DUAL ROLE OF CALCIUM IN STEROIDOGENESIS IN THE ISOLATED ADRENAL CELL OF RAT

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Summary

Corticosterone synthesis in isolated rat adrenal cells in response to ACTH and dibutyryl cyclic-AMP (dcAMP) and formation of cyclic-AMP (cAMP) from prelabeled 8-^{14}C-adenine have been studied in the presence of varying Ca^{++} concentrations. At physiologic concentrations of ACTH steroidogenesis is proportional to Ca^{++}. In the absence of Ca^{++}, 10^4 higher concentrations of ACTH is necessary. cAMP formation is detectable only at supraphysiologic concentrations of ACTH. Maximum corticosterone formation in response to dcAMP is dependent upon Ca^{++} concentration. Action of Ca^{++} may be both prior to and following elaboration of the second messenger; other compounds besides cAMP may be the mediators of ACTH action at physiologic concentrations.

Introduction

Although calcium (Ca^{++}) has been shown as early as 1953 to play an important role in steroidogenesis (1) the exact mechanism of its action is not clear. Several workers have reported a Ca^{++} requirement for ACTH action (2,3,4,5). Lefkowitz, et al demonstrated that ACTH could bind to subcellular membrane particles from a mouse adrenal tumor in the absence of Ca^{++} suggesting a Ca^{++}-insensitive as well as a Ca^{++}-sensitive action of ACTH (6). The availability of a highly homogenous preparation of isolated adrenal cells (7) which respond to physiologic concentrations of ACTH (8) permitted us to investigate the effects of varying concentrations of Ca^{++} on ACTH and dibutyryl cyclic-AMP (dcAMP)-induced steroidogenesis as well as conversion of 8-^{14}c-adenine to cyclic-AMP (cAMP). Based on our data a hypothesis is offered regarding the role of Ca^{++} in ACTH-induced steroidogenic formation of cAMP.

Materials and Methods

The adrenal cell preparation previously described (9) was modified as follows: For each experiment adrenals from twenty-four rats were digested with trypsin. 2 mM of Ca^{++} was present at this stage since the omission of Ca^{++} during trypsin digestion resulted in poor cell yield. After digestion the cells were centrifuged and re-suspended in Krebs-Ringer-Bicarbonate buffer, pH 7.4, containing 4% albumin and 0.2% glucose (KRB-AG). Lima bean trypsin inhibitor, 2 mM EGTA and 50 μCi of 8-^{14}C-adenine were added to the cell suspension. Since these cell preparations are devoid of cyclic 3'5' AMP (cAMP) phosphodiesterase activity (10) no methylxanthine was added to the incubation mixture. After a thirty minute incubation, the cells were again centrifuged and re-suspended in Ca^{++}-free KRB-AG buffer. Aliquots of 0.8 ml cells plus 0.2 ml ACTH or test material and desired Ca^{++} concentration in vehicle were incubated for two hours. Corticosterone production was measured by fluorometric method (11). Results are expressed as micrograms of corticosterone produced per ml of cell suspension per two hour incubation. Adenyl cyclase activity was measured by extracting the incubation mixture with 100 μl of 30% perchloric acid containing 35 mM non-labeled cAMP. An aliquot of the extract was applied to PEI-cellulose plates. The plates were chromatographed using 95% ethanol:1.15 M ammonium acetate buffer (74:26). The area corresponding to cAMP was scraped from each plate and counted in a scintillation counter. Results are reported as picomoles of ^{14}C-cAMP formed from labeled precursor per two hour incubation. Preliminary experiments demonstrated incorporation of the radioactive adenine into ATP within the cells which was then transferred to newly-formed cAMP upon addition of ACTH.

Results

The experiments depicted in Figures 1A and 1B were designed to contrast ACTH with dcAMP-induced steroidogenesis in varying Ca^{++} concentrations.

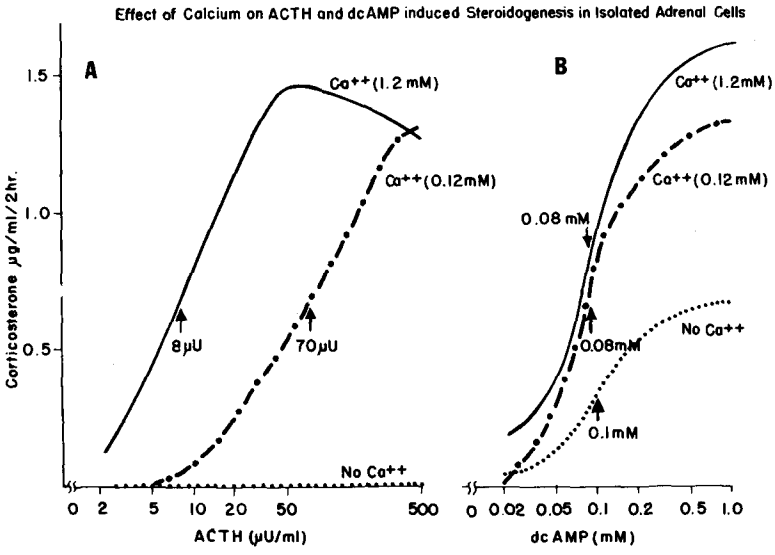

Figure 1: Depicts concentration response curve for steroidogenesis to ACTH (Figure 1A) or dcAMP (Figure 1B) at varying concentrations of Ca^{++}. Results represent the mean of 3 experiments in duplicate.

Figure 1A demonstrates that as the Ca^{++} concentration is decreased there is a marked shift in the concentration of ACTH necessary to achieve half maximum steroidogenesis. The half maximum ACTH concentration shifts from 8 μU with 1.2 mM of Ca^{++} to 70 μU with 0.12 mM of Ca^{++}. In the absence of Ca^{++}, steroidogenesis is not detected at up to 500 μU of ACTH. This pattern of steroid response may be contrasted with that of dcAMP shown in Figure 1B where there is a decreasing maximum steroidogenic response to dcAMP with decreasing concentrations of Ca^{++}. The concentration of dcAMP necessary to achieve half maximum steroid response, however, does not change with decreasing Ca^{++} concentrations. In the absence of Ca^{++}, dcAMP still induces steroidogenesis but it is reduced by 50%.

The experiments depicted in Figures 2 and 3 were designed to measure simultaneous steroidogenesis and ^{14}C-cAMP formation in response to ACTH at varying concentrations of Ca^{++} respectively. The marked effect of Ca^{++} on the steroid response at physiologic concentration (5 μU) of ACTH is evident in Figure 2. As the Ca^{++} concentration is increased with 5 μU of ACTH,

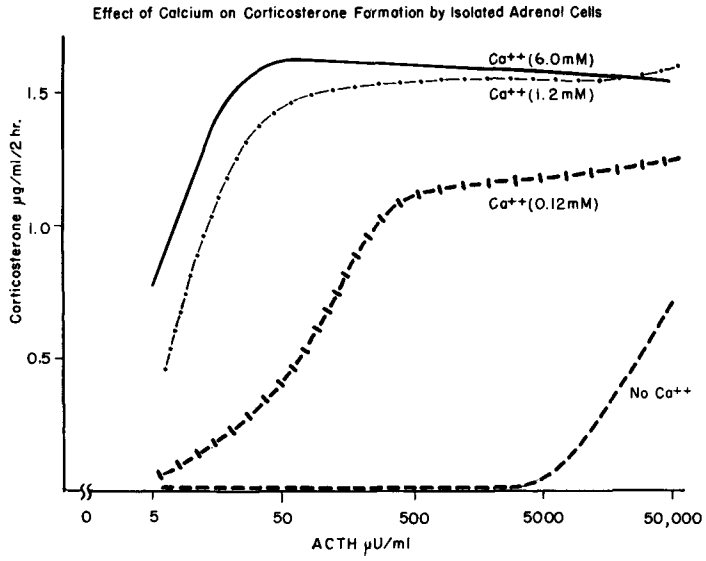

Figure 2: Steroid response to ACTH at varying concentrations of Ca^{++}. Results are the mean of 3 experiments.

there is increased steroid production. In this set of experiments no steroidogenic response was detected up to 500 µU of ACTH in the absence of Ca^{++}. However, by increasing levels of ACTH, steroidogenesis was detectable. Figure 3 depicts that ^{14}C-cAMP formation is also Ca^{++}-dependent. The concentration of ACTH necessary to achieve half maximum ^{14}C-cAMP formation increases as the concentration of Ca^{++} is lowered. This pattern of response resembles that seen for steroid production. However, the concentration of ACTH at which ^{14}C-cAMP formation becomes detectable in our system is considerably higher than the level at which steroid response is detected. At 1.2 mM Ca^{++}, no ^{14}C-cAMP is formed until ACTH exceeds far beyond 50 µU, a level at which maximum steroidogenesis has already been achieved. At 50,000 µU of ACTH, ^{14}C-cAMP is detectable in the absence of Ca^{++} in the suspending media.

In the presence of 2 mM Ca^{++}, the omission of magnesium from the suspending medium had no effect on the steroidogenic response to ACTH. (Data not shown).

Discussion

Our studies demonstrate that a) at physiologic concentrations of ACTH,

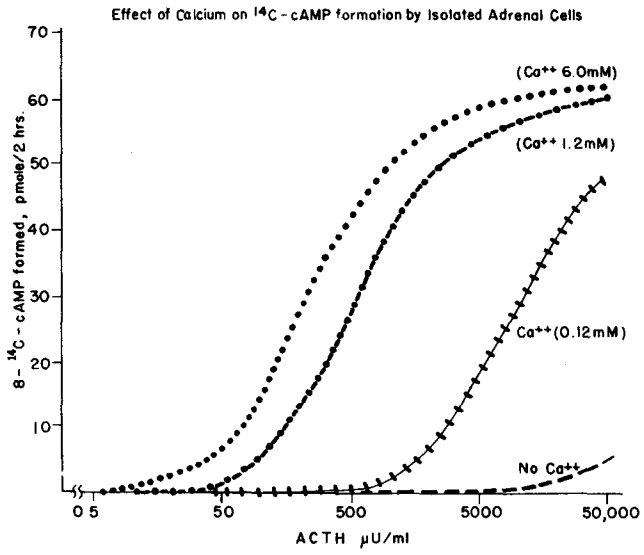

Figure 3: ^{14}C-cAMP formation in response to ACTH at varying concentration of Ca^{++}. Results are the mean of 3 experiments.

there is an absolute requirement for Ca^{++} to induce steroidogenesis; b) in the absence of Ca^{++} in the suspending media a 10,000 fold higher concentration of ACTH is necessary to induce steroidogenesis; c) steroid response to dcAMP is also Ca^{++}-dependent but the requirement is not absolute; d) ^{14}C-cAMP formation is not apparent until concentrations of ACTH exceed that necessary for maximum steroidogenesis; e) at these supraphysiologic concentrations of ACTH, ^{14}C-cAMP is Ca^{++}-dependent.

The observation that both cAMP formation and steroidogenesis are Ca^{++}-dependent indicates an important role for Ca^{++} at the cell membrane. Others have also observed a Ca^{++} dependency for cAMP and steroidogenesis but speculated that Ca^{++} is required primarily at the step between binding of ACTH and activation of adenyl cyclase (12). Haksar and Peron, using a less sensitive system, compared the Ca^{++} requirements for the steroidogenic action of ACTH and dcAMP; however, they did not study formation of cAMP (13).

Our studies with dcAMP suggest a role for Ca^{++} both before and beyond cAMP formation as well as a direct effect of calcium on cAMP formation. A possible

site of Ca^{++} could be protein synthesis, a necessary step for steroidogensis which has been demonstrated to be Ca^{++}-sensitive (14,15).

The lack of correlation between cAMP formation and steroidogensis with physiologic levels of ACTH is consistent with earlier studies in our and other laboratories (9,16). It is tempting to postulate that other compounds besides cAMP may mediate the initial action of ACTH. This could be either intracellular cationic alteractions (17) and/or formation of other cyclic nucleotides (9,18).

ACKNOWLEDGEMENTS

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