Aldosterone and Growth Hormone: Influence of Diet and Hypophysectomy on Rat Renal Response[1] (34564)

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Several recent reports have indicated that either growth hormone or some other principle(s) derived from the hypophysis must be present for aldosterone to exert its classical antinatriuretic effect in the rat (1–3). However, profound aldosterone-induced antinatriuresis has been reported in the hydropenic, hypophysectomized rat (4). This finding suggests that hypophyseal hormones need not be present for aldosterone to exert a powerful antinatriuretic effect. It suggests, rather, that the renal response to aldosterone may be conditioned by the water and electrolyte status of the test animal. Evidence supporting this hypothesis is presented below, along with further observations on some features of the renal interactions of aldosterone and growth hormone.

Methods and Materials. Male albino rats were used in all studies. Hypophysectomy was accomplished by retropharyngeal approach (Hormone Assay Labs). All rats were maintained on their respective diets for 7 preexperimental days. The high sodium diet ($>$ 0.13 meq of Na/g of mixture) was Purina lab chow, mixed with condensed milk. The low sodium diet ($<$ 0.002 meq of Na/g of mixture) was obtained from Nutritional Biochemicals and was mixed 1:1 with distilled water. Animals accustomed to handling and gavage were given 2.5 % of their body weight of distilled water by gavage, 2 hr after carrier or hormone injection (i.m., 0.2 ml). Urine was collected under oil for the next 2 hr, with a clearing of the bladder by gentle suprapubic pressure at the beginning and end of each collection period. Autopsy was performed and hypophyseally-dependent organ weights were taken to confirm the absence of the hypophysis.

Experiments were arranged as a series of crossover tests with a minimum of 72 hr between trials.

Urinary sodium and potassium concentrations were determined by flame photometry (Baird). D-Aldosterone was obtained from Ciba. Porcine growth hormone was obtained from Nutritional Biochemicals. Results were analyzed for significance of differences between the means by Student's t test; p values of less than 0.05 were assigned statistical significance.

Results. Unoperated rats, high sodium diet. Aldosterone was antinatriuretic and antidiuretic in unoperated, water-loaded rats. The hormone also lowered the sodium: potassium excretory ratio. Thus, the response was the one now classically associated with aldosterone administration. The administration of growth hormone alone resulted in antidiuresis. The combined administration of growth hormone and aldosterone resulted in summative antinatriuresis, antidiuresis, and lowering of the sodium to potassium excretory ratio when a high dosage (50 μg/100 g of rat) of aldosterone was administered (Table I).

Hypophysectomized rats, high sodium diet. Aldosterone was profoundly antinatriuretic and antidiruetic in hyophysectomized, water-loaded rats maintained on a diet containing a significant quantity of sodium. The hormone also lowered the sodium:potassium excretory ratio. Growth hormone was antinatriuretic, antikaliuretic and antidiuretic. The combined administration of aldosterone and growth hormone resulted in profound, summative anti-

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TABLE I. Renal Responses to Aldosterone (Aldo) and Growth Hormone (GH) as Influenced by Hypophysectomy and Diet.

Hormone (μg/100 g rat)	n	Excretion/100 g/hr[a] V (ml)	Na (μeq)	K (μeq)	Na/K
		Unoperated, high sodium diet			
None	19	1.58 ± 0.18	12.9 ± 3.29	27.3 ± 11.08	0.47 ± 0.13
Aldo, 50	17	0.88 ± 0.13[b]	5.6 ± 1.57[b]	31.1 ± 6.29	0.18 ± 0.06[b]
GH, 100	17	0.73 ± 0.28[b]	11.7 ± 3.13	13.4 ± 3.32	0.81 ± 0.06[b]
Aldo, 4 + GH, 100	12	0.59 ± 0.13[b]	9.4 ± 2.65	39.0 ± 8.17	0.24 ± 0.04
Aldo, 50 + GH, 100	10	0.43 ± 0.01[b]	1.2 ± 0.74[b]	39.2 ± 19.91	0.03 ± 0.01[b]
		Hypophysectomized, high sodium diet			
None	28	0.88 ± 0.09	32.7 ± 6.26	16.7 ± 2.65	1.96 ± 0.46
Aldo, 4	17	0.54 ± 0.10[b]	6.0 ± 2.56[b]	14.6 ± 3.41	0.41 ± 0.06[b]
GH, 100	21	0.56 ± 0.08[b]	6.8 ± 1.62[b]	5.8 ± 1.07[b]	1.18 ± 0.32
Aldo, 4 + GH, 100	16	0.72 ± 0.09	1.5 ± 0.23[b]	13.6 ± 1.75	0.11 ± 0.04[b]
		Hypophysectomized, low sodium diet			
None	30	0.57 ± 0.07	1.5 ± 0.60	20.4 ± 2.57	0.07 ± 0.04
Aldo, 4	30	0.80 ± 0.10[b]	1.4 ± 0.41	21.4 ± 2.19	0.07 ± 0.02
GH, 100	12	0.35 ± 0.07[b]	0.4 ± 0.05[b]	11.9 ± 2.52[b]	0.03 ± 0.01
Aldo, 4 + GH, 100	12	0.52 ± 0.08	0.5 ± 0.09	15.1 ± 2.09	0.04 ± 0.01

[a] Values are the means ± SEM; p derives from application of Student's t test to the differences between the means.

[b] $p < 0.05$.

natriuresis and lowering of the sodium:potassium excretory ratio.

Hypophysectomized rats, low sodium diet. In the face of very low control sodium excretory rates, aldosterone alone failed to significantly influence sodium excretory rates though its administration was associated with diuresis. Growth hormone, contrarily, was antinatriuretic, antikaliuretic, and antidiuretic. The combination of growth hormone and aldosterone did not lead to any decreased sodium excretion.

Discussion. The observation of principal interest in this report involves the marked effect of previous salt intake on the response to aldosterone in the hypophysectomized rat. Those hypophysectomized rats maintained on a high sodium diet responded to aldosterone with a profound antinatriuresis. In fact, the same animals showed a greater sensitivity, in terms of fractional decrease in sodium excretory rate, to 4 μg of aldosterone than did the unoperated rats to 50 μg. This observation is not consonant with the observations of Lockett and Roberts which led to their statment that ". . . the sodium-retaining action of aldosterone is dependent on the presence of growth hormone. . ." (2). The results are also in contrast to those reported by Croxatto *et al.*, which the authors found to be ". . . in agreement with the conclusion of Lockett and Roberts that the Na retaining effect of aldosterone requires the presence of growth hormone." (3)

On the other hand, the results in the sodium-restricted hypophysectomized rats are in better agreement with the observations of Lockett and Roberts (2) and Croxatto *et al.* (3) in that aldosterone failed to produce an antinatriuretic effect while producing a diuretic one.

When these observations are considered together with the observation of Bauman and Earls that ". . . aldosterone. . . exerted a profound sodium retaining effect in the [hypophysectomized][2] hydropenic rat." (4), it seems clear that neither the hypophysis nor growth hormone need be present for aldos-

[2] Authors' insertion.

terone to exert its classical, antinatriuretic effect. Still, under some conditions, the hypophysectomized rat appears refractory to the sodium-retaining action of aldosterone. That this refractoriness does not derive from addition of hormone to high endogenous levels which might be exerting maximal effect is indicated by the *in vivo* studies of Palmore and Mulrow (5) and the *in vitro* studies of Lee *et al.* (6). These workers found that salt deprivation in the hypophysectomized rat does not stimulate aldosterone production, and that blood levels are lower than in unoperated rats. Consideration of the observations here reported, along with those cited above, suggest that the response to aldosterone administration in the hypophysectomized rat is preconditioned by its water and electrolyte status. Differences in that status may be responsible for the differing results of different laboratories. The control sodium excretory rates reported in the studies of Lockett and Roberts ranged from 2.6 ± 1.11 to 8.2 ± 2.37 μeq/100 g/hr (1). These values are low compared to the high salt diet controls in our study (32.76 ± 6.26). Presumably, these differences in excretory rate reflect different states of sodium and water balance. The observations of Croxatto *et al.* (3) derived from experiments on animals previously maintained with DOCA and 0.5 % saline, which were challenged with 5 % of body weight of 0.5 % saline. Such animals should be in a different state of sodium and water balance than those reported in this paper where maintenance and challenge were without hormone or saline. The animals of Bauman and Earls (4) had been made hydropenic and were presumably in yet another state of salt and water balance.

Clearly, the data do not permit speculation as to the nature of the mechanism leading to the natriuretic and/or diruetic response to aldosterone which is sometimes seen in the hypophysectomized rat. However, it does not appear to be a function of hypophysectomy nor the absence of hypophyseal hormones per se.

The proposition that growth hormone itself may be antinatriuretic and antidiuretic is borne out by these experiments. Micropuncture results (unpublished) from this laboratory indicate the presence of a proximal tubular site for growth hormone-stimulated sodium reabsorption. This finding confirms the stop-flow findings of De Lima and Lockett (7).

Hence, the summative effects of growth hormone and aldosterone on sodium and water reabsorption do not appear to mean that aldosterone is dependent on growth hormone in order to exert its antinatriuretic and/or diuretic functions. The two hormones might summate, when they do, by operating on separate mechanisms either in parallel along the nephron, or perhaps serially, with a predominance of growth hormone activity proximally, and a predominance of aldosterone activity distally. Alternately, growth hormone might influence an underlying factor governing the operational capacity of the kidney for both nonaldosterone-dependent and aldosterone-dependent reabsorption. This could explain growth hormone's ability to stimulate reabsorption alone, to summate in aldosterone-induced reabsorption, and to reverse the direction of aldosterone-induced natriuresis and diuresis. Further work seeking to elucidate the nature of the interaction is in progress in this laboratory.

Summary. Experiments in hypophysectomized rats indicated that though growth hormone and aldosterone could produce summative antinatriuresis when given together, neither growth hormone nor the hypophysis appeared necessary for aldosterone to exert a profoundly antinatriuretic effect. Previous dietary history, however, may modify the response of the hypophysectomized rat to aldosterone. In sodium-replete rats, the response was antinatriuretic and antidiuretic, while in sodium-depleted rats, the response was diuretic and did not significantly modify sodium excretion.

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