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THE EFFECT OF CHANGES IN POTASSIUM CONCENTRATION ON THE MAXIMAL STEROIDOGENIC RESPONSE OF PURIFIED ZONA GLOMERULOSA CELLS TO ANGIOTENSIN II

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SUMMARY

Alterations in potassium concentration change the corticosterone output of purified zona glomerulosa cells. These were prepared by enzymic digestion of capsular tissue from rat adrenals followed by unit gravity sedimentation. These changes occur for both unstimulated and angiotensin maximally stimulated cells (800 μg/ml angiotensin II). For all experiments, corticosterone output was minimal at the lowest K^+ concentrations used (2 mM) and then increased (3.6 and 5.9 mM) until a maximum output was obtained (8.4 mM). The output then decreased at 13 mM. Similar results were previously obtained when maximal stimulation was achieved with cyclic AMP.

The corresponding changes in aldosterone output as a function of potassium concentration with both unstimulated and angiotensin maximally stimulated cells had the same characteristics. However the responses were greater (stimulated/basal output) than for corticosterone.

The overall results confirm that a simple second messenger hypothesis concerning a correlation between K^+ and cyclic AMP concentrations cannot be accepted for zona glomerulosa cells. These *in vitro* data may be relevant to results in man which indicate sensitization of the actions of angiotensin on the adrenal by salt deprivation.

INTRODUCTION

In previous work [1, 2], the effect of changes in K^+ concentration on the steroidogenesis of rat adrenal glomerulosa cells, maximally stimulated by serotonin and cyclic AMP, was studied. It was intended to show whether there were any effects of K^+ concentration on steroidogenesis other than those due to alterations in cyclic AMP concentration. As certain changes in K^+ concentration alter the cyclic AMP output of the zona glomerulosa cells, maximum stimulation with serotonin and cyclic AMP at any particular K^+ concentration was employed. Any effect of K^+ concentration would then presumably be due to the modification of the action of cyclic AMP or to mechanisms not involving the nucleotide. It was found that alterations in steroidogenesis did occur with changes in K^+ concentrations using maximum stimulation with serotonin or cyclic AMP [2].

Angiotensin II used at high enough concentrations has also been shown to stimulate both steroidogenesis and the cyclic AMP output of pure zona glomerulosa cells [2, 3]. It was therefore of interest to study the effect of K^+ concentration on the steroidogenesis of zona glomerulosa cells maximally stimulated with angiotensin II. Apart from the relevance to previous experiments with serotonin and cyclic AMP, there was the possibility of this *in vitro* system being a useful model for certain *in vivo* effects. In particular the comparison of such *in vitro* results with the additional or modifying effects of sodium deprivation on the stimulation of adrenals with angiotensin II could be instructive. In order to make such comparisons more valid, both aldosterone and corticosterone outputs were measured.

The concentration of angiotensin II required to stimulate zona glomerulosa cells maximally also notably increases the steroidogenesis of zona fasciculata cells under our conditions. It was therefore necessary to employ adrenal cells purified by 1 g sedimentation after enzymic dispersion of capsular tissue. This preparation of zona glomerulosa cells contained no significant numbers of contaminating zona fasciculata cells [3]. For the same reason, corresponding experiments with cyclic AMP had also to be carried with a purified cellular preparation [2].

EXPERIMENTAL

Methodology. Dispersed zona glomerulosa cells were prepared by collagenase (Worthington Biochemical Corporation, batch No CLS 44 A 181) treatment of adrenal capsular tissue from female Sprague–Dawley rats, 150–200 g, as previously described [3]. The cells were purified from contaminating fasciculata-reticularis cells by 1 g sedimentation [3]. The peak fractions containing zona glomerulosa cells were bulked, centrifuged and redispersed in Krebs–Ringer

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bicarbonate buffer containing 3.8 mmol K^+, 2 g glucose and 40 g BSA (Pentex Fraction V) per litre, (KRBGA). The cell concentration of the final solution was determined using an improved Neubauer Haemocytometer.

Incubations were carried out in 10-ml Teflon beakers in a total vol. of 1 ml comprising 0.45 ml of the cell suspension, containing approximately 1.4×10^5 cells, 0.05 ml normal saline and 0.5 ml KRBGA. The K^+ content of the KRBGA was adjusted to give a final concentration of 2, 3.6, 5.9, 8.4 and 13 mmol K^+/l. Duplicate incubations were carried out at each K^+ concentration. Angiotensin, valine-5-angiotensin II amide, hypertensin, Ciba was added in 0.05 ml normal saline to one of the duplicate beakers to give a final concentration of 800 μg/ml.

Incubation was for 2 h in a metabolic shaker at 37 C under 95% O_2, 5% CO_2. At the end of the incubation, the medium and cells were diluted to 10 ml with distilled water, 3 ml from each sample was used for corticosterone and 7 ml for aldosterone measurement. 15,000 d.p.m. 1,2-^{3}H corticosterone, 68 pg and 10,000 d.p.m. [1, 2, 6, 7-^{3}H]-aldosterone, 16, pg were added to the appropriate samples as recovery indicators and the total vol. adjusted to 10 ml with distilled water.

Extraction was carried out by the method of Tait *et al.* [4]. The dried extract was dissolved in a suitable vol. of ethanol and corticosterone and aldosterone measured by radio-immunoassay using antibodies against corticosterone 21-hemisuccinate and aldosterone 18, 21-disuccinate albumin conjugates developed in sheep [5]. The method used was a modification of that described by Tait *et al.* [1] whereby the vol. of diluted antiserum and Dextran-coated charcoal per assay tube were decreased from 0.5 to 0.2 ml. This increased the sensitivity of the assay to 250 pg for corticosterone and 25 pg for aldosterone. Results were calculated as previously described [1].

RESULTS

The results of the three experiments with purified zona glomerulosa cells maximally stimulated with angiotensin II are shown in Fig. 1. The effect on corticosterone output was previously achieved with 200 μg/ml angiotensin II at 3.6 mM K^+ [2]. Therefore a minimum dose of 400 μg/ml angiotensin II was used routinely to ensure maximum stimulation at all K^+ concentrations. In the three experiments shown in Fig. 1, angiotensin II concentrations of 400 and 800 μg/ml at 3.6 mM K^+ were both employed and these doses stimulated steroid output to an equivalent and therefore maximal extent.

In every experiment, changes in K^+ concentration markedly affected corticosterone output. This occurred not only with no additional stimulation, as reported previously [1, 2, 6], but also with maximum stimulation by angiotensin II at any particular K^+ concentration. Data from all experiments showed corticosterone production increasing from 2 to 8.4 mM K^+ and then decreasing at 13 mM K^+. The effect on aldosterone output is even more marked but showed again a maximum output at 8.4 mM K^+ with both zero and maximum angiotensin stimulation.

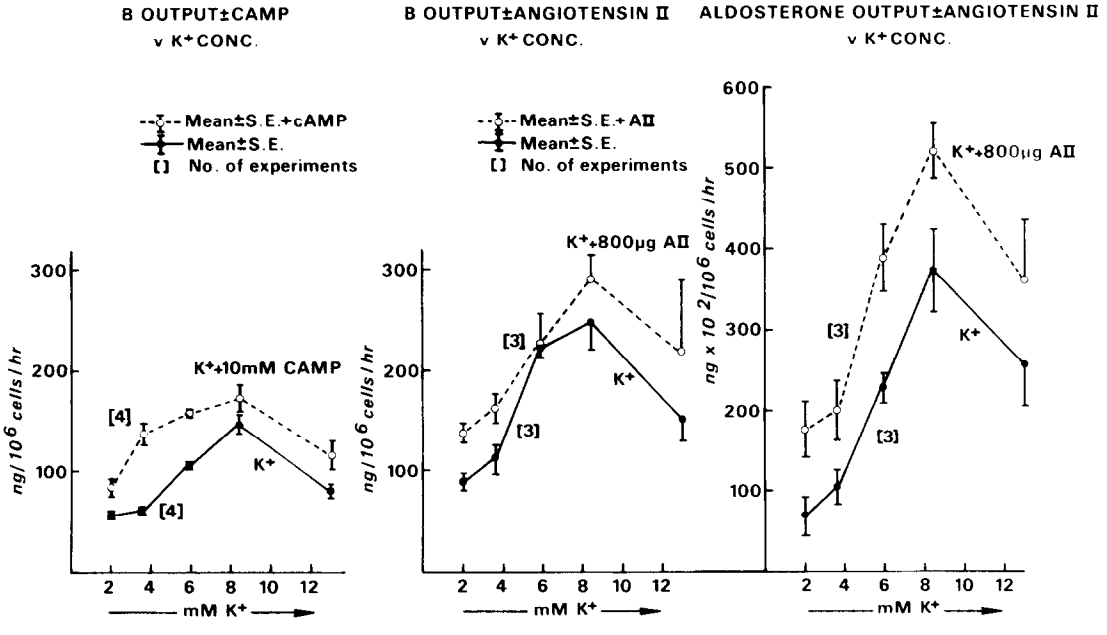

Fig. 1. Effect of changes in potassium concentration of the incubation medium on corticosterone and aldosterone outputs from purified zona glomerulosa cells with and without maximal stimulation by angiotensin II, 800 μg/ml and cyclic AMP 10 mM. Steroid output is expressed as ng per 10^6 cells per h.

Corresponding results from four previous experiments [2] with the same pure preparations of cells showing the effects of K^+ concentration on steroidogenesis maximally stimulated by cyclic AMP, are also presented in Fig. 1. The basal production of corticosterone is lower than in the present experiments but the maximal stimulation (expressed as a ratio of stimulated to basal output) at various K^+ concentrations is similar using either cyclic AMP or angiotensin II. There is a maximum output of corticosterone at 8.4 mM K^+ for both stimuli.

DISCUSSION

The present and previous results (Fig. 1) show that alterations in potassium concentration can change the steroid output of zona glomerulosa cells, maximally stimulated with either cyclic AMP or angiotensin II. The peptide, at the high concentration used, increases the production of cyclic AMP with maximum effectiveness at any particular K^+ concentration. The data therefore indicate that steroidogenesis can be affected by changes in K^+ concentration without alterations in effective cyclic AMP concentrations. The mechanisms for such effects may be to modify the action of cyclic AMP or they may act entirely independently of the nucleotide. The former type of mechanism would include modification of nucleotide–receptor interaction. However, alterations in the internal rate of transport of cyclic AMP to its site of action, rate limited in a particular manner, must be considered a possibility. The second type includes postulated effects of changes in internal calcium distribution which may be affected by external potassium concentrations through interactions between the Na–K and Na–Ca exchange systems [7], and may then act independently of cyclic AMP [8]. Also included in this type of mechanism could be the effects of changes in cyclic GMP levels. As discussed in detail in a previous publication [2], it is extremely difficult to decide between these two possible types of mechanism particularly as a specific inhibitor of cyclic AMP action is not generally available.

Although angiotensin II at high doses increases cyclic AMP output, at lower concentrations steroidogenesis can be stimulated almost to its maximum effect without any significant increase in cyclic AMP output. This type of result has been reported in the stimulation of most steroid producing tissue with concomitant measurement of cyclic AMP outputs [9]. It has also been previously noted for the effects of changes in K^+ concentration on the corticosterone output of zona glomerulosa cells [3]. The major objection to the obvious interpretation of this dichotomy in cyclic AMP and corticosterone outputs is that the measurements of nucleotide may not be related to the biologically effective concentrations. The results of the experiments reported here using both cyclic AMP and angiotensin II stimulation, when presumably the nucleotide has a maximally biologically effective concentration at the site of action and yet changes in K^+ can still modify steroid output, would seem to overcome this objection. At least under these conditions of maximal stimulation, the single second messenger hypothesis regarding the correlation of K^+ effects and cyclic AMP concentrations must be modified [8]. Whether the mechanisms acting other than through cyclic AMP, at high doses of angiotensin with alterations in K^+ concentrations are the same as those operating at lower doses of angiotensin at a particular concentration of K^+, remains to be established. It should be noted that although large doses of angiotensin (400 μg/ml) are required to ensure maximal steroidogenic responses in all situations, significant increases in corticosterone output occur with 0.25 ng/ml in this preparation (unpublished observations).

Aldosterone also responds to alterations in K^+ concentration with angiotensin maximally stimulated cells (Fig. 1). The responses to this steroid (stimulated/basal output) are actually greater than for the corresponding corticosterone outputs. In previous studies it was found that under the conditions of the incubations described here, aldosterone output was a power function of corticosterone production [10]. This was because not only were there the expected effects on aldosterone output of alterations in the production of a major precursor, corticosterone, but the % conversion of corticosterone to aldosterone was also affected by corticosterone concentrations in some type of cooperative phenomenon.

The exaggerated effects of changes in K^+ concentration on aldosterone compared to corticosterone output may be entirely due to this phenomenon. A different experimental design will be necessary to investigate a separate specific effect on the conversion of corticosterone to aldosterone independent of the variations of corticosterone output.

It has been claimed that in man alterations in salt intake can modify the action of angiotensin II in stimulating aldosterone production [11]. Some of the doses of the peptide used could be nearly maximally effective in both salt deplete and replete states but nevertheless the aldosterone output differs in the two stimulated conditions [11]. There has been considerable discussion as to whether this represents a modification of the action of angiotensin or an independent effect. The analogy with the present results and their possible interpretation is evident particularly as Mulrow and co-workers [12] believe that in the rat, salt deprivation increases aldosterone output through alterations in plasma K^+ concentration. However, in man and other species, there is evidence that this is not the primary mechanism [13]. Nevertheless these *in vitro* results may be related to the *in vivo* effects and in particular modification of the action of cyclic AMP by salt deprivation must be considered, as a possible mechanism for any sensitization of the action of angiotensin.

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