EFFECT OF ACTH ON ^{125}I-LABELED ANGIOTENSIN II BINDING AND RESPONSE BY RAT ADRENAL GLOMERULOSA CELLS

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1. Introduction

Angiotensin (Ag II) is a potent stimulator of aldosterone production in a number of experimental animals and humans [1–5]. Studies using dog and rat adrenal glomerulosa cells have shown that these cells are highly responsive to physiological concentrations of Ag II [2,3]. The demonstration of specific Ag II receptors in glomerulosa cells of dog and rat adrenals, with a correlation between ^{125}I-labeled Ag II binding and aldosterone production further established the importance of Ag II [2,3]. However, studies with primary monolayer cultures of rat adrenal glomerulosa cells [6,7] showed that Ag II at 1 or 100 μg/ml failed to stimulate aldosterone production. A transient stimulation was noticed only with the addition of ACTH. It was also shown that under the influence of ACTH the mitochondrial cristae of cultured cells changed from the characteristic tubular form to vesicular form typical of fasciculata cells. Earlier studies [8] have shown that when endogenous steroid secretion was examined in glomerulosa cell cultures, under long term treatment, the pattern of steroid secretion changed from glomerulosa cells to fasciculata type. The cells produced more corticosterone than aldosterone, which is characteristic of glomerulosa cells. Similar changes in steroid secretion pattern and suppression of plasma aldosterone, after ACTH treatment for 4 days were reported [9]. Based on these studies [7,9] it was concluded that during prolonged ACTH treatment the glomerulosa cells may be converted to a functional fasciculata type of cell. Studies using ^{125}I-labeled Ag II [2,3] have shown that specific AG II receptors are localized mainly in the glomerulosa cells and not in the fasciculata cells. It is well established now that the first event in mediation of peptide hormone action is binding of the hormone to target cell [10]. In view of the significant morphological and functional changes induced by ACTH in glomerulosa cells it was considered important to examine whether ACTH treatment also induces changes at the Ag II receptor level. In this study we report the original observation that treatment of adult male rats with synthetic ACTH for 4–8 days resulted in a significant decrease in binding of ^{125}I-labeled Ag II by adrenal glomerulosa cells compared to the untreated groups. Along with this a parallel significant decrease in plasma concentration of aldosterone and in vitro response of the cells to both ACTH and Ag II was noticed.

Abbreviations: ACTH, adrenocorticotropic hormone; Ag II, angiotensin II; BSA, bovine serum albumin

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2. Materials and methods

Adult hooded black and white male rats (300–350 g body wt) were used. For the first two experiments the animals were maintained in groups of 5, and in the third experiment, they were kept in individual cages.

Synthetic ACTH (synacten or cortrosyn) were obtained from Ciba-Geigy Ltd, Canada and synthetic Ag II from Peninsula Labs., San Carlos, CA. Hormones

were administered in 0.5% gelatin by i.p. route for 4–8 days at 50–150 $\mu g \cdot rat^{-1} \cdot day^{-1}$.

Twenty four hours after the last injection the animals were sacrificed by stunning and trunk blood collected plasma separated and stored at –20°C until further processing. Cell suspensions from the pooled capsular tissue from each group were prepared by the method in [11].

Binding studies were by the method in [3]. Briefly, suspension of cells ($5–10 \times 10^4$) in medium 199 (Gibco) with 2 mg bovine serum albumin/ml and 5 mM potassium were incubated in the presence of $\sim 10^5$ dpm of ^{125}I-labeled Ag II in polypropylene vials for 45 min at 37°C under 95% O_2 and 5% CO_2. The total incubation volume was 1 ml. Non-specific binding was determined in the presence of 10^{-6} M non-radioactive Ag II. The separation of bound ^{125}I-labeled Ag II from free hormone was achieved by filtration on Millipore filters HAWP (0.45 μm) nitrocellulose filters. The filters were washed twice with 10 ml cold, phosphate buffered saline (0.15 M NaCl, 0.01 M sodium phosphate (pH 7.4) and after air drying radioactivity was determined in a Beckman Model 9000 γ-counter. Non-specific binding was generally <1%. Values are reported as fg Ag II bound/ 10^5 cells.

In one experiment, $\sim 5 \times 10^4$ glomerulosa cells/ tube were incubated with or without Ag II or ACTH in medium 199 for 2 h at 37°C under 95% O_2 and 5% CO_2. After the incubation the tubes were stored at –20°C.

Plasma or cell suspensions were extracted with dichloromethane, the extract evaporated to dryness under a stream of air and reconstituted in 0.1 M phosphate buffer (pH 7.2) containing 5% bovine serum albumin. Suitable aliquots were used for determination of aldosterone by radioimmunoassay [12]. Recovery was monitored using [^{3}H]aldosterone and was generally >90%. Statistical significance was tested by Student's 't'-test.

3. Results

The regulation of function of zona glomerulosa cells appears to be under the control of several factors. Available evidence based on in vivo and in vitro experiments suggests that ACTH plays a significant role. The results obtained in the present study lend support to this conclusion. Administration of ACTH to male rats for 4–8 days caused a significant decrease in the quantity of ^{125}I-labeled Ag II bound by the glomerulosa cells compared to the cells from control group (table 1), suggesting a partial loss of one of the features characteristic of glomerulosa cells. The decrease which was observed in all the three experiments ranged from 19–54% and seems to be influenced by the duration and/or dose of ACTH treatment.

Table 1
Effect of in vivo ACTH treatment on ^{125}I-labeled Ag II binding by rat adrenal glomerulosa cells

	fg Ag II bound/10^5 cells		
	Expt 1	Expt 2	Expt 3
Control	1004 ± 58	1648 ± 26	964 ± 42
ACTH treated	812 ± 33[a]	1010 ± 111[b]	444 ± 17[b]
Decrease (%)	19	39	54

[a] $P = 0.05–0.025$
[b] $P < 0.001$

Expt 1, 50 μg ACTH $\cdot rat^{-1} \cdot day^{-1}$ for 4 days, $N = 5$; expt 2, 100 μg ACTH $\cdot rat^{-1} \cdot day^{-1}$ for 8 days, $N = 9$; expt 3, 150 μg ACTH $\cdot rat^{-1} \cdot day^{-1}$ for 8 days, $N = 8$. All values mean ± SE. N = number of determinations

The plasma aldosterone concentration indicates that not only a decrease in binding but also a functional change has been induced. There is a significant decrease in the plasma aldosterone compared to the control group. The decrease in corticosterone levels, however is not statistically significant. The results presented in table 2 are from one experiment

Table 2
Effect of ACTH treatment on plasma aldosterone and corticosterone concentrations

	Aldosterone (ng/dl)	Corticosterone (μg/dl)
Control	14.6 ± 1.66	1.08 ± 0.45
ACTH-treated	8.2 ± 1.07[a]	0.66 ± 0.19[b]

[a] $P < 0.001$
[b] P, not significant

Data from expt 3 of table 1. Values represent mean ± SE of 8 determinations

Table 3
Effect of in vivo ACTH treatment on in vitro response of rat adrenal glomerulosa cells to Ag II and ACTH

Group	ng aldosterone/10^5 cells	
	Control	Treated
No hormone	2.03 ± 0.06	0.80 ± 0.14
Ag II 1×10^{-9} M	4.34 ± 0.09	0.71 ± 0.07
Ag II 1×10^{-8} M	9.00 ± 0.61	0.89 ± 0.05
Ag II 1×10^{-7} M	5.00 ± 0.03	0.71 ± 0.14
ACTH 0.3×10^{-12} M	2.38 ± 0.12	0.42 ± 0.10
ACTH 0.3×10^{-11} M	7.19 ± 0.90	0.76 ± 0.15
ACTH 0.3×10^{-10} M	12.30 ± 1.32	1.33 ± 0.15

Approximately 5×10^4 cells/tube were incubated with or without hormone for 2 h at 37°C under 95% O_2 and 5% O_2. Aldosterone were determined by radioimmunoassay after extraction of cell suspension with dichloromethane. Values are mean ± SE of triplicate determinations

where the rats were caged individually in order to minimize the stress. In the other two experiments a significant decrease in plasma aldosterone was also noticed although the absolute values in the control and treated group were higher than those presented.

The results of the in vivo ACTH treatment on the in vitro response of the glomerulosa cells to Ag II and ACTH are presented in table 3. It can be seen that while the cells from control group exhibited the expected response, to both hormones, the response of cells from treated group is greatly diminished.

4. Discussion

The differentiation of adrenal cortical cells into specific glomerulosa and fasciculata cell types and its regulation is poorly understood. The role of ACTH in differentiation of cortical cells in tissue culture of human fetal adrenals has been investigated [13]. They observed that cortical cells capable of proliferation in culture had the ultrastructure of the permanent zone cells of the fetal adrenal or adult zona glomerulosa type. ACTH stimulation induced a differentiation of these cells into zona fasciculata like cells. Similar results have been reported [7–9]. Although Ag II plays a significant role in regulation of aldosterone production by glomerulosa cells, ACTH appears to have a dominating influence over the morphological and functional differentiation of these cells. In view of this it is logical to expect that ACTH treatment should induce a change at the receptor level. To investigate this, advantage has been taken of the fact that Ag II receptors are primarily localized in the glomerulosa cells. The results of this study show that in addition to the functional change induced by ACTH as indicated by decreased plasma aldosterone and response to Ag II a change at the receptor level has also been induced. The long term effects of ACTH on plasma Ag II or response of adrenals to Ag II have not yet been studied in detail. Available studies show that the kidney renin content was found to be markedly diminished in rats bearing an ACTH growth hormone and prolactin secreting tumor [14]. Plasma renin activity was found to be decreased in dogs after long-term ACTH treatment [15]. It is not clear whether the structural and functional changes induced by ACTH administration are mediated directly at the adrenal level or by its action on the renin–angiotensin system.

The regulation of one hormone receptor by homologous or heterologous hormones has been reported [10]. Thus, hCG administration to rats causes a decrease in LH binding by rat Leydig cell [16]. The phenomenon of desensitization has been implicated in order to explain this. The observed decreased response to ACTH by glomerulosa cells could also be due to the desensitization phenomenon. The fact that the differentiation induced by ACTH can be observed at the receptor ultrastructural and functional level makes the glomerulosa cell system unique.

The concentration of rat plasma corticosterone was not increased after 8 days of ACTH treatment (table 2). In this particular experiment, rats received their last ACTH injection in the morning at 11.00 h and were sacrificed 24 h later. The low corticosterone concentration found may be tentatively explained by the assumption that at the time, the plasma samples were taken, the adrenals could have been in a period of replenishment of the precursors necessary for the synthesis of corticosterone. However, more work is needed to clarify this point.

Ths high dose of ACTH administered was chosen based on the studies [9] and on the reported very short half-life of ACTH [17]. The use of ACTH tumor bearing rats with chronic high levels of ACTH

would be a suitable model to investigate the action of ACTH at the glomerulosa cell level.

In conclusion, our results are in agreement with the observations of [7,9] showing that decreased production of aldosterone is due to a morphological change of glomerulosa to fasciculata cell forms.

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