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ADRENOCORTICAL FUNCTION IN RATS WITH INHERITED HYPOTHALAMIC DIABETES INSIPIDUS (BRATTLEBORO STRAIN)

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SUMMARY

Steroidogenic function was studied in cells isolated from the zonae glomerulosa and fasciculata/reticularis of normal and sodium deficient Brattleboro rats. Compared with those of non-DI rats, z. glomerulosa cells of rats with diabetes insipidus (DI) produced more corticosterone and were less sensitive to *in vitro* stimulation by angiotensin II, potassium and ACTH while their aldosterone secretory response was more sensitive to dietary sodium changes. These differences of DI and non-DI z. glomerulosa function have been analyzed with regard to quantitative and qualitative changes in enzymes responsible for cholesterol cleavage and for the conversion of corticosterone to aldosterone. Z. fasciculata/reticularis cells from DI rats were less responsive to ACTH than those of non-DI cells.

INTRODUCTION

The condition of hypothalamic diabetes insipidus (DI) as exemplified by the genotypically homozygous form of Brattleboro rat[1, 2] is a unique model with which to elucidate adrenocortical function in the absence of endogenous vasopressin. The DI rat moreover has a deficient CRF-ACTH reserve[3], and apparently impaired adrenocortical function[4, 5]. The latter may result from the former, although it is likely that other factors associated with the DI condition—polyuria, potassium wasting, possible sodium deficiency[6]—impinge on adrenocortical autonomy.

With these considerations in mind, the present studies examine characteristics of isolated adrenocortical cells from Brattleboro rats with (DI) and without (non-DI) endogenous vasopressin. Further a description is given of enzymic and biosynthetic capacity of the cells free of the many endogenous secondary factors.

MATERIALS AND METHODS

Male Brattleboro rats aged 4–6 months (250–300 g body weight) were bred in the Department of Zoology, University of Sheffield. The DI condition was diagnosed on the basis of daily water intake between ages of 8 and 10 days. The following groups were studied:

1. (a) DI and (b) non-DI: two groups of rats of each type were fed a normal diet containing 47.7 mmol Na and 213 mmol K/kg. These animals provided adrenals for incubation.

2. (a) DI and (b) non-DI: one group of six rats of each type were fed a diet containing 141 mmol Na and 225 mmol K/kg. The water and electrolyte balances of these rats were examined.

3. (a) DI and (b) non-DI: three groups of six rats of each type were fed a sodium deficient diet containing 3.1 mmol Na and 225 mmol K/kg. All rats were used for adrenal incubation studies but one group of each type had previously been the subject of metabolism cage studies.

Methodology for the metabolic studies is given in Kenyon *et al.*[7] where full details of tissue preparation, cell incubations and steroid analysis can also be found. Briefly cells were isolated from z. glomerulosa strippings and z. fasciculata/reticularis tissue by collagenase digestion. Isolated z. glomerulosa cells were suspended in a final volume 10 ml Krebs' bicarbonate ringer containing albumin and glucose (KBRA), and incubated under the following conditions: (a) control, (b) with 10.6 mM K^+ (c) with 50 μg angiotensin II/ml, (d) with 0.1 U ACTH/ml, (e) with 10^{-4} M corticosterone. Isolated z. fasciculata/reticularis cells were incubated with and without 0.1 U ACTH/ml. Aldosterone and corticosterone were measured by radioimmunoassay after paper chromatography.

RESULTS

Metabolic studies

The pattern of change in electrolyte and fluid balances of Brattleboro rats in response to sodium deprivation is broadly similar to that in Long Evans rats[7]. Sodium deficiency in the DI rat increased the water balance (dietary intake/urine output $\times 100$) from 81.1 $\pm$ 5.3% to 93.4 $\pm$ 0.6% ($P < 0.05$), reduced the sodium balance from 64.5 $\pm$ 6.1% to 39.3 $\pm$ 3.54% ($P < 0.01$) and increased the potassium balance from 63.7 $\pm$ 4.8 to 104.1 $\pm$ 6.7% ($P < 0.001$). The balances of water, sodium and potassium for the non-DI rat did not change in response to sodium

Table 1. The effect of dietary sodium restriction on aldosterone synthesis (ng/10^6 cells/2h) by isolated zona glomerulosa cells of DI and non-DI rats

	DI Diet		Non-DI Diet	
Cell Treatment	Control	Sodium deficient	Control	Sodium deficient
Basal	(5) 1.21 ± 0.19	(8) 21.25 ± 5.43	(6) 1.30 ± 0.32	(8) 5.33 ± 1.45
Potassium	(6) 2.67 ± 0.53	(9) 29.45 ± 8.51	(6) 3.66 ± 0.96	(9) 12.42 ± 2.19
Angiotensin	(6) 4.45 ± 1.16	(9) 28.62 ± 6.65	(5) 1.84 ± 0.3	(9) 7.86 ± 1.52
ACTH	(6) 8.42 ± 0.90	(9) 103.9 ± 32.4	(6) 5.8 ± 0.75	(9) 46.2 ± 8.2
Corticosterone	(6) 173.9 ± 17.3	(9) 946.7 ± 195.4	(6) 166.6 ± 39.6	(7) 426.4 ± 77.3

( ) no. of incubations.
± S.E.M.

deprivation, although the mean sodium balance increased from 66.8 ± 9.3% to 83.4 ± 18.9%. Some of the DI and non-DI rats were potassium wasting when given a sodium deficient diet.

Incubation studies

Non-DI rats. Aldosterone synthesis (Table 1) in z. glomerulosa cells of control diet rats was increased threefold by potassium ($P < 0.05$) and more than four fold by ACTH ($P < 0.001$); angiotensin had no effect. Corticosterone synthesis (Table 2) was stimulated fourfold by angiotensin ($P < 0.01$), threefold by potassium (N.S.) and sevenfold by ACTH ($P < 0.001$). Dietary sodium restriction had little effect on *in vitro* stimulation of steroidogenesis but basal aldosterone synthesis was four times greater ($P < 0.001$) and the amount of added corticosterone converted to aldosterone was trebled ($P < 0.02$). Basal corticosterone synthesis (Table 3) by z. fasciculata/reticularis cells and responsiveness to ACTH was unchanged by dietary sodium levels; ACTH stimulated corticosterone synthesis seventeenfold in cells of both dietary types ($P < 0.001$).

DI rats: Aldosterone synthesis by z. glomerulosa cells of control diet rats was doubled by potassium ($P < 0.05$), quadrupled by angiotensin ($P < 0.05$) and ACTH increased it by a factor of seven ($P < 0.001$). The decreased corticosterone synthesis in incubations with potassium and angiotensin and its increase with ACTH were not statistically significant. Sodium deprivation increased basal aldosterone synthesis more than 17 times ($P < 0.001$) without affecting corticosterone synthesis. Only ACTH stimulated z. glomerulosa cells of sodium deficient rats (aldosterone fivefold, $P < 0.05$; corticosterone fourfold, $P < 0.05$). Five times more aldosterone was converted from added corticosterone in cells of sodium deficient rats ($P < 0.05$).

Basal corticosterone synthesis by z. fasciculata/reticularis cells was increased 7 fold ($P < 0.05$) by sodium

Table 2. The effect of dietary sodium restriction on corticosterone synthesis (ng/10^6 cells/2 h) by isolated zona glomerulosa cells of DI and non-DI rats

	DI Diet		Non-DI Diet	
Cell Treatment	Control	Sodium deficient	Control	Sodium deficient
Basal	(6) 354.8 ± 133.1	(9) 367.7 ± 118.5	(5) 49.5 ± 7.7	(9) 71.0 ± 10.7
Potassium	(6) 297.7 ± 90.1	(9) 518.4 ± 149.7	(6) 148.5 ± 51.8	(9) 135.2 ± 18.4
Angiotensin	(6) 156.8 ± 36.1	(9) 571.3 ± 147.5	(6) 206.1 ± 32.7	(9) 110.1 ± 14.8
ACTH	(6) 655.2 ± 82.2	(9) 1301.2 ± 280.9	(6) 382.9 ± 87.5	(9) 564.6 ± 93.8

( ) no. of incubations.
± S.E.M.

Table 3. Effect of dietary sodium restriction on corticosterone synthesis (ng/10^6 cells/2 h) by isolated zona fasciculata and zona reticularis cells of DI and non-DI rats

	DI Diet		Non-DI Diet	
Cell Treatment	Control	Sodium deficient	Control	Sodium deficient
Basal	(6) 225.9 ± 66.4	(9) 1470.5 ± 440.6	(6) 154.5 ± 56.2	(9) 165.7 ± 62.5
ACTH	(6) 810.1 ± 190.7	(9) 5210.9 ± 959.7	(6) 2573 ± 404	(9) 2969 ± 510

( ) no. of incubations.
± S.E.M.

deprivation although responsiveness to ACTH was not affected.

DISCUSSION

Control of steroidogenic function in the z. glomerulosa of Brattleboro rats may be analysed allowing the following assumptions:

1. Two control points in the aldosterone biosynthetic pathway exist [8]; the side chain cleavage of cholesterol and the conversion of corticosterone to aldosterone. These may operate independently.

2. The enzymes 18-hydroxylase and 18-hydroxydehydrogenase convert corticosterone to aldosterone. *De novo* synthesis of these enzyme systems has been correlated with dietary sodium and potassium manipulations[9, 10]. This has been confirmed with z. glomerulosa cells incubated with 10^{-4} M corticosterone which saturates the converting enzymes[7]. Differences in aldosterone synthesis under these conditions thus reflect the quantity of converting enzymes present.

3. In addition to quantitative changes induced by chronic factors such as diet, *in vitro* treatment with potassium, angiotensin, ACTH and even corticosterone may acutely stimulate corticosterone to aldosterone conversion [11, 12]. The term activation describes acute stimulation and does not imply induced enzyme synthesis. Activation is seen where aldosterone synthesis is stimulated without change in corticosterone output.

4. Cholesterol side chain cleavage is a key step in the control of steroid biosynthesis. The mechanism of ACTH action on cholesterol cleavage in the z. fasciculata/reticularis has been intensively studied [13, 14]. The acute stimulation of cholesterol cleavage by ACTH involves protein synthesis but may be justifiably considered in this discussion as an activation. Activation of cholesterol cleavage in z. glomerulosa cells increases corticosterone and aldosterone synthesis in parallel.

5. Aldosterone synthesis by ACTH-treated z. glomerulosa cells of Long Evans rats is 15% of the aldosterone synthesised by cells of the same pool treated with 10^{-4} M corticosterone[7]. This percentage was not changed by diet; thus under maximal ACTH stimulation, the conversion of corticosterone to aldosterone is rate limiting and maximal cholesterol cleavage capacity is not influenced by dietary sodium or potassium. An increased percentage would have suggested a greater maximum potential of the cholesterol cleavage complex, an effect considered here as a quantitative enzyme change.

These somewhat speculative assumptions allow analysis of adrenocortical steroidogenic control in the Brattleboro rat.

Zona glomerulosa cells of DI and non-DI rats given a control diet synthesise similar amounts of aldosterone from added corticosterone; there is no difference in converting enzyme quantity. Basal aldosterone synthesis is also similar but seven times more corticosterone is synthesised by DI than by non-DI cells. DI cleavage activity is higher and converting activity lower therefore. Neither of these differences suggests quantitative changes in the control enzymes (ACTH-stimulated aldosterone synthesis as a percentage of aldosterone converted from added corticosterone is similar for DI and non-DI cells). A high cleavage activity coupled with low converting enzyme activity are compatible with observations [6] that DI rats have high plasma angiotensin levels but low plasma potassium. Moreover DI rats may be potassium wasting and indeed a potassium deficient diet reduces the specific activity of the aldosterone product of rat z. glomerulosa strippings incubated with [^{3}H]-corticosterone [10].

In vitro stimulation of DI cells generally increased aldosterone synthesis more than corticosterone. If basal cholesterol cleavage is high, activation of converting enzymes may occur, especially as the potential for corticosterone conversion is great. Non-DI z. glomerulosa cells respond to *in vitro* stimulation with increases in corticosterone synthesis which are at least as great as increases in aldosterone; cholesterol cleavage is activated.

Basal aldosterone synthesis in DI cells is increased 17 fold by sodium deprivation which is partly derived from a quantitative increase in converting enzyme

since five times more aldosterone was produced from added corticosterone. The quantity of cholesterol cleavage complex may also change. Aldosterone synthesised by ACTH-stimulated cells of sodium deficient rats was 10.8% of the aldosterone converted from added corticosterone compared with 3.8% in cells of control diet rats. Sodium deprivation of DI rats increased z. glomerulosa responsiveness to ACTH; twice as much corticosterone was synthesised.

The basal aldosterone synthesis response by cells of non-DI rats to sodium deprivation is smaller. Four times more aldosterone was synthesised and most of this increase reflects the quantity of converting enzymes (3 times more aldosterone was converted from added corticosterone). However the ratio of ACTH-stimulated aldosterone synthesis to aldosterone converted from added corticosterone was three times greater for cells of sodium deprived rats than it was for cells of control diet rats, indicating a quantitative difference in cleavage complex. The responsiveness of non-DI cells to *in vitro* stimulants was not greatly altered by sodium deprivation.

The present results agree with previous observations that DI rats are less responsive to stress[4, 5], in that ACTH stimulated z. fasciculata/reticularis cells of DI rats to a lesser extent than cells of non-DI rats. However Yates *et al.*[3] found that while stress produced a smaller increase in plasma corticosterone in DI than in non-DI rats, ACTH injections had similar effects and concluded that CRF activity in DI rats was impaired. The present studies confirm observations[5] that the reduced stress response also concerns ACTH action on the adrenal cortex.

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