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THE EFFECTS OF DIETARY SODIUM RESTRICTION AND POTASSIUM SUPPLEMENTATION AND HYPOPHYSECTOMY ON ADRENOCORTICAL FUNCTION IN THE RAT

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SUMMARY

Aldosterone and corticosterone were measured in incubations of isolated zona glomerulosa cells prepared from the following groups of rats: control; sodium deficient; potassium supplemented; sodium deficient–potassium supplemented; hypophysectomised. In each case the *in vitro* effects of potassium ions, angiotensin, ACTH and corticosterone on the secretory responses were examined. Corticosterone synthesis was measured in equivalent zonae fasciculata/reticularis cells incubated with and without ACTH.

Aldosterone synthesised by zona glomerulosa cells was increased 200% and 300% by sodium deficient and sodium deficient–potassium supplemented diets respectively. Potassium supplementation and sodium restriction increased the *in vitro* responsiveness of cells to potassium and ACTH and more corticosterone was converted to aldosterone under these conditions. Corticosterone synthesis was reduced by 55% in untreated zona glomerulosa cells of sodium deficient–potassium supplemented rats. Cells from hypophysectomised rats were more responsive to potassium ions and had a reduced rate of corticosterone synthesis. Changes in corticosterone synthesis in response to *in vitro* stimulation did not always parallel changes in aldosterone synthesis. The latter observation is considered when describing a model for the intracellular control of aldosterone synthesis.

In cells isolated from zonae fasciculata/reticularis of sodium deficient, sodium deficient–potassium supplemented and hypophysectomised rats, corticosterone synthesis was reduced by at least 80%. Hypophysectomy did not affect responsiveness to ACTH although cells of sodium deficient rats were more responsive.

Changes in steroidogenic function have been correlated with sodium, potassium and water fluxes in whole animals. The effects of dietary manipulation on zone widths of the adrenal cortex have been studied.

INTRODUCTION

It is well established that mammals maintained on a sodium deficient or a potassium supplemented diet have a higher than normal plasma aldosterone level[1–3]. These latter changes result from a multifactorial system that influences both adrenocortical structure and function. So that aldosterone secretion may be seen to be influenced by the renin-angiotensin system[4–6]; changes in the number of functional z. glomerulosa cells[7, 8]; hyperkalemia[9, 10]; hypophysial factors[11, 12]; altered adrenocortical sensitivity to trophic factors[13–16].

Such a system of control is clearly difficult to decipher using *in vivo* methods alone since there are so many inter-dependent features. The present studies attempt to define a control system using data obtained *in vitro* from incubations of isolated adrenocortical cells which are then related to zonal dimensions of the adrenal cortex and the water and electrolyte status of the whole animal.

MATERIALS AND METHODS

(a) *Animals*

Male Long Evans rats, weighing 140–190 g and bred in the Department of Zoology, University of Sheffield were maintained on 12 h light 12 h dark regime and fed the appropriate diet for at least 4 weeks.

(b) *Experimental groups*

Control rats were fed Coopers R & M No. 1 diet (47.7 mmol Na/kg and 225 mmol K/kg) and given tap water to drink.

Potassium supplemented. Coopers diet; 0.154 M potassium chloride to drink.

Sodium deficient. Tekland test diet (3.1 mmol Na/kg and 225 mmol K/kg); distilled water to drink.

Sodium deficient–potassium supplemented. Tekland diet; 0.154 M potassium chloride to drink.

Hypophysectomised. Coopers diet; tap water to drink. Rats were hypophysectomised under ether anaesthesia when they were aged 14–16 weeks and left for 14 days by which time, eating and renal function had stabilised and growth had ceased[17]. The completeness of the hypophysectomy was verified *post mortem* by examination of the base of the brain; adrenal weights had decreased 70%.

(c) *Metabolic studies*

Six rats of each dietary type were kept individually

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in all-glass metabolism cages (Jencons, Hemel Hempstead, England) and allowed to adapt for 5–7 days. Food and fluid intake and urinary output were measured for the next 5 days and urine samples stored at −20°C for subsequent analysis. After the metabolic examination, the rats were housed in ordinary cages with the dietary regimes continued.

(d) *Isolated cell preparation*

Rats were killed by cervical fracture; adrenal glands, free of adnexa, were placed in ice-cold Krebs' bicarbonate Ringer's solution containing 4% bovine serum albumin (Sigma, London) and 0.2% glucose (KBRA).

Under a dissecting microscope three regions of the adrenal glands were separated; the zona glomerulosa + tissue capsule (z. glomerulosa stripping), the zonae fasciculata/reticularis and the medulla. The latter was discarded. The pooled z. glomerulosa strippings and the z. fasciculata/reticularis were incubated separately in 5 ml KBRA with 3 mg/ml collagenase (Worthington Biochemicals) in an atmosphere of 95% oxygen and 5% carbon dioxide at 37°C in an apparatus described by Lowry *et al.*[18]. After 50 min, the cells were gently disaggregated by sucking the suspension up and down a 1.0 ml Eppendorf pipette thirty times. The cell preparations were filtered through 45 μm nylon gauze (Henry Simon Ltd., Stockport, Cheshire) into polythene tubes and centrifuged at 1000 *g* for ten min at 4°C. The supernatant was decanted and the cells resuspended in 5 ml KBRA. The cells were similarly washed twice more and finally suspended in 10 ml KBRA. The number of cells/ml was estimated using a Thoma haemacytometer and phase contrast microscopy. Z. glomerulosa strippings from six rats yielded between 150,000 to 250,000 cells/ml and z. fasciculata/reticularis, 250,000 to 500,000 cells/ml.

(e) *Incubation*

Z. glomerulosa cells were incubated in: (i) KBRA; (ii) KBRA with 10.6 mM K^+; (iii) KBRA with 50 μg angiotensin II/ml (Hypertensin, CIBA); (iv) KBRA with 0.1 U ACTH/ml (Ferring, Stockholm, Sweden); (v) KBRA with 10^{-4} M corticosterone (Sigma, London). Z. fasciculata/reticularis cells were incubated with and without 0.1 U ACTH/ml. All incubations were carried out in triplicate in 15 cm polystyrene tubes (Henleys Medical Supplies Ltd.). The final incubation vol. was 1.0 ml (0.5 ml KBRA with the appropriate factors + 0.5 ml of cell suspension). The tubes were gassed with 95% O_2, 5% CO_2, stoppered and incubated in a shaking water bath (80 cycles/min) at 37°C for 2 h.

(f) *Assay of steroids*

(i) *Radioactive steroids.* ([1,2-^{3}H]-aldosterone S.A. = 50 Ci/mmol and [1,2-^{3}H]-corticosterone, S.A. 33 Ci/mmol) were obtained from the Radiochemical Centre, Amersham. Steroids were obtained from Sigma London and other chemicals from Koch Light Laboratories Ltd. All solvents (B.D.H. Ltd.) were A.R. grade and redistilled before use. Antiserum to aldosterone was a gift from N.I.H. and diluted on receipt by a factor of 50,000 using 0.5 M borate buffer (pH = 8.0). The antiserum was divided into 0.1 ml aliquots and stored deep frozen. Antiserum to corticosterone was a gift from Dr. H. Gross, Department of Mental Health, New York and was diluted to 1/75,000 with 0.9% NaCl containing 0.1% B.S.A.

(ii) *Radioactive counting.* Radioactive samples were counted in a Packard Liquid Scintillation Counter 3375. The corticosterone samples were counted as 0.5 ml aqueous samples in 10 ml scintillation fluid (14 g PPO, 1.2 g POPOP, 1 litre ethoxy ethanol and 1.6 litres toluene) and those of aldosterone as a 0.1 ml aqueous solution in 10 ml scintillation fluid (14 g PPO, 1.2 g POPOP, 200 g naphthalene, 0.5 litres ethoxy ethanol and 2 litres toluene). The efficiencies of counting were of the order of 20% for corticosterone and 30% for aldosterone.

(iii) *Radioimmunoassays: aldosterone.* An internal recovery marker (0.1 ml of a 0.1 μCi/ml solution of [^{3}H]-aldosterone) was added to 0.5 ml of each zona glomerulosa incubation mixture prior to extraction with 2 × 5 ml dichloromethane. The pooled extracts were evaporated to dryness, transferred with three washes of acetone to methanol-washed Whatman No. 1 chromatography paper (2 cm × 40 cm strips) and run in a Bush B5 system for 10 h. The tritiated aldosterone areas were located using a Packard Radiochromatogram scanner and eluted with 10 ml aqueous ethanol (ethanol:water = 95:5). Eluates were evaporated to dryness. Ethanol (1 ml) was added to the dried extract and 0.1 ml fractions were counted for 20 min in 10 ml scintillation mixture to estimate recovery. Varying aliquots of the remaining sample were assayed for aldosterone by radioimmunoassay[19]. Standards (5–100 pg) were prepared by pipetting appropriate vols of an aldosterone solution into Eppendorf microtubes. Tritiated aldosterone (0.1 ml of 0.02 μCi/ml) was added to each standard tube. From the sample recoveries the vol. of this same solution required to bring the total radioactivity up to that in the standard tubes was calculated and added before evaporating all tubes to dryness under nitrogen. Tubes were then placed in a vacuum oven at 35°C for 1 h. The antiserum was further diluted to 50 ml with borate buffer containing 0.5% BSA and 0.1 ml of this finally diluted solution was added to standards and samples. The tubes were vortexed and left overnight at 4°C. The following day 0.1 ml of ice-cold dextran-charcoal suspension (0.25% charcoal and 0.25% dextran T-70 in borate buffer) were then added to each tube. After vortexing, the tubes were left for 10 min at 4°C before centrifugation (2,000 *g* for 10 min at 4°C). Half of the supernatant was counted for 20 min and a standard curve constructed using the free:bound [^{3}H]-aldosterone ratio.

Corticosterone. An internal recovery marker (0.1 ml

of a 0.5 μCi/ml solution of [^{3}H]-corticosterone) was added to 0.5 ml of incubation mixture. The samples were dried, extracted and spotted onto paper as described for the aldosterone samples. The chromatograms were run in a Bush B1 system, scanned and the radioactive areas eluted with methanol to a final vol. of 10 ml. A fraction of the eluate (1/10) was counted for recovery and aliquots of the remaining samples were assayed. Duplicate standards (0.1–4.0 ng corticosterone) and samples were pipetted into 10 ml conical centrifuge tubes and evaporated to dryness. A solution of [^{3}H]-corticosterone (0.5 ml of 0.1 μCi/ml [^{3}H]-corticosterone in 0.9% NaCl containing 0.1% BSA) was added to each tube. After vortexing, 0.5 ml of diluted antiserum was added to each tube and the tubes vortexed again. The tubes were allowed to stand at room temperature for 30 min and then transferred to an ice bath for 10 min. Ice-cold dextran-charcoal suspension (0.5 ml of 0.5% dextran T-70 and 0.5% charcoal in 0.9% NaCl) was added to each tube which was vortexed before returning to the ice-bath for 25 min. The tubes were centrifuged (1750 *g* for 20 min at 4°C) and 0.5 ml of the supernatant then counted. A standard curve was constructed using the free to bound ratio of [^{3}H]-corticosterone.

(*g*) *Histology*

Adrenal glands with adnexa were rapidly removed and placed in Bouin's fluid at 4°C for 24 h then transferred to 70% ethanol containing lithium carbonate. All groups were processed at the same time to afford closer comparison. Sections were cut at 5 μm and stained with Ehrlich's haematoxylin and eosin. Four median sections from each right gland were measured using a Zeiss Ultraphot microscope with a corrected graticule.

RESULTS

Table 1 presents the results of metabolic studies. The intake of potassium by potassium supplemented rats was 40% higher than that of the normal diet rats ($P < 0.001$) though sodium intake was unchanged. Fluid intake and urine output were lowered by about 25% ($P < 0,01$ in both cases) compared with controls. The increased urinary potassium equalled the extra potassium imbibed. This gave a difference of 18% between potassium balances (intake/output × 100; [17]) of normal and potassium supplemented rats ($P < 0.05$). Sodium-deficient rats although their diet contained similar amounts of K to normal, actually took in 25% less K ($P < 0.001$) and 40% less water ($P < 0.001$) and concomitantly the urine volume was reduced ($P < 0.001$). The urinary K was 50% higher ($P < 0.01$) despite reduced intake of this ion.

Sodium deficient–potassium supplemented rats had unchanged drinking rates compared with sodium deficient rats. The extra potassium taken in was again equal to the increase in urinary excretion of the ion. The potassium balance was 25% higher than that for animals on a sodium deficient diet alone ($P < 0.05$). This diet was not followed by a changed urinary sodium concentration although the sodium balance was 45% greater ($P < 0.05$).

Histology

Dietary manipulations changed the mean widths of the histological zones of the adrenal cortex (Table 2). The zona glomerulosa increased in width by different amounts depending on the diet: potassium supplementation produced a 14% increase compared with controls; sodium deficiency diet a 46% increase and sodium deficient–potassium supplemented diet a 65% increase. Other zone widths were not significantly changed except that the zona fasciculata of potassium supplemented rats was reduced by 9%.

Steroidogenesis (Tables 3, 4 and 5)

(a) *Control diet. In vitro* ACTH increased aldosterone production nine times and corticosterone production six times ($P < 0.02$) in zona glomerulosa cells; corticosterone production was five times greater in z. fasciculata/reticularis cells. Addition of angiotensin II and extra potassium were without effect. Untreated z. glomerulosa cells produced 70 times more corticosterone than aldosterone as measured in ng/10^6

Table 1. Metabolic studies: the effects of dietary sodium restriction and potassium supplementation on food, fluid and electrolyte balances in male Long Evans rats ($n = 12 \pm$ S.E.M.)

Diet	Na^+ intake (μEq/100 g BW/24 h)	K^+ intake (μEq/100 g BW/24 h)	Water intake (ml/100 g BW/24 h)	Urine (ml/100 g BW/24 h)	Urine $[Na^+]$ (mEq/l)	Urine $[K^+]$ (mEq/l)	Water balance (%)	Na^+ balance (%)	K^+ balance (%)
Control	247.7 ± 10.1	1102.5 ± 45.1	8.13 ± 0.46	3.96 ± 0.32	9.93 ± 1.1	184.6 ± 15.9	48.52 ± 2.1	16.84 ± 2.68	63.7 ± 4.05
High potassium	253.2 ± 15.5	1543.6 ± 64.4	6.22 ± 0.39	2.7 ± 0.15	12.88 ± 1.73	460.8 ± 29.0	45.42 ± 4.17	12.62 ± 1.8	81.88 ± 7.38
Low sodium	11.12 ± 0.66	807.4 ± 48.2	5.08 ± 0.19	2.27 ± 0.19	0.87 ± 0.19	283.8 ± 23.2	45.34 ± 2.0	16.94 ± 3.48	79.34 ± 7.01
Low-sodium–high potassium	10.6 ± 0.79	1372 ± 93.7	6.22 ± 0.66	3.59 ± 1.66	2.86 ± 1.13	299.9 ± 17.6	56.7 ± 3.33	63.87 ± 22.36	99.48 ± 5.57

Table 2. The effect of dietary sodium restriction and potassium supplementation on the zonal widths of adrenal cortices of rats

Diet	Zona glomerulosa	Zona fasciculata	Zona reticularis
Control	(24)	(24)	(24)
	67.7 ± 2.0	509.7 ± 16.7	315.8 ± 18.4
High potassium	(20)	(20)	(20)
	77.0 ± 1.8	464.3 ± 10.2	339.6 ± 14.7
	$P < 0.001$	$P < 0.05$	(N.S.)
Low sodium	(24)	(24)	(24)
	98.8 ± 3.9	523.7 ± 17.3	326.6 ± 23.0
	$P < 0.001$	(N.S.)	(N.S.)
Low sodium–high potassium	(28)	(28)	(28)
	111.7 ± 2.9	508.9 ± 13.5	392.1 ± 21.9
	$P < 0.001$	(N.S.)	(N.S.)

Results expressed in μm ± S.E.M. Number of observations in parentheses. Values are compared statistically with those of control diet rats using Students *t*-test; N.S. not significant.

cells/2 h. Z. fasciculata/reticularis cells under the same conditions produced three times more corticosterone than z. glomerulosa cells.

(b) *Potassium supplemented diet.* Untreated z. glomerulosa and z. fasciculata/reticularis cells produced similar amounts of aldosterone and corticosterone to those from rats fed the control diet. Addition of potassium to the incubation medium induced four times greater aldosterone production ($P < 0.001$) though corticosterone was unaffected. Added ACTH was a most potent stimulant to aldosterone synthesis (28 times, $P < 0.001$). Significantly corticosterone production was increased only seven fold ($P < 0.05$). In quantitative terms the ACTH-stimulated output of aldosterone by z. glomerulosa cells of potassium supplemented rats was twice that of cells from control rats ($P < 0.001$). ACTH action on the z. fasciculata/reticularis cells gave a 12 fold increase in corticosterone synthesis ($P < 0.01$).

(c) *Sodium deficient diet.* Untreated z. glomerulosa cells synthesised 50% more aldosterone ($P < 0.05$) than similar cells of control rats. *In vitro* potassium increased aldosterone production four fold ($P < 0.05$) and corticosterone over 3 fold ($P < 0.01$). Addition of ACTH produced a seven fold increase in corticosterone synthesis ($P < 0.001$) and a 14 fold increase in aldosterone synthesis ($P < 0.001$). The capacity to convert corticosterone to aldosterone was increased by 140% ($P < 0.001$).

Untreated z. fasciculata/reticularis cells synthesised 85% less corticosterone than cells of control diet rats, ($P < 0.01$). Added ACTH stimulated corticosterone synthesis over 24 fold ($P < 0.001$).

(d) *Sodium deficient-potassium supplemented diet.* The z. glomerulosa preparations produced three times more aldosterone than the control ($P < 0.001$) and twice that of cells from rats on a low sodium diet ($P < 0.001$). Addition of potassium and ACTH gave further stimulations; two fold for the former ($P < 0.01$) and 11 fold for the latter ($P < 0.001$). Potassium *in vitro* did not affect corticosterone synthesis but ACTH increased it seven fold ($P < 0.001$). Untreated z. gomerulosa cells synthesised half as much corticosterone as the controls ($P < 0.02$). When corticosterone was added to the incubation medium, the output of aldosterone was 50% higher than that of z. glomerulosa cells of sodium deficient rats ($P < 0.001$) and 250% higher than that of sodium replete animals.

Untreated z. fasciculata/reticularis preparations

Table 3. The effects of various substances on the capacity of isolated zona glomerulosa cells to synthesise aldosterone

Substance added to incubation medium		Experimental animal groups				
		Control	Potassium supplemented	Sodium deficient	Sodium deficient–potassium supplemented	Hypophysectomised
(a)	Control	3.98 (8) ± 0.62	2.69 (9) ± 0.54	5.88 (8) ± 0.63	12.19 (9) ± 1.66	3.93 (9) ± 0.69
(b)	Potassium	4.49 (9) ± 0.84	11.65 (9) ± 1.81	24.38 (9) ± 4.25	22.01 (9) ± 2.66	6.71 (9) ± 0.95
(c)	Angiotensin II	4.49 (9) ± 0.59	4.27 (9) ± 0.73	7.66 (8) ± 0.83	13.2 (9) ± 1.78	3.57 (9) ± 0.37
(d)	ACTH	34.09 (9) ± 2.84	74.52 (9) ± 9.59	81.55 (9) ± 8.25	134.7 (8) ± 20.8	29.13 (9) ± 3.15
(e)	Corticosterone	218.1 (9) ± 22.3	477.5 (9) ± 29.1	527.1 (9) ± 59.3	766.9 (9) ± 44.5	134.1 (3) ± 19.6

Each group was made up of six male rats of the Long Evans strain. Aldosterone (ng/10^6 cells/2 h) measured by radioimmunoassay. The results are given as the mean ± standard error incubations (*n*) of cells from z. glomerulosa strippings of three groups of animals.

Table 4. Effect of dietary potassium and sodium on corticosterone synthesis by isolated zona glomerulosa cells

Substance added to incubation medium		Experimental animal groups				
		Control	Potassium supplemented	Sodium deficient	Sodium deficient–potassium supplemented	Hypophysectomised
(a)	Control	(8) 285.4 ± 53.2	(9) 369.9 ± 87.4	(7) 206.2 ± 34.1	(8) 125.0 ± 38.0	(9) 154.6 ± 21.5
(b)	Potassium	(9) 218.2 ± 35.5	(9) 477.4 ± 79.2	(8) 552.8 ± 78.1	(8) 214.7 ± 68.8	(9) 529.1 ± 211.6
(c)	Angiotensin	(8) 596.0 ± 140.7	(9) 262.9 ± 39.2	(6) 502.9 ± 189.7	(8) 158.9 ± 21.7	(9) 239.4 ± 47.0
(d)	ACTH	(8) 1645.0 ± 554.5	(9) 2593.2 ± 915.0	(9) 1439.5 ± 244.3	(9) 842.0 ± 158.1	(9) 757.3 ± 189

Corticosterone (ng/10^6 cells/2 h) was measured by radioimmunoassay.

synthesised similar amounts of corticosterone to those from rats on a sodium deficient diet but nearly five times less than preparations taken from control rats ($P < 0.01$). The z. fasciculata/reticularis preparations were particularly reactive to added ACTH; corticosterone synthesis was increased by 39 fold ($P < 0.001$).

(e) *Hypophysectomy.* Basal corticosterone synthesis by z. fasciculata/reticularis cells is 16% of that by cells of normal control rats ($P < 0.01$). ACTH increased corticosterone synthesis five fold in cells of both hypophysectomised and control rats ($P < 0.001$). Corticosterone synthesis by untreated z. glomerulosa cells was reduced by 45% after hypophysectomy ($P < 0.05$); aldosterone synthesis was unaffected. Increasing the *in vitro* potassium concentration had no effect on steroidogenesis by z. glomerulosa cells of control rats but aldosterone synthesis was raised three fold ($P < 0.001$) and corticosterone two fold ($P < 0.01$) in cells of hypophysectomised rats. ACTH had similar effects on cells of control and hypophysectomised rats; aldosterone was increased approximately eight fold ($P < 0.001$) and corticosterone five fold ($P < 0.02$). Angiotensin in the doses employed had no effect on steroidogenesis. Aldosterone converted from added corticosterone by z. glomerulosa cells of hypophysectomised rats was about half that of cells from intact rats, an insignificant difference.

DISCUSSION

The use of cells from known areas of the adrenal cortex to study the control of steroidogenesis has certain advantages. Firstly, access and egress of stimulants and steroid products are facilitated. Secondly, changes in steroidogenesis can be related to cell numbers. Thirdly, steroid production can be assigned accurately to cell types; thus aldosterone is not the product of conversion of corticosterone from the zona fasciculata/z. reticularis. Purification of cell types by sedimentation[20] would have been ideal but the effects of diet on steroidogenesis are such that the z. glomerulosa cells from sodium deficient rats are of very similar size to z. fasciculata cells.

The cell suspension technique, when combined with histological measurements of zonal widths, has proved to be a powerful tool in the study of adrenocortical function. It was found, for example, that synthesis is increased by both changes in z. glomerulosa cell numbers (as measured by changes in zonal widths) and also by increased unit cell production. Thus, whilst potassium supplementation gave no change in basal synthesis of aldosterone but a 14% increase in z. glomerulosa width, a sodium deficient diet increased the width of the z. glomerulosa by 50% and increased the unit cell synthesis of aldosterone. Further, the sodium deficient–potassium supple-

Table 5. Effect of dietary potassium and sodium on corticosterone synthesis by isolated zonae fasciculata/reticularis cells

Substance added to incubation medium		Experimental animal groups				
		Control	Potassium supplemented	Sodium deficient	Sodium deficient–potassium supplemented	Hypophysectomised
(a)	Control	(6) 968.3 ± 238.0	(9) 736.6 ± 253.0	(9) 149.9 ± 36.7	(9) 209.9 ± 27.7	(7) 161.7 ± 56.3
(b)	ACTH	(6) 5170.0 ± 569.0	(9) 9050.0 ± 2460.0	(9) 3675.0 ± 857.0	(9) 8285.0 ± 585.0	(9) 875.0 ± 138.0

Cells were incubated in KBRA with (b) and without (a) 0.1 I.U. ACTH/ml. For other details see legend to Table 4.

mented diet gave a 65% increase in the width of the z. glomerulosa with a 300% increase in the rate of aldosterone synthesis.

Cells change their responsiveness to various treatments. Increases in the potassium concentration of the incubation medium from 3.6 to 8.5 mM stimulated aldosterone synthesis in quartered adrenals[7] and in z. glomerulosa strippings[15]; equal responsiveness was found in tissues from sodium replete and deficient rats. Potassium, added to the incubation medium, had no effect on tissues from rats on a potassium deficient diet. On the other hand, in the present studies a change in the potassium concentration from 5.9 to 10.6 mM had no effect on cells from rats on the control diet, while a potassium supplemented or a sodium deficient diet allowed a four fold stimulation of aldosterone synthesis but a sodium deficient–potassium supplemented diet had only a two fold effect. These findings that sodium deprivation affects the response of the adrenal cortex to acute potassium treatment differ from those of Müller and others[21, 22] but they agree with Dluhy *et al.*[9]. In brief, increased dietary potassium heightens cellular responsiveness affected by dietary sodium[3].

Previous studies on adrenocortical tissue from sodium deficient rats both *in vitro* and *in vivo*, agree with our finding that there is an increased aldosterone secretory response to ACTH[13, 16, 22–24]. Similarly potassium supplementation increases adrenocortical responsiveness to ACTH[7, 14, 15] a response tempered by dietary sodium. Thus ACTH stimulated z. glomerulosa cell aldosterone synthesis by a factor of eight in tissues from control rats but by a factor of 28 in cells from rats on a high potassium diet; yet it was less in cells of sodium deficient–potassium supplemented rats compared with those of sodium deficient rats.

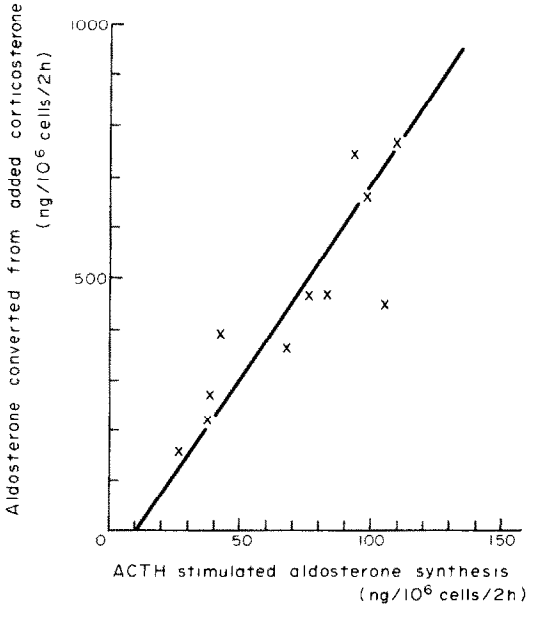

Fig. 1. Aldosterone converted from added corticosterone as a function of ACTH-stimulated aldosterone synthesis by isolated z. glomerulosa cells from rats fed different dietary sodium and potassium regimes.

In a consideration of the aldosterone biosynthetic pathway two control points have been identified; the side chain cleavage of cholesterol to pregnenolone and the conversion of corticosterone to aldosterone. The intracellular events leading to the activation of cholesterol side chain cleavage are well documented[25, 26], particularly the mechanism of action of ACTH in the z. fasciculata/reticularis. It is also established that increased dietary potassium or reduced dietary sodium induce synthesis of the mitochondrial corticosterone to aldosterone converting enzymes[27]. This is confirmed by z. glomerulosa cells incubated with saturating concentrations of corticosterone. The ability to convert added corticosterone to aldosterone was found to increase in the dietary regimes from control to potassium supplemented to sodium deficient and to sodium deficient–potassium supplemented. It is interesting to note that aldosterone synthesis by ACTH-stimulated z. glomerulosa cells is closely correlated with the amounts of corticosterone converted to aldosterone (Fig. 1). The ACTH-stimulated aldosterone production is only 15% of the capacity of the z. glomerulosa cells to convert corticosterone which suggests that, under conditions of maximal ACTH stimulation, the conversion of corticosterone is the rate-limiting step in aldosterone synthesis. On the assumption that ACTH exerts its maximal effect on the z. glomerulosa cells, it might be concluded that the capacity for cholesterol cleavage is not affected by dietary sodium or potassium. Such a conclusion is supported by our observations that there are similar percentage increases in corticosterone synthesis by z. glomerulosa cells from rats on different dietary regimes when treated with ACTH *in vitro*.

The conversion of corticosterone to aldosterone can be acutely stimulated in the presence of bovine serum albumin by ACTH, angiotensin, potassium and corticosterone[16, 28] probably reflecting an activation of pre-existing enzymes rather than the quantitative changes discussed above. The present study suggests that when the *in vitro* stimulation of aldosterone synthesis exceeds that of corticosterone, then corticosterone converting enzymes have been activated. For example, *in vitro* potassium stimulated z. glomerulosa cells from rats fed a potassium supplemented diet to produce four times more aldosterone, yet corticosterone synthesis was unaffected. Thus if the extra *in vitro* potassium had activated the cholesterol cleavage complex, then corticosterone and aldosterone output would have been increased in parallel. Activation of the corticosterone converting enzymes might result in more aldosterone being produced with a consequent reduction in corticosterone output: However, the ratio of corticosterone:aldosterone synthesised by the z. glomerulosa cell is 140:1 so that

a quadrupling of the converting enzyme activity would produce a drop of the order of 6% in corticosterone output which could not be detected.

Major changes in dietary intake may also change converting enzyme activity without necessarily affecting enzyme quantity. In this context, a comparison between unstimulated synthesis of aldosterone and the conversion of corticosterone to aldosterone in response to dietary sodium restriction is relevant. Aldosterone synthesised by untreated z. glomerulosa cells was increased by 200% but the conversion was only 50% greater. It might be supposed that dietary potassium activated the converting enzymes. There is some support for this thesis, in that there is a difference in responsiveness to *in vitro* potassium between z. glomerulosa cells of sodium deficient rats and those of sodium deficient-potassium supplemented rats. Z. glomerulosa cells of sodium deficient rats were stimulated to produce twice as much corticosterone and four times the amount of aldosterone. The increase in aldosterone synthesis is due therefore to a doubling in cholesterol side chain cleavage activity, plus a similar increase in converting enzyme activity. In z. glomerulosa cells from rats on a sodium deficient–potassium supplemented diet, aldosterone and corticosterone synthesis were both doubled by *in vitro* potassium treatment. In this case, it can be argued that *in vitro*, potassium affects only cholesterol cleavage activity. Potassium supplement of the sodium deficient diet fully activated the converting enzymes, blanketing the possibility of an *in vitro* effect. It follows that the amount of aldosterone synthesised by z. glomerulosa cells of sodium deficient rats must be similar to that of cells of sodium deficient–potassium supplemented rats when treated with potassium *in vitro*.

Hypophysectomy reduces adrenocortical responsiveness to ACTH[29] within four days[30]. Z. fasciculata/reticularis cells of both intact and hypophysectomised rats give similar increases in corticosterone output after ACTH, although the basal production rates are much lower in the hypophysectomised series. The latter observation probably reflects tissue degeneration. Whole adrenal glands of rats superfused four days after hypophysectomy failed to respond to ACTH[30], and this was considered to result from an absence of *de novo* messenger RNA synthesis activating cholesterol side chain cleavage. The present studies suggest, however, that, with sensitive radioimmunoassays, and when ACTH has free access to adrenocortical cellular receptors corticotrophin can be effective at least fourteen days post-hypophysectomy. Z. glomerulosa steroidogenesis is also changed by hypophysectomy: corticosterone synthesis fell and *in vitro* responsiveness to potassium increased. The lower corticosterone output indicates that, without ACTH, adrenocortical cells have a reduced cholesterol cleavage activity. Hypophysectomy did not affect aldosterone synthesis which implies that later steps in the aldosterone biosynthetic pathway must compensate the reduced cleavage activity. Quantitative changes in corticosterone to aldosterone converting enzymes are not the compensating factors because similar amounts of aldosterone were produced in incubations with added corticosterone. Activation of preexisting converting enzymes may occur. Baumann and Müller's observations support this contention[31], in that aldosterone synthesis was higher and corticosterone lower, in incubations of z. glomerulosa strippings of hypophysectomised rats fed a potassium deficient diet as compared with intact rats. Further, the synthesis of deoxycorticosterone, the immediate precursor of corticosterone, was halved by hypophysectomy.

Presumably the lower basal cholesterol cleavage activity is sensitive to potassium *in vitro*. We found that corticosterone and aldosterone synthesis were increased to the same extent by *in vitro* potassium. Cells of intact rats were not affected.

The cellular adrenocorticosteroidogenetic characteristics are clearly influenced by dietary electrolyte intake and overall balance. Many of the changes seen *in vivo* are sustained *in vitro* particularly those that involved amounts of enzyme and the cellular responsiveness the stimulants. These latter may reflect altered receptor numbers which are maintained tonically by ACTH or angiotensin for example, while the former possibly results from endogenous substrates synthesised as result of cellular electrolyte content[3].

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