METABOLIC REGULATION OF STEROIDOGENESIS IN ISOLATED ADRENAL CELLS OF THE RAT. ACTH REGULATION OF cGMP AND cAMP LEVELS AND STEROIDOGENESIS

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1. Introduction

The model of isolated adrenal cell system [1–3] permits direct studies of ACTH[1] action on isolated target tissue cells. These cells a) respond to microunit amounts of ACTH [1] in the synthesis of corticosterone from endogenous precursors, b) have undetectable levels of phosphodiesterase activity [4] and c) can convert the exogenously added precursors, (20S)-20-hydroxycholesterol, pregnenolone, progesterone and deoxycorticosterone to corticosterone [5–8]. The levels of cAMP produced in response to ACTH have been measured in this preparation [9] but the nucleotide levels and corticosterone production lack a perfect correlation [10]. It is thus possible that ACTH control of steroidogenesis is not entirely due to its presumed second messenger cAMP [5]. The present studies provide evidence that ACTH control of steroidogenesis may be due to both cyclic nucleotides, cAMP and cGMP. Preliminary results of these observations have been presented elsewhere [11,12].

* This investigation constitutes a part of the requirement of the partial fulfillment of the doctoral dissertation of Nahed K. Ahmed.

[1] The trivial names and abbreviations used are: ACTH – adrenocorticotropic hormone; cAMP – adenosine cyclic 3′:5′-monophosphate; cGMP – guanosine cyclic 3′:5′-monophosphate; pregnenolone – 5-pregnen-3β-ol-20-one; progesterone – 4-pregnen-3,20-dione; deoxycorticosterone – 21-hydroxy-4-pregnen-3,20-dione; corticosterone – 11β, 21-dihydroxy-4-pregnen-3,20-dione.

2. Materials and methods

Isolated adrenal cell preparation and the method of incubation with ACTH, cAMP, and cGMP was that already described [1–3,7]. In general, for each isolated adrenal cell preparation, the adrenal glands from 16 rats were used, and the cells from each adrenal (approximately 2×10^6 cells) were resuspended in 0.8 ml of Krebs–Ringer–bicarbonate buffer, pH 7.4, containing 4% albumin and 0.2% glucose. The incubation experiments were conducted in quadruplicate, two of the samples being used for the determination of corticosterone [13] and the other two for the measurement of cAMP and cGMP concentrations as follows:

After 1 hr of incubation 5 ml of 1 M $HClO_4$ and 0.05 ml of 1 M Tris were added to the two vessels in which cyclic nucleotide levels were measured. To each vessel was then added amounts of [^{14}C] cGMP and [^{3}H] cAMP equivalent to 5000 cpm to correct for the recoveries of the two cyclic nucleotides. After homogenizing for 5 min the samples were centrifuged, the supernatant solutions were transferred to a second set of tubes and the pH of the mixtures adjusted to 9 by the dropwise addition of 10 M KOH. The $KClO_4$ precipitate was removed by centrifugation and the supernatant solution was transferred to 1.2–1.5 ml of QAE-Sephadex (acetate form) contained in a glass column, 0.6 cm in diameter. The resin was washed first with 7 ml of distilled water and the eluate was discarded. This was followed by 7 ml of 0.5 M ammonium acetate to elute preferentially the cyclic nucleotides. This se-

cond eluate was then collected and lyophilized to dryness.

The cAMP and cGMP in the above lyophilized residues were separated from each other by the modified method of Kuo and Greengard [14] using columns containing QAE-Sephadex A-25 in the formate form. Elution of the above column with 7 ml 0.5 N formic acid yielded cAMP. Elution with 7 ml of 4 N formic acid gave cGMP. These two fractions were used for the measurement of cAMP and cGMP concentrations. That the separation of the two nucleotides was complete, was assured by counting the aliquots of the two fractions for ^{3}H and ^{14}C cpm, there being no ^{14}C radioactivity in the cGMP fraction and no ^{3}H radioactivity in the cAMP fraction.

The determination of cAMP and cGMP was then accomplished by the method of Gilman [15] using the cAMP binding protein isolated from bovine kidney [16] and the cGMP binding protein isolated from lobster muscle [14].

3. Results and discussion

In order to determine the correlation between the synthesis of corticosterone and the endogenous levels of cAMP and cGMP formed in response to the varying concentrations of ACTH, isolated adrenal cells were incubated with a series of concentrations of ACTH and the levels of corticosterone, cAMP and cGMP measured (fig. 1). Figs. 2 and 3 are the standard curves for the assay of cAMP and cGMP respectively. The results show that in the intact isolated adrenal cell 2.5–10 μunits of ACTH does not raise the level of

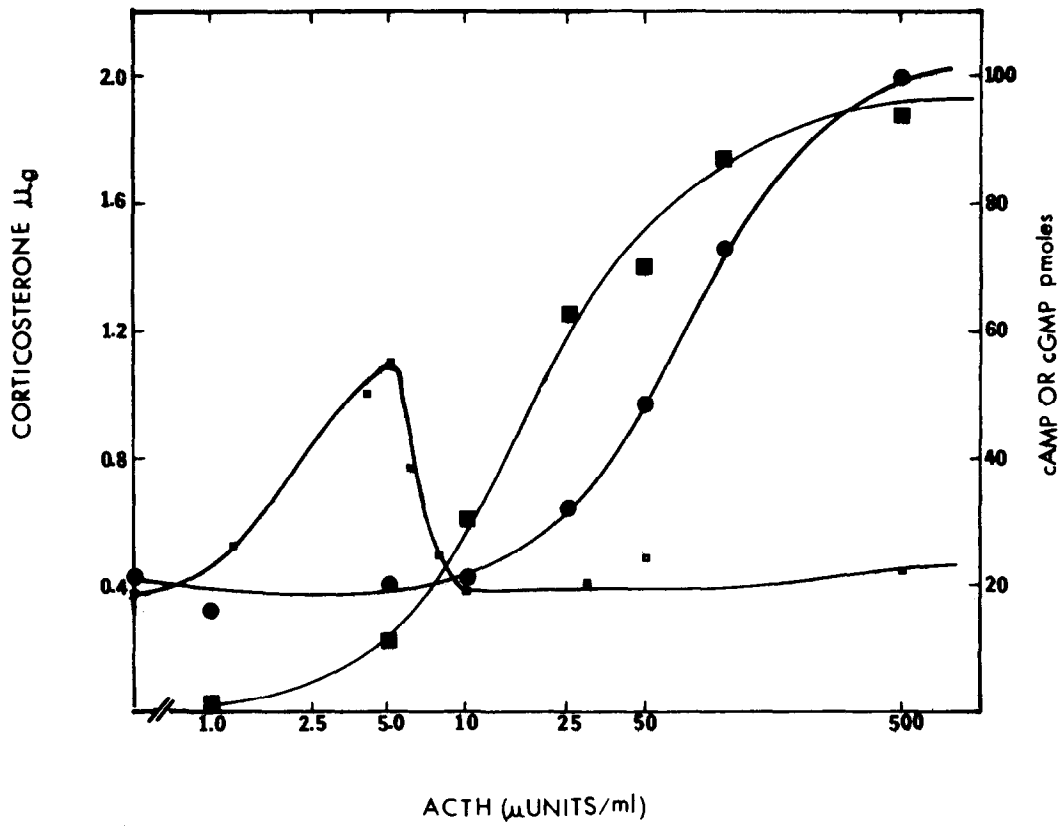

Fig. 1. Dose-response curve for production of cAMP (•—•) cGMP (□—□) and for corticosterone (■—■) in response to increasing concentrations of ACTH. Incubation system: adrenal cell suspension, 0.8 ml; reagents dissolved in 0.2 ml vehicle. Total volume of incubation 1 ml. The data summarize the results of three separate experiments, each of which contained duplicate cell incubation mixtures.

cAMP but does stimulate steroidogenesis. It is only above a concentration of 10 μunits of ACTH that a significant increase in the cAMP level is observed. Although 100 μunits of ACTH yields the maximum amount of steroidogenesis, the cAMP levels above this concentration of the hormone continue to rise. In contrast to the amounts of cAMP synthesized in response to the adrenal hormone, 5 μunits of ACTH

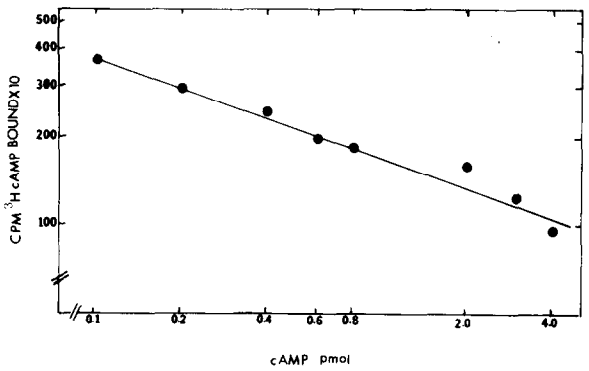

Fig. 2. A standard curve for assay of cAMP. The incubation mixture contained 40 μg of cAMP-dependent protein kinase, 5 pmol [³H] cAMP and various amounts of cAMP in 0.1 ml of 50 mM sodium acetate buffer (pH 4.0).

stimulates the peak synthesis of cGMP with a concomitant rise in corticosterone. Higher than 5 μunits of ACTH causes a dramatic fall in the level of cGMP but the synthesis of corticosterone continues to rise.

These results suggest that levels of ACTH less than 5 μunits stimulate steroidogenesis by altering the levels of cGMP, but not cAMP. At higher concentrations of ACTH (more than 10 μunits), there is no stimulation of the synthesis of cGMP, but there is hormone concentration-dependent synthesis of cAMP, which in turn stimulates corticosterone production. This seems to suggest very strongly that the stimulation of steroidogenesis by ACTH at low concentrations is mediated by cGMP, but not cAMP. At higher concentrations of ACTH (more than 10 μunits), there is no stimulation of the synthesis of cGMP, but there is hormone concentration-dependent synthesis of cAMP which in turn stimulates corticosterone production. This would imply that ACTH-stimulated steroidogenesis is mediated by both cGMP and cAMP, the former nucleotide mediating the ACTH control of corticosterone synthesis at low concentrations of the hormone and the latter at concentrations aboe 10 μunits. The fact that maximal stimulation of cGMP synthesis is achieved at 5 μunits of ACTH and the complete lack of stimulation at 7.5 μunits suggests possible allosteric inhibition of its own action upon cellular guanyl cyclase at the higher ACTH concentration.

Previously [11] it was demonstrated that the cGMP-stimulated steroidogenic step cholesterol to (20 S)-20-hydroxycholesterol is under the transcriptional control of the nucleotide. In view of the present finding that the physiologic concentrations of ACTH elevate cGMP levels without affecting the level of cAMP, it would suggest that cGMP plays a crucial role in the mediation of ACTH-stimulated steroidogenesis in the isolated adrenal cell.

Although to 'ate cGMP has been implicated as an intracellular messenger of various agents [17–22], the present study indicates that it, as well as cAMP, plays a role as media'o of ACTH-stimulated steroidogenesis.

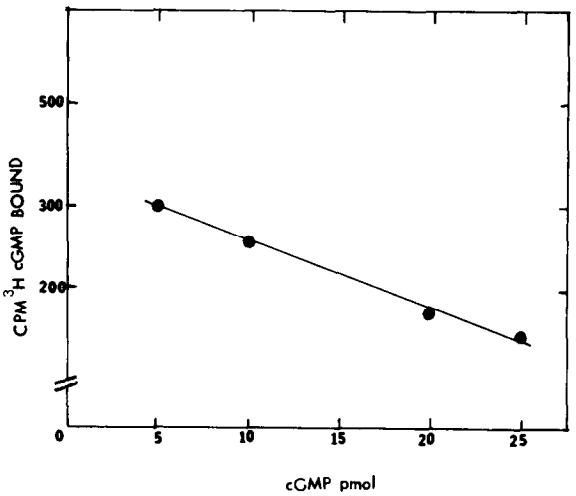

Fig. 3. A standard curve for assay of cGMP. The incubation mixture contained 350 μg of cGMP-dependent protein kinase, 6 pmol of [³H] cGMP and various amounts of cGMP in 0.1 ml of 50 mM sodium acetate buffer (pH 4.0).

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