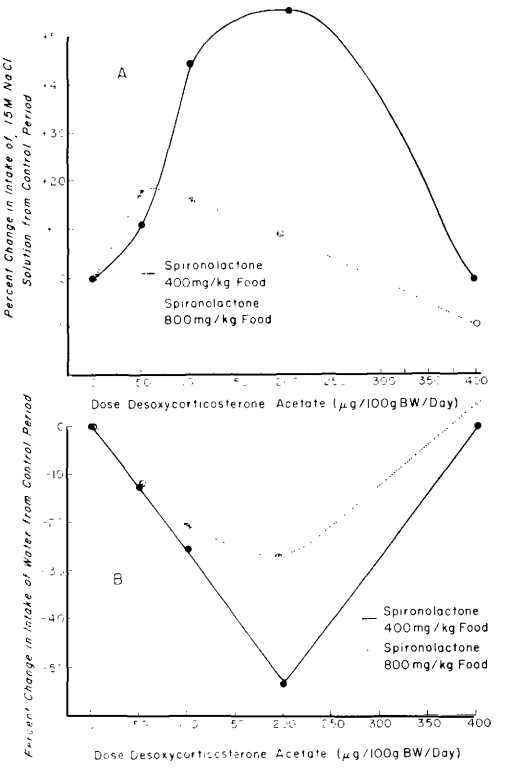

FIG. 2A. Effect of spironolactone administered in food on percent change in intake of 0.15M NaCl solution from control period by adrenalectomized rats given graded doses of desoxycorticosterone acetate. 2B. Effect of spironolactone on percent change in intake of water from control period by the same rats shown in A.

TABLE I. Effect of Dietary Administration of Spironolactone (400 mg/kg Food) on Spontaneous Intakes of 0.15M NaCl Solution, Water and Food by Adrenalectomized Rats Receiving Graded Dose Levels of Desoxycorticosterone Acetate.

Daily drug dose	Days given drug	Measurements made	Control period (ml or g/100 g BW/day)	Experimental period (ml or g/100 g BW/day)	Mean % change from control period
Adrex + oil	4	.15M NaCl soln. intake	13.9	11.9	−14.5
		Water intake	2.4	3.8	+58.5
		Food intake	6.8	6.5	− 4.9
		Δ Body wt (g/4 days)			+ 3
Adrex + 50 μg DOCA/100 g BW	4	.15M NaCl soln. intake	8.9	8.5	− 4.4
		Water intake	2.6	3.5	+35.7
		Food intake	6.2	5.7	− 7.7
		Δ Body wt (g/4 days)			+ 6
Adrex + 100 μg DOCA/100 g BW	4	.15M NaCl soln. intake	5.3	6.9	+30.0
		Water intake	3.6	4.8	+32.9
		Food intake	6.1	5.3	−12.3
		Δ Body wt (g/4 days)			+ 5
Adrex + 200 μg DOCA/100 g BW	4	.15M NaCl soln. intake	6.3	8.9	+41.4
		Water intake	3.3	3.5	+ 5.2
		Food intake	5.8	5.7	− 1.8
		Δ Body wt (g/4 days)			+ 2
Adrex + 400 μg DOCA/100 g BW	4	.15M NaCl soln. intake	9.4	8.0	−14.8
		Water intake	2.4	3.8	+58.7
		Food intake	5.9	5.8	− 1.3
		Δ Body wt (g/4 days)			+ 2

Analysis of variance (mean % change):

Source	df	.15M NaCl soln. MSS	F	P	Water MSS	F	P	Food MSS	F	P
Treatment	4	3176.26	4.42	<.01	2444.35	1.05	>.05	104.71	.36	>.05
Linear	1	1017.90	1.42	<.05						
Quadratic	1	7381.05	10.27	<.01						
Error	20	718.86			2323.88			287.36		
Total	24									

400 mg spironolactone per kg food (Fig. 2A) can be explained on the basis of the fact that intake of NaCl solution (ml/100 g body weight/day) increased relation to controls at all doses of DOCA administered with the exception of the highest dose. Administration of spironolactone tended to flatten the NaCl intake curve shown in Fig. 1. For this reason, the greatest percentage change in NaCl intake from control level occurred at doses of DOCA ranging from 100 to 200 μg/100 g body weight/day. In contrast, the percentage change in water intake accompanying administration of spironolactone was V-shaped but was approximately a mirror image of the effect on NaCl intake (Fig. 2B).

Table I gives mean percentage changes in intakes of 0.15 M NaCl solution, water and food for all groups during the period 400 mg spironolactone was mixed into each kg of food. There is a significant ($P<0.01$) effect of treatment with 400 mg spironolactone/kg food on percentage change in intakes of 0.15 M NaCl solution detectable by analysis of variance. The relationship between drug dose and percentage change in intake of 0.15 M NaCl solution can be expressed by a quadratic regression. Use of the pooled variance and "t" test reveals that only the 100 and 200 μg doses of DOCA differ significantly ($P<0.05$) from control(12). There is no significant effect of treatment on percentage change in either water or food intake. The amount of spironolactone ingested daily by the rats was calculated from their food intake and was approximately 8 mg/100 g body weight.

Increasing the level of spironolactone to 800 mg/kg food was also accompanied by an increase in percentage change in NaCl intake but of lesser magnitude than that of the lower

TABLE II. Effect of Dietary Administration of Spironolactone (800 mg/kg Food) on Spontaneous Intakes of 0.15M NaCl Solution, Water and Food by Adrenalectomized Rats Receiving Graded Dose Levels of Desoxycorticosterone Acetate.

Daily drug dose	Days given drug	Measurements made	Control period (ml or g/100 g BW/day)	Experimental period (ml or g/100 g BW/day)	Mean % change from control period
Adrex + oil	4	.15M NaCl soln. intake	13.5	11.7	—13.0
		Water intake	2.3	2.8	+19.9
		Food intake	6.6	6.4	— 2.8
		Δ Body wt (g/4 days)			+ 4
Adrex + 50 μg DOCA/100 g BW	4	.15M NaCl soln. intake	7.3	7.7	+ 5.0
		Water intake	2.9	3.1	+ 5.2
		Food intake	5.8	5.9	+ 2.2
		Δ Body wt (g/4 days)			+ 2
Adrex + 100 μg DOCA/100 g BW	4	.15M NaCl soln. intake	3.3	3.4	+ 3.0
		Water intake	4.1	4.1	— .5
		Food intake	5.7	5.6	— 2.1
		Δ Body wt (g/4 days)			0
Adrex + 200 μg DOCA/100 g BW	4	.15M NaCl soln. intake	8.4	8.1	— 3.7
		Water intake	2.6	2.4	— 7.3
		Food intake	6.1	5.7	— 6.9
		Δ Body wt (g/4 days)			+ 3
Adrex + 400 μg DOCA/100 g BW	4	.15M NaCl soln. intake	9.0	6.8	—24.9
		Water intake	2.7	2.0	+24.5
		Food intake	5.9	5.6	— 5.1
		Δ Body wt (g/4 days)			+ 1

Analysis of variance (mean % change):

Source	df	.15M NaCl soln. MSS	F	P	Water MSS	F	P	Food MSS	F	P
Treatment	4	763.77	.80	>.05	907.18	.31	>.05	59.25	.50	>.05
Error	20	956.95			2917.63			118.37		
Total	24									

level (Fig. 2A). The reduction in percentage change in water intake was also less in magnitude than that observed with 400 mg/kg (Fig. 2B). Table II reveals that the effect of the treatment on percentage change in intakes of NaCl solution, water and food was not significant statistically. The daily amount of spironolactone ingested by these rats was approximately 16 mg/100 g body weight.

Discussion. The spontaneous NaCl intake of adrenalectomized rats is affected by administration of DOCA. A U-shaped dose-response relationship exists between intake and dose of DOCA administered (Fig. 1). A similar relationship was demonstrated earlier by Wolf(14) and by us(4,5). On the basis of similar studies using aldosterone, we have interpreted the shape of the curve to suggest that: (a) intake of NaCl solution is controlled by the same hormone controlling renal sodium loss and (b) intact rats normally control NaCl intake near the minimal portion of the curve since their daily aldosterone secretion rate is similar to the dose of aldosterone required to reduce NaCl intake of adrenalectomized rats to a minimal level(3). Thus, either an increase or a decrease in aldosterone secretion rate may be expected to increase NaCl intake above the level observed in the normal rat. The shape of the curve also explains the opposite response of adrenalectomized and intact rats to injections of the same dose of DOCA. Adrenalectomized rats reduce(11), while intact rats increase, their NaCl intake when 1.0 to 2.5 mg DOCA is administered(1,9). The different response to the same dose levels of DOCA apparently depends on the initial position of the rat on the dose-response curve.

The specificity of the salt intake response to mineral corticoids was tested by administration of other steroid hormones(4,5). It appears at present that only those hormones with mineralocorticoid-like properties influ-

ence the spontaneous NaCl intake of adrenalectomized rats. Cortisone acetate, testosterone propionate, estrone and thyroxine were tested earlier and shown to be without effect. The present study, designed as a further test of the specificity of the mineralocorticoid effect, utilized spironolactone, the mineralocorticoid antagonist(7,13). Simultaneous administration of both DOCA and spironolactone to adrenalectomized rats altered the shape of the NaCl intake curve observed with DOCA alone by returning NaCl intake toward that of untreated, adrenalectomized controls (Fig. 1). Thus when spironolactone was administered to DOCA-treated rats, a greater percentage increase in NaCl intake was observed for those dose levels of DOCA which initially reduced NaCl intake to the greatest extent (Fig. 2).

The smaller effect of the higher dose of spironolactone on percentage change in NaCl intake may be related to the mineralocorticoid-like effect of this class of compounds reported to occur at doses exceeding 9 mg/day(7). The DOCA-like effect is also apparent from data presented in Table I and II in which it can be seen that spironolactone decreased spontaneous NaCl intake of otherwise untreated, adrenalectomized rats by 13 to 15%.

The effect of spironolactone in increasing the NaCl intake of DOCA-treated, adrenalectomized rats is considered to be a further suggestion that the mineralocorticoid effect on NaCl intake is specific.

Summary. The increased intake of 0.15 M NaCl solution by adrenalectomized rats was reduced progressively by subcutaneous administration of increasing doses of the mineralocorticoid hormone, desoxycorticosterone acetate (DOCA). When the administered dose exceeded 100 μg/100 g body weight/day, the spontaneous NaCl intake returned toward that of control, adrenalectomized rats. Thus, a U-shaped dose-response relationship was observed between NaCl intake and dose of DOCA administered. Dietary administration of spironolactone, the mineralocorticoid antagonist (400 mg/kg food), simultaneously with graded doses of DOCA, inhibited the effect of DOCA on spontaneous NaCl intake of adrenalectomized rats. The results of this experiment provide further indirect evidence for the specificity of control of spontaneous NaCl intake by blood level of mineralocorticoid hormone.

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