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SOME EFFECTS OF GROWTH HORMONE ON WATER DIURESIS IN RATS

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Intramuscular injections of aldosterone increased the urinary excretion of sodium and the rate of elimination of a water load by hypophysectomized rats. The sodium retaining action of aldosterone could, however, be restored in these animals either by the administration of growth hormone, or by adrenalectomy, or by removal of that part of the diencephalon which underlies the pineal stalk (Lockett & Roberts, 1963*a*).

The present work is a study of the modification of the renal effects of growth hormone by hypophysectomy in rats.

METHODS

Female Wistar rats, 140–180 g, drank freely and were fed on diet 86 (M.R.C. Animal Laboratory Bureau, 1952); this was crushed and was made into a stiff mash for all hypophysectomized animals. The drinking water supplied to adrenalectomized animals contained 0·6 % NaCl. All were maintained at a room temperature of 23–25° C.

Operations were performed under light pentobarbitone anaesthesia, deepened with ether as necessary. The transpharyngeal route was used for hypophysectomy (Burn, 1952); a mid-dorsal incision was employed for adrenalectomy. The usual operation for *total hypophysectomy* was modified: the sharp edge of the capillary sucker was held against the adenohypophysis in such a way that approximately one-sixth of the adenohypophysis was cut off and remained detached within the pituitary fossa as the rest of the gland was removed. This small portion of the adenohypophysis presumably atrophied, for the adrenal weights fell to one third of normal in 3 weeks (Table 1). The advantages of this modification in technique were great, for adequate water diurises were sustained for long enough to permit the use of cross-over tests, from the fifth to the fifteenth day, for measurement of the effect of hormones. In this and the following paper, *subtotal hypophysectomy* implies that this operation was used. *Neurohypophysectomy* was effected by passing a fine capillary sucker through the right side of the adenohypophysis before very gentle suction was applied; this suction was just sufficient to remove the posterior lobe but almost always produced slight bleeding. Four weeks later, however, these animals showed brisk responses to water loads, a fivefold increase in sensitivity to the antidiuretic hormone and a water intake greatly in excess of normal. All experiments were conducted during this phase, and ended in the middle of the third post-operative month. Sensitivity to the antidiuretic hormone had begun to decrease by the end of the third month, probably because that residual part of the pars nervosa which extends into the infundibular process had hypertrophied. These

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animals were subjected to post mortem examination: their adrenal glands had not atrophied (Table 1) for their weights fell in the upper part of the normal range.

Rats were accustomed to handling before use. Each experiment was designed as a series of cross-over tests in which every animal received each treatment in individual sequence determined by deliberate randomization; equal numbers of each treatment were allotted to each day. Tests were made every other day and began with a 2 hr period of starvation during which rats were deprived of solid food. The oral water load, equivalent to 2·5 % body weight, was given at the end of the starvation period immediately before each animal was put into a separate cage for the collection of all urine entering the bladder in the next hour. This collection period was extended to 2 hr for all adrenalectomized animals. Since, after practice periods, almost every rat micturated spontaneously when held gently and

TABLE 1. Weights of pairs of adrenal glands from normal and operated rats. The values shown for adrenal and body weights are means ± the standard errors of these means

Operated state	No. of rats	Post-operative time (days)	Body weight (g)	Adrenal weights (mg/100 g body weight)
Unoperated	19	—	149 ± 2·18	30·9 ± 1·24
Subtotally hypophysectomized	11	26	175 ± 4·24	11·1 ± 0·98
Neurohypophysectomized	11	87	177 ± 5·92	33·4 ± 1·19

firmly under restraint for administration of a water load, it was necessary to use suprapubic pressure to empty the bladders solely to terminate urinary collections, Since the rate of excretion of sodium by rats has been 2·1 ± 0·23 (11) times as great at 11 a.m. as at 4 p.m. and has fallen continuously throughout the day, all cross-over tests which have constituted single experiments were made at a time of day fixed for each experiment.

Injections of D-aldosterone (Ciba Laboratories Ltd.) in 0·1 ml. arachis oil, or of pure bovine growth hormone (kindly supplied by Professor Wilhelmi) freshly dissolved in 0·1 ml. 0·9 % NaCl which had been brought to pH 9·0, were made intramuscularly 2 hr before administration of the water load. In some experiments 3 ml. 5 % inulin in 0·9 % NaCl was injected subcutaneously into each rat 1 hr before hydration (Botting, Farmer & Lockett, 1961).

The diphenylamine method was used for the estimation of inulin (Chasis, Ranges, Goldring & Smith, 1938), and concentrations of sodium and potassium in urine were determined by means of an EEL flame photometer.

RESULTS

The influence of growth hormone on the excretion of water, sodium and potassium during water diuresis in normal, subtotally hypophysectomized and neurohypophysectomized rats. Growth hormone alone usually caused a small decrease in the urine volume and in the excretion of sodium and potassium by rats during water diuresis (Table 2). In normal animals (Expts. 1–3), adrenalectomized (Expts. 4–6) and neurohypophysectomized animals (Expt. 9) the urine volumes and rates of excretion of sodium and potassium fell to 70–80 % of the control values for each cross-over test in response to injections of 40, 80 and 160 μg of growth hormone. The effects were not obviously greater with the larger doses, and were, over-all, smaller in subtotally hypophysectomized (Expts. 7 and 8) than in normal animals.

TABLE 2. Actions of growth hormone on the urinary excretion of water and electrolytes by rats.

Operated state	No.	Body weight (g)	Urinary excretion/100 g rat/1 or 2 hr: Water (ml.)	Na (μ-equiv.)	K (μ-equiv.)	Na/K	Inulin (mg)	Treatment (μg bovine growth hormone/rat I.M.)		Expt. no.
			Rates/hr							
Unoperated	6	144 ± 2·3	1·91 ± 0·163	31·4 ± 7·62	16·8 ± 4·9	2·3 ± 0·39	—	None	water laden	1
			1·55 ± 0·136	36·9 ± 12·81	22·3 ± 6·8	1·6 ± 0·25**	—	40 μg		
			1·75 ± 0·258*	19·7 ± 9·17**	12·2 ± 3·0*	1·6 ± 0·51**	—	80 μg		
			1·42 ± 0·196*	24·7 ± 9·82	14·3 ± 5·7	2·0 ± 0·42	—	160 μg		
	6	144 ± 2·3	1·50 ± 0·140	583·6 ± 8·00	193·6 ± 2·2	3·2 ± 0·41	—	None	loaded with 4% NaCl	2
			1·23 ± 0·223	537·6 ± 7·64*	165·6 ± 1·0	3·2 ± 0·32	—	40 μg		
			1·23 ± 0·163*	538·8 ± 5·90*	183·4 ± 1·4	3·3 ± 0·33	—	80 μg		
			1·00 ± 0·132*	582·8 ± 3·44	189·4 ± 2·0	3·0 ± 0·28	—	160 μg		
	6	148 ± 2·5	1·11 ± 0·201	223·1 ± 10·81	82·1 ± 7·4	2·8 ± 0·37	35·0 ± 5·02	None	loaded with 1% NaCl	3
			1·00 ± 0·126	177·5 ± 8·85	67·3 ± 1·6	3·1 ± 0·50	39·0 ± 5·98	40 μg		
			0·60 ± 0·062**	114·7 ± 7·46*	44·9 ± 5·8*	2·9 ± 0·28	38·0 ± 4·62	80 μg		
			0·66 ± 0·164**	128·4 ± 11·74*	44·8 ± 12·3*	3·3 ± 0·69	29·7 ± 5·64	160 μg		
			Rates/2 hr							
Adrenalectomized (salt maintained)	6	140 ± 2·9	1·30 ± 0·139	122·2 ± 15·60	41·6 ± 4·5	2·6 ± 0·31	—	None		4
			1·40 ± 0·140	85·6 ± 14·83**	31·1 ± 7·9	2·3 ± 0·41*	—	40 μg		
			0·80 ± 0·211	60·3 ± 17·42**	26·8 ± 8·8	2·0 ± 0·29*	—	80 μg		
			0·95 ± 0·306	55·3 ± 24·49**	25·4 ± 9·1	2·4 ± 0·69	—	160 μg		
	6	149 ± 4·4	1·78 ± 0·275	127·5 ± 8·87	45·9 ± 5·8	3·0 ± 0·42	—	None		5
			1·06 ± 0·240	75·1 ± 9·81**	34·1 ± 7·9	2·2 ± 0·64*	—	20 μg		
			0·76 ± 0·228*	53·2 ± 17·31**	31·6 ± 5·7	1·7 ± 0·27*	—	40 μg		
			1·14 ± 0·300	91·3 ± 22·00	20·0 ± 4·1	2·8 ± 0·57	—	80 μg		
			1·32 ± 0·440	92·1 ± 19·72	36·2 ± 8·3	2·5 ± 0·11	—	160 μg		
	6	163 ± 5·7	1·58 ± 0·251	209·2 ± 24·73	53·7 ± 7·0	4·0 ± 0·15	26·4 ± 2·42	None		6
			1·39 ± 0·163	195·2 ± 29·99	59·7 ± 7·2	3·4 ± 0·15**	26·4 ± 0·69	40 μg		
			0·97 ± 0·115*	141·1 ± 24·78**	49·4 ± 7·6*	2·9 ± 0·24**	25·5 ± 0·68	80 μg		
			1·23 ± 0·180	143·5 ± 24·15**	52·0 ± 7·4*	2·6 ± 0·27**	27·5 ± 0·97	160 μg		
			Rates/1 hr							
Subtotally hypophysectomized	7	174 ± 5·0	1·60 ± 0·141	28·6 ± 4·96	18·4 ± 2·9	1·6 ± 0·24	—	None		7
			1·76 ± 0·180	24·5 ± 5·73	16·6 ± 3·1	1·5 ± 0·62	—	40 μg		
			1·40 ± 0·132	23·7 ± 4·89	16·2 ± 3·3	1·4 ± 0·40	—	80 μg		
			1·83 ± 0·122	23·1 ± 4·14	18·1 ± 2·4	1·4 ± 0·26	—	160 μg		
	6	177 ± 4·1	1·70 ± 0·187	22·1 ± 5·24	16·1 ± 3·1	1·4 ± 0·14	—	None		8
			1·52 ± 0·158	24·1 ± 4·87	17·0 ± 6·5	1·7 ± 0·28	—	40 μg		
			1·61 ± 0·196	17·2 ± 3·73	16·9 ± 4·7	1·3 ± 0·46	—	80 μg		
			1·71 ± 0·131	29·4 ± 5·74	19·5 ± 2·9	1·6 ± 0·25	—	160 μg		
Neuro-hypophysectomized	5	148 ± 3·1	1·84 ± 0·202	27·7 ± 5·65	22·2 ± 4·4	1·5 ± 0·41	—	None		9
			1·68 ± 0·261	21·4 ± 2·11	16·0 ± 4·2	1·8 ± 0·55	—	40 μg		
			1·36 ± 0·133*	15·6 ± 5·63*	16·8 ± 4·7	1·2 ± 0·26	—	80 μg		
			1·80 ± 0·111	10·3 ± 2·88**	12·2 ± 2·9**	0·9 ± 0·13*	—	160 μg		

The values shown are means ± the standard errors of these means. The significance of differences between means has been examined by *t* test and is indicated by asterisks; one, $P = < 0{\cdot}05$; two, $P = < 0{\cdot}01$.

Modification by growth hormone of the urinary actions of a fixed dose of aldosterone. Although 40–80 μg growth hormone had no significant action itself (Table 2, Expts. 7 and 8) on the urine excreted by subtotally hypophysectomized rats, these and lower doses converted the diuretic natriuretic effect of aldosterone in subtotally hypophysectomized animals into one of antidiuresis accompanied by retention of both sodium and potassium; the sodium:potassium ratio fell (Table 3, Expts. 10–12). Neurohypophysectomized animals (Expt. 13) responded to growth hormone in the presence of aldosterone as did subtotally hypophysectomized animals. Growth hormone, in doses of 20–80 μg, intensified the sodium retention and antagonized the extrusion of potassium caused by aldosterone in adrenalectomized rats (Expt. 14). This action of growth hormone was maximal at 40 μg and disappeared when the dose level had been raised to 160 μg.

DISCUSSION

The amounts of growth hormone used, 20–160 μg per 150 to 200 g body weight, are likely to have produced physiological concentrations (1·0–2·5 μg per ml., Contopoulos & Simpson, 1957) in the blood stream, for the molecular weight of growth hormone (bovine) was given as 47,886 (Li, Clauber, Fuss-Bech, Levy, Condliffe & Papkoff, 1955) and so large a molecule could not be expected to diffuse with great rapidity from intramuscular sites of injection. The reductions in the urinary excretion of water, sodium and potassium which are caused by 20–80 μg growth hormone in rats (Table 2) resemble the effects of single injections of growth hormone on the urine of man (Biglieri, Watlington & Forsham, 1961), and are attributable to direct effects of growth hormone on the renal tubular cells (Lockett & Roberts, 1963*b*), since they are also demonstrable on the isolated perfused kidney.

These doses of growth hormone converted the diuretic natriuretic effect of aldosterone in hypophysectomized (Lockett & Roberts, 1963*a*), subtotally hypophysectomized (Table 3) and neurohypophysectomized (Table 3) rats into an antidiuretic effect which was accompanied by retention both of sodium and potassium; the sodium:potassium ratio fell. Similarly, physiological concentrations of growth hormone (Lockett & Roberts, 1963*b*) replace the diuretic natriuretic action of aldosterone on the cat kidney perfused with blood from headless or hypophysectomized donors (Davey & Lockett, 1960) by the antidiuretic sodium and potassium retaining action of aldosterone observed in these preparations when they are perfused with blood from intact donor animals. The interaction of growth hormone and aldosterone in subtotally hypophysectomized and in neurohypophysectomized rats (Table 3) may therefore be considered to occur within the kidney.

TABLE 3. Modification of the renal actions of aldosterone by growth hormone (bovine) in adrenalectomized and in subtotally hypophysectomized rats

Operated state	No.	Body weight (g)	Urinary excretion/100 g rat/1 or 2 hr: Water (ml.)	Na (μ-equiv.)	K (μ-equiv.)	Na/K	Treatment/rat, by I.M. injection aldosterone (Ald.) growth hormone (GH)	Expt. no.
			Rates/hr					
Subtotally hypophysectomized	7	169 ± 3·4	1·93 ± 0·170	27·7 ± 2·57	16·7 ± 2·74	2·0 ± 0·28	None	10
			2·10 ± 0·107*	38·1 ± 2·97*	27·6 ± 3·82*	1·7 ± 0·21	4 μg Ald.	
			1·76 ± 0·202†	21·8 ± 3·08†	20·0 ± 4·77	1·3 ± 0·17	4 μg Ald. and 20 μg GH	
			1·71 ± 0·109†	10·0 ± 2·38††	14·4 ± 3·98††	0·9 ± 0·21†	4 μg Ald. and 40 μg GH	
			1·61 ± 0·138††	12·2 ± 2·72††	25·2 ± 2·71	1·1 ± 0·18†	4 μg Ald. and 80 μg GH	
			1·25 ± 0·492††	38·8 ± 11·50	47·8 ± 16·82	1·0 ± 0·10†	4 μg Ald. and 160 μg GH	
	6	145 ± 2·1	1·90 ± 0·312	47·2 ± 9·55	25·8 ± 5·80	1·9 ± 0·131	None	11
			1·99 ± 0·063	70·0 ± 6·15**	27·9 ± 5·98	2·9 ± 0·445*	4 μg Ald.	
			1·60 ± 0·303	35·6 ± 8·89††	19·2 ± 4·47†	2·0 ± 0·448	4 μg Ald. and 20 μg GH	
			1·73 ± 0·355	15·1 ± 3·41††	14·8 ± 3·92††	1·2 ± 0·302†	4 μg Ald. and 40 μg GH	
			1·62 ± 0·160†	19·8 ± 3·71††	19·0 ± 2·16†	1·3 ± 0·334†	4 μg Ald. and 80 μg GH	
			1·58 ± 0·376	41·6 ± 18·87†	23·9 ± 6·48	1·9 ± 0·498	4 μg Ald. and 160 μg GH	
	6	176 ± 4·2	1·97 ± 0·274	28·3 ± 6·32	16·2 ± 2·52	1·9 ± 0·231	None	12
			1·85 ± 0·187	38·4 ± 5·43*	22·6 ± 3·76*	1·8 ± 0·262	4 μg Ald.	
			1·85 ± 0·173†	30·4 ± 8·12†	19·0 ± 3·16	1·8 ± 0·480	4 μg Ald. and 20 μg GH	
			1·62 ± 0·204†	12·2 ± 4·24††	14·3 ± 2·52††	0·9 ± 0·681††	4 μg Ald. and 40 μg GH	
			1·47 ± 0·163†	14·8 ± 4·12††	17·3 ± 2·33†	0·8 ± 0·495††	4 μg Ald. and 80 μg GH	
			1·40 ± 0·204††	41·7 ± 9·27	41·0 ± 12·78††	1·6 ± 0·472	4 μg Ald. and 160 μg GH	
Neuro-hypophysectomized	7	164 ± 4·6	1·77 ± 0·158	32·6 ± 2·68	18·3 ± 2·66	2·1 ± 0·252	None	13
			2·02 ± 0·133*	45·2 ± 6·79**	23·5 ± 6·70	2·6 ± 0·511	4 μg Ald.	
			1·54 ± 0·218*†	20·8 ± 5·70††	14·0 ± 3·43†	1·5 ± 0·203†	4 μg Ald. and 20 μg GH	
			1·73 ± 0·266†	9·2 ± 2·78††	7·8 ± 2·24††	1·3 ± 0·252†	4 μg Ald. and 40 μg GH	
			1·78 ± 0·146†	14·1 ± 1·60††	13·7 ± 2·93†	1·3 ± 0·282†	4 μg Ald. and 80 μg GH	
			1·63 ± 0·320††	27·2 ± 4·01††	27·2 ± 6·96	1·3 ± 0·311†	4 μg Ald. and 160 μg GH	
			Rates /2 hr					
Adrenalectomized	6	154 ± 4·1	0·87 ± 0·382	86·6 ± 13·10	28·5 ± 5·91	3·7 ± 0·872	None	14
			1·13 ± 0·144*	36·3 ± 8·98**	44·9 ± 6·29*	1·0 ± 0·259**	4 μg Ald.	
			0·90 ± 0·188	22·9 ± 5·11†	33·9 ± 2·62	0·7 ± 0·150	4 μg Ald. and 20 μg GH	
			0·61 ± 0·211†	16·2 ± 4·45†	35·2 ± 8·33	0·6 ± 0·357	4 μg Ald. and 40 μg GH	
			0·61 ± 0·267†	23·3 ± 8·87†	37·4 ± 12·73	0·8 ± 0·321	4 μg Ald. and 80 μg GH	
			1·03 ± 0·060	51·1 ± 14·03†	45·0 ± 11·63	1·3 ± 0·222	4 μg Ald. and 160 μg GH	

The values shown are means ± their standard errors. Test for significance of differences between means, as for Table 1. Significant differences are indicated by asterisks for the effect of aldosterone and daggers for modification of the action of aldosterone by growth hormone: one, $P = < 0{\cdot}05$; two, $P = < 0{\cdot}01$.

It is well known that the sodium retention caused by aldosterone in normal and in adrenalectomized animals is accompanied by an increase in the rate of excretion of potassium. By contrast, the sodium retention caused by aldosterone in subtotally hypophysectomized rats which have received growth hormone (Table 3) is accompanied by retention of potassium. Search for an adenohypophyseal hormone which permits aldosterone to stimulate exchange of sodium for potassium, predominantly in the distal nephron, is therefore in progress.

SUMMARY

1. Growth hormone, 40–80 μg, caused retention of water, sodium and potassium without change in urinary sodium:potassium ratios during water diuresis in normal, neurohypophysectomized and adrenalectomized rats. These effects of growth hormone appeared reduced in subtotally hypophysectomized animals.

2. The diuretic, natriuretic and kaluretic action of aldosterone in both neurohypophysectomized and subtotally hypophysectomized rats was converted by 20–40 μg growth hormone to an antidiuretic effect accompanied by retention both of sodium and potassium.

3. Growth hormone, 20–80 μg, intensified the sodium retention and antagonized the potassium extrusion caused by aldosterone in adrenalectomized rats.

4. Increase in these doses of growth hormone decreased the modification of the effects of 4 μg aldosterone described in 2 and 3.

REFERENCES

Biglieri, E. G., Watlington, C. O. & Forsham, P. H. (1961). Sodium retention with human growth hormone and its subfractions. *J. clin. Endocrinol.* **21**, 361–370.

Burn, J. H. (1952). *Biological Standardization*, 2nd ed. P. 272. Oxford: University Press.

Botting, R., Farmer, J. B. & Lockett, M. F. (1961). The effect of subcutaneous adrenaline, noradrenaline and isoprenaline on the excretion of electrolytes by rats. *Arch. int. Physiol. Biochem.* **69**, 203–211.

Contopoulos, A. N. & Simpson, M. E. (1957). Increased growth promoting substance in the plasma of pregnant rats. *Endocrinol.* **61**, 765–773.

Chasis, H., Ranges, H. A., Goldring, W. & Smith, H. W. (1938). The control of renal blood flow and glomerular filtration in normal man. *J. clin. Invest.* **20**, 655–662.

Davey, M. J. & Lockett, M. F. (1960). Actions and interactions of aldosterone monacetate and neurohypophysial hormones on the isolated cat kidney. J. Physiol. **152**, 206–219.

Li, C. H., Clauber, H., Fuss-Bech, P., Levy, A. L., Condliffe, P. G. & Papkoff, H. (1955). Hypophyseal growth hormone as a protein. In *The Hypophyseal Growth Hormone, Nature and Actions*. Pp. 70–90. Ed. R. W. Smith, O. H. Gabler and C. N. H. Long, New York: McGraw Hill Book Co. Inc.

Lockett, M. F. & Roberts, C. N. (1963*a*). Hormonal factors affecting sodium excretion in the rat. *J. Physiol.* **167**, 581–590.

Lockett, M. F. & Roberts, O. N. (1963*b*). Some actions of growth hormone on the perfused cat kidney. *J. Physiol.* **169**, 879–888.

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