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ISOLATED GUINEA-PIG ADRENAL CELLS: EFFECTS OF PHYSIOLOGICAL CONCENTRATIONS OF ACTH, ANGIOTENSIN II AND POTASSIUM CHLORIDE

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SUMMARY

The effects of physiological concentrations of ACTH, angiotensin II (A II) and potassium chloride (KCl) on dispersed guinea-pig adrenal cells obtained by tryptic digestion have been investigated.

ACTH was fully effective on glucocorticoid output at a concentration of 0.85×10^{-12} M whereas some production was also observed with 2.4×10^{-6} M A II and 10 mM KCl. With regard to aldosterone output, the minimum ACTH and A II doses required were 0.85×10^{-11} M and 2.4×10^{-12} M, respectively. The addition of 0.3 mM KCl was sufficient to alter the aldosterone level significantly. The maximal steroid response was obtained with either 0.85×10^{-8} M ACTH or 10.6 mM KCl while high doses of A II were comparatively less effective.

INTRODUCTION

Following the establishment of the collagenase method by Haning *et al.*[1] and the trypsin technique by Sayers *et al.*[2], *in vitro* experiments on isolated adrenal cells have been developed. The control of adrenal steroid biosynthesis has since been extensively studied in various animal species and the most recent papers describe sensitive adrenal cell systems obtained from the dog or rat [3–6]. Although the guinea-pig has been used infrequently [7], its adrenals would seem to provide a model closer to the human adrenals than other animals in view of the fact that the main circulating glucocorticoid is cortisol [8, 9] and the mineralocorticoid is aldosterone [10]. Steroidogenesis presumably follows the same route as in man with two distinct pathways culminating in either cortisol or aldosterone.

In the present paper, we describe a sensitive system, using dispersed guinea-pig adrenal cells, for studying the control of these two hormones by physiological concentrations of ACTH, angiotensin II (A II) and potassium chloride (KCl).

MATERIALS AND METHODS

α-1 cosyntropin and angiotensin II amide (Synacthen and Hypertensin) were obtained from Ciba Pharmaceuticals.

Cell dispersions and incubations were performed in Eagle's minimum essential medium with Earle's saline solution (MEM, Eurobio Laboratories). Five or six tricoloured guinea-pig males (500–700 g) were used for each experiment. They were anaesthetized by intraperitoneal injection of sodium barbital (6 mg/100 g body wt.) before sacrifice by incision of the abdominal aorta. The adrenals were removed, freed of fat and extraneous tissue and minced into 0.8–1.0 mm fragments with a tissue chopper. The fragments were washed three times with MEM and treated five or six times with 20 ml trypsin solution according to the protocol of Lowry *et al.*[11], with some minor modifications. The trypsin concentration (Trypsin 2×, Precibio) was reduced to 0.2 g per 100 ml MEM and the first 20 ml of the trypsin digest discarded. The combined harvests were centrifuged at 4°C after a slow acceleration to 200 *g*. The pellet was washed with 20 ml MEM containing 0.1% lima bean trypsin inhibitor (Worthington Biochemical Corp.) (MEM–LB1). After a second centrifugation, the pellet was re-suspensed in 20 ml MEM-LB1 and filtered through nylon gauze (100 μm pore size). 8×10^5–1.6×10^6 cells per adrenal were thus obtained. The cell suspension was adjusted to a final concentration of 3×10^5–5×10^5 cells/ml with MEM–LB1.

ACTH, A II and KCl were diluted in 0.9% NaCl solution containing 0.5% bovine serum albumin (Miles laboratory) and acidified to pH 3.5 with 0.1 N HCl. 0.9 ml cell suspension was added to 0.1 ml of each dilution to be tested in a Teflon beaker. All experiments were carried out in triplicate.

Cell suspensions were incubated for 90 min at 37°C under an atmosphere of 95% O_2 and 5% CO_2 in a Dubnoff shaker. They were then stored at −20°C until the steroid assay.

1 ml of the steroid incubate was extracted with 10 ml dichloromethane. 1 ml of extract was used for glucocorticoid determination by protein binding analysis [12] and 0.5 ml for the radio-immunoassay of aldosterone [13].

The proportions of corticosterone and cortisol among the glucocorticoids produced were established by chromatography of the extracts on LH 20 Sephadex (Pharmacia) using a dichloromethane-methanol system (98:2, v/v)[14] and collection of 1 ml fractions. The samples were then submitted to competitive protein-binding analysis.

RESULTS

Both basal and stimulated steroid levels varied from one experiment to another. Basal glucocorticoid outputs ranged from 15 to 40 ng/0.9 ml cell suspension/90 min and basal aldosterone outputs from 220 to 1230 pg/0.9 ml cell suspension/90 min. Nevertheless, in the different experiments, the ratios of steroid output stimulated by any one dose of stimulant to the corresponding basal level were comparable. The results are therefore expressed in terms of the ratio between stimulated and basal outputs.

ACTH stimulation

Full glucocorticoid production started under the influence of 0.85×10^{-12} M ACTH which provoked an output of 262% of the basal output. Maximum production (800%) was obtained with 0.85×10^{-9} M ACTH. The ACTH concentration inducing half maximum production (Km) was found to be 0.78×10^{-11} M. (Fig. 1).

In one experiment carried out with 6×10^5 cells per beaker, the sensitivity was lowered to 0.51×10^{-12} M ACTH. The maximum output was 3500% and the K_m 2×10^{-11} M.

The minimum ACTH concentration effective for aldosterone production was 0.85×10^{-11} M. Maximum production was seen with 0.85×10^{-8} M

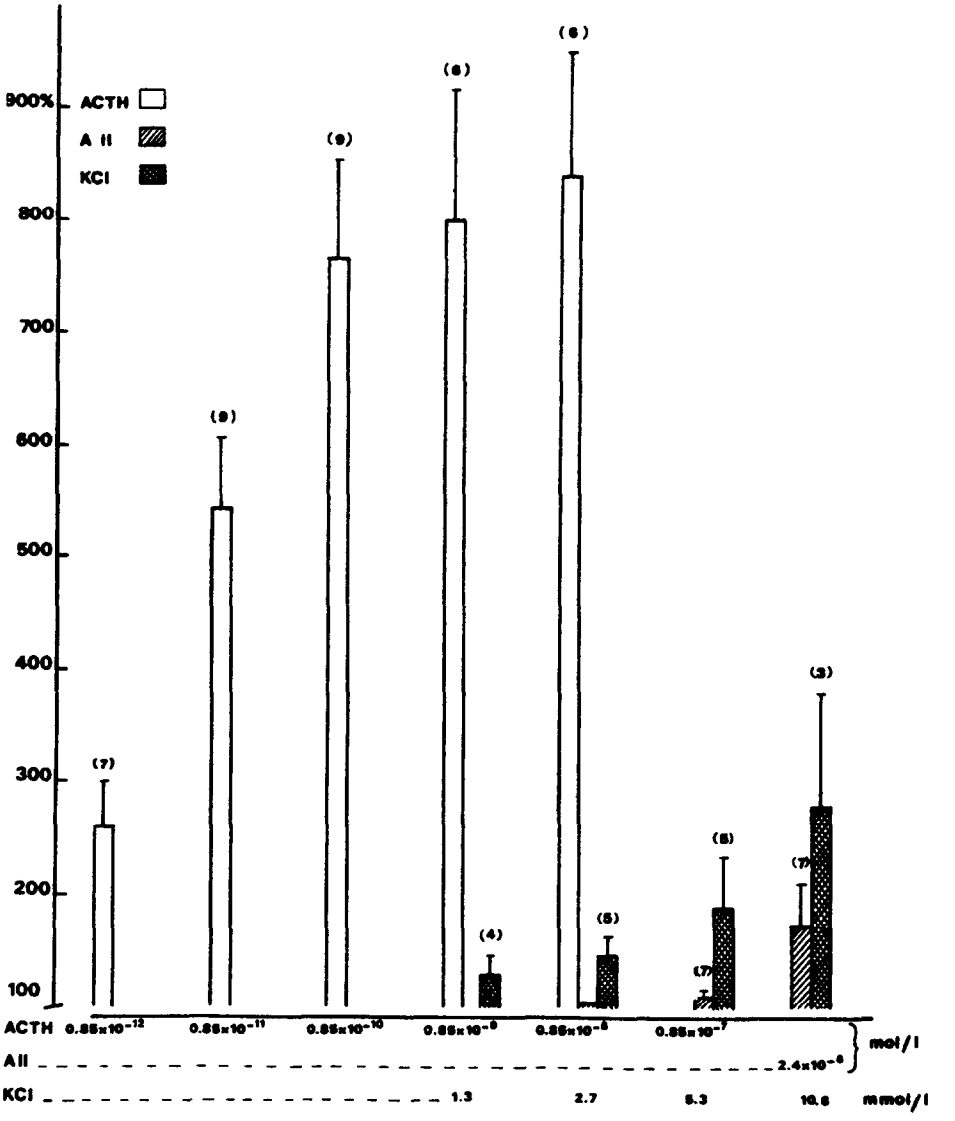

Fig. 1. Glucocorticoid outputs induced by ACTH, angiotensin II and potassium chloride. Potassium and sodium concentrations of the medium equal 5.3 mM and 142 mM respectively. Columns represent the mean ± SE and numbers in brackets indicate the number of experiments.

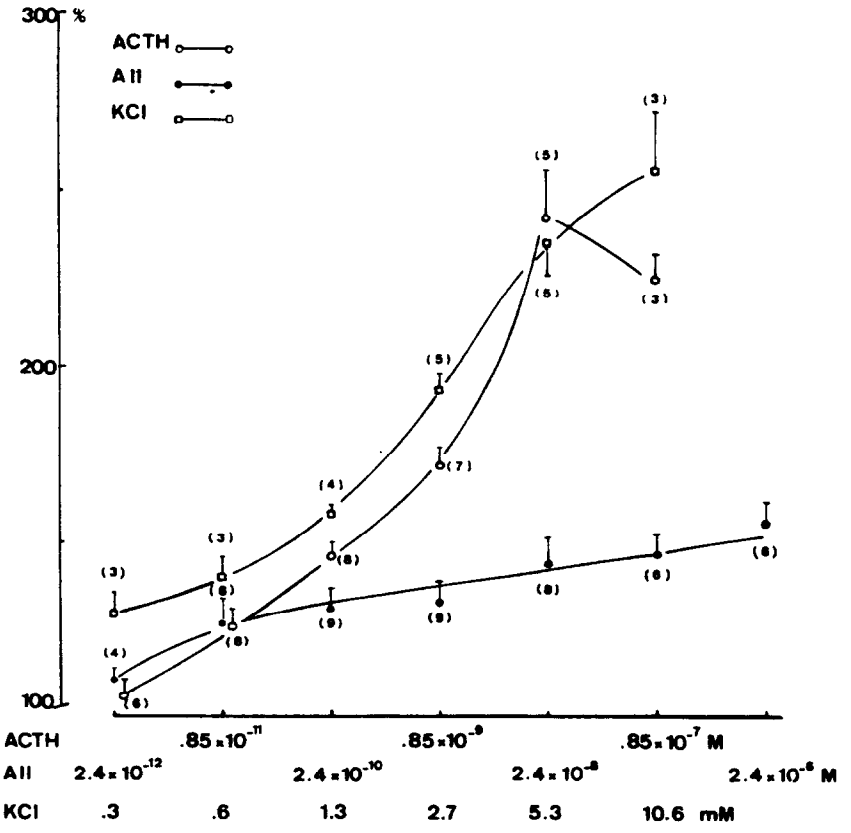

Fig. 2. Effects of ACTH, angiotensin II and potassium chloride on aldosterone output. Potassium and sodium concentrations of the medium equal 5.3 mM and 142 mM respectively. Points represent the mean ± SE and numbers in brackets indicate the number of experiments.

ACTH and the concentration provoking half maximum production was 2.62×10^{-10} M. (Fig. 2).

Angiotensin II stimulation

Some glucocorticoid production could be observed with 2.4×10^{-6} M A II (Fig. 1). Aldosterone output began with 2.4×10^{-12} M A II. The response increased very slowly only reaching a ratio of 156% ± 6.6 with 2.4×10^{-6} M A II (Fig. 2). The Km, calculated from the first five points, was 2.22×10^{-10} M.

Potassium chloride stimulation

Glucocorticoid output was obtained when 5.3 mM KCl was added to medium which already contained 5.3 mM. This effect was, however, variable (Fig. 1). The corticosterone to cortisol ratio was 9%, whereas with ACTH stimulation was 3%.

A significant increase in aldosterone production was seen with the addition of as little as 0.3 mM KCl to the medium and a plateau was observed at a final concentration of 10.6 mM (Fig. 2).

DISCUSSION

The control of adrenal steroidogenesis by physiological concentrations of ACTH, A II and KCl has been investigated using a *in vitro* system which is the most sensitive known to date.

The patent role of ACTH in glucocorticoid production is quite clear as 0.85×10^{-12} M was sufficient to induce fully the glucocorticoid response. This concentration corresponds to the lowest detectable level (radio-immunoassay) of ACTH in man [15].

The action of A II proved of particular interest. On its own, AII increased glucocorticoid output, but only from a concentration of 2.4×10^{-6} M. This confirms the findings of McKenna *et al.* who used isolated human adrenal cells [16].

The increased glucocorticoid output observed under the influence of KCl was unexpected. Increased K^+ concentrations are known to stimulate steroidogenesis in glumerulosa cells, but not in fasciculata cells [2, 17]. However, as KCl affects the transformation of cholesterol to pregnenolone [18, 19] in the course of aldosterone biosynthesis and this step is also part of cortisol synthesis, it could be imagined that an excess of pregnenolone might lead to cortisol formation. Stimulation by KCl was variable and occured only at a final concentration of 10.6 mM. In this case the corticosterone to cortisol ratio was higher than that seen after ACTH stimulation. This would fit in with the notion of preferential stimulation by KCl of the aldosterone synthesis pathway.

The minimum dose of A II required to start aldosterone production was 2.4×10^{-12} M. To date, this has been the lowest concentration found to provoke a significant in vitro aldosterone response. The threshold sensitivity of aldosterone to ACTH was predictably, although only moderately higher (0.85×10^{-11} M) but this level is still within the range of physiological concentrations [15]. The maximum steroidogenic response was induced by ACTH and not by A II, as has already been reported, both *in vivo* [20] and *in vitro* [6]. This discrepancy would imply a de-sensitization of the glomerulosa cells to A II, which has been suggested by Bing and Schulster[6] and which may be caused by the A II itself. The response was marked at 2.4×10^{-12} M and 2.4×10^{-11} M but failed to increase much with higher concentrations. However, the possibility of a massive destruction of A II receptors by the enzymatic treatment should not be ruled out.

The effects of KCl on aldosterone output could be seen with the addition of as little as 0.3 mM. The maximum steroidogenic response, reached at 10.6 mM (i.e. twice the initial concentration), was of the same order of magnitude as that provoked by 0.85×10^{-8} M ACTH. This emphasizes the importance of physiological changes in K^+ concentrations and confirms the rôle of extracellular potassium in the control of aldosterone secretion which has already been underlined by other authors [21–24].

It is evident that in the guinea-pig, ACTH plays a major part and the importance of extra-cellular potassium must be stressed as physiological variations in these two stimuli could cause significant changes in aldosterone responses. A II acted at low concentrations, strictly comparable to circulating levels, but at high concentrations, it was less effective than ACTH as a stimulus for aldosterone production.

REFERENCES

1. Haning R., Tait S. A. S. and Tait J. F.: *In vitro* effects of ACTH, angiotensins, serotonin and potassium on steroid out-put and conversion of corticosterone to aldosterone by isolated adrenal cells. *Endocrinology* **87** (1970) 1147–1166.
2. Sayers G., Swallow R. L. and Giordano N. D.: An improved technique for the preparation of isolated rat adrenal cells: a sensitive, accurate and specific method for the assay of ACTH. *Endocrinology* **88** (1971) 1063–1068.
3. Peytreman A., Nicholson W. E., Brown R. D., Liddle G. W. and Hardman J. G.: Comparative effects of angiotensin and ACTH on cyclic AMP and steroidogenesis in isolated bovine adrenal cells. *J. clin. Invest.* **52** (1973) 835–842.
4. Peach M. J. and Chiu A. T.: Stimulation and inhibition of aldosterone biosynthesis in vitro by angiotensin II and analogs. *Circ. Res. Supp.* **I**, XXXIV–XXXV (1974) 7–13.
5. Fredlund P., Saltman S. and Catt K. J.: Aldosterone production by isolated adrenal glomerulosa cells: stimulation by physiological concentrations of angiotensin II. *Endocrinology* **97** (1975) 1577–1586.
6. Bing R. F. and Schulster D.: Steroidogenesis in isolated rat adrenal glomerulosa cells: response to physiological concentrations of angiotensin II and effects of potassium, serotonin and (Sar[1], Ala[8])-angiotensin II. *J. Endocr.* **74** (1977) 261–272.
7. Kolanowski J., Ortega N. and Crabbé J.: Réponse des cellules corticosurrénales isolées de rat et de cobayes à la corticotropine et à l'AMP cyclique. *Ann. Endocr. (Paris)* **35** (1974) 501–507.
8. Kolanowski J., Pizzaro M. A., Crabbé J. and Sayers G.: A lasting effect of ACTH on adrenocortical steroidogenesis: *in vivo* and *in vitro* studies on guinea-pig. *Acta endocr. Copenh. suppl.* **177** (1973) 86.
9. Dalle M. and Delost P.: Changes in the concentrations of cortisol and corticosterone in the plasma and adrenal glands of the guinea-pig from birth to weaning. *J. Endocr.* **63** (1974) 483–488.
10. Malinowska K. W. and Nathanielsz P. N.: Plasma aldosterone concentration in the new-born guinea-pig. *Endocrinology* **59** (1973) XXXII.
11. Lowry P. J., McMartin C. and Peters: Properties of a simplified bioassay for adrenocorticotrophic activity using the steroidogenic response of isolated adrenal cells. *J. Endocr.* **59** (1973) 43–55.
12. Pham-Huu-Trung M. T.: Dosage du cortisol plasmatique par liaison compétitive à la transcortine. *Ann. Biol. Clin.* **28** (1970) 145–152.
13. Pham-Huu-Trung M. T. and Corvol P.: A direct determination of plasma aldosterone. *Steroids* **24** (1974) 587–598.
14. Murphy B. E. P.: Sephadex column chromatography as an adjunct to competitive protein binding assays of steroids. *Nature (New Biol.)* **232** (1971) 21–24.
15. Pham-Huu-Trung M. T., Gourmelen M., Raux-Eurin M. C. and Girard F.: Pituitary adrenal axis activity in treated congenital adrenal hyperplasia: static and dynamic studies. *J. Clin. Endocr. Metab.* **47** (1978) 422–427.
16. McKenna T. J., Island D. P., Nicholson W. E. and Liddle G. W.: Angiotensin stimulates cortisol biosynthesis in human adrenal cells *in vitro*. *Steroids* **32** (1978) 127–136.
17. Kaplan N. M.: The biosynthesis of adrenal steroids: effects of angiotensin II, adrenocorticotropin and potassium. *J. clin. Invest.* **44** (1965) 2029–2039.
18. Williams G. H. and Dluhy R..G.: Aldosterone biosynthesis. Interrelationship of regulatory factors. *Am. J. Med.* **53** (1972) 595–605.
19. Laragh J. H., Sealey J. and Brunner H. R.: The control of aldosterone secretion in normal and hypertensive man: abnormal renin-aldosterone patterns in low renin hypertension. *Am. J. Med.* (1972) 649–663.
20. Rayyis S. S. and Horton R.: Effect of angiotensin II on adrenal and pituitary function in man. *J. clin. Endocr.* **32** (1971) 539–546.
21. Himathongkam T., Dluhy R. G. and Williams G. H.: Potassium–aldosterone–renin interrelationships. *J. clin. Endocr. Metab.* **41** (1975) 153–159.
22. Tait J. F. and Tait A. S.: The effect of changes in potassium concentration on the maximal steroidogenic response of purified zôna glomerulosa cells to angiotensin II. *J. steroid. Biochem.* **7** (1976) 687–690.
23. Fredlund P., Saltman S., Kondo T., Douglas J. and Catt K. J.: Aldosterone production by isolated glomerulosa cells: modulation of sensitivity to angiotensin II and ACTH by extra-cellular potassium concentration. *Endocrinology* **100** (1977) 481–486.
24. Williams G. H. and Braley L. M.: Effects of dietary sodium and potassium intake and acute stimulation of aldosterone output by isolated human adrenal cells. *J. clin. Endocr. Metab.* **45** (1977) 55–64.