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REGULATION OF SERTOLI CELL CYCLIC ADENOSINE 3':5' MONOPHOSPHATE PHOSPHODIESTERASE ACTIVITY BY FOLLICLE STIMULATING HORMONE AND DIBUTYRYL CYCLIC AMP

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SUMMARY

Total phosphodiesterase activity was measured in Sertoli cell culture after exposure to isobutyl-methyl-xanthine, dibutyryl cyclic AMP and FSH. After 24 hr of incubation both FSH and dibutyryl cAMP caused a significant increase in total phosphodiesterase activity of Sertoli cell homogenates (control: 66 ± 16 pmoles/min/mg protein; FSH: 291 ± 25 pmoles/min/mg protein; dibutyryl cAMP: 630 ± 70 pmoles/min/mg protein). FSH stimulation was potentiated by isobutyl-methyl-xanthine. Both in the presence and absence of xanthine, the induction of phosphodiesterase was dependent on the FSH concentration, with maximal stimulation achieved with 0.5-1.0 μg FSH/ml. The induction of phosphodiesterase activity by hormone was abolished by cycloheximide treatment. The data suggest that FSH regulates phosphodiesterase activity via changes of cAMP levels in Sertoli cell in culture.

INTRODUCTION

The Sertoli cell is a target cell for FSH, testosterone and adrenergic agonists (1). FSH stimulates Sertoli cell adenylate cyclase activity, increases cyclic AMP levels (2) and consequently enhances cyclic AMP dependent protein kinase activity (3). Although no specific FSH dependent phosphorylation has been demonstrated in the Sertoli cell, distal events to cyclic AMP dependent cascade have been described (1). These include activation of testosterone aromatization (4), stimulation of total protein and DNA synthesis (2,5), and synthesis and secretion of specific proteins such as ABP (6), plasminogen activator (7), FSH inhibiting factor (8) and, more recently, synthesis of protein kinase inhibitor(9)

During testis maturation, the Sertoli cell becomes refractory to hormonal stimulation (1) and this state is reverted by hypophysectomy (10); it has been suggested that phospho-

ABBREVIATIONS : MIX, 3-isobutyl-1-methyl-xanthine; db-cAMP, 6-N,2-O-dibutyryl adenosine 3':5' monophosphate; cAMP, adenosine 3':5' cyclic monophosphate; FSH, Follicle Stimulating Hormone; PMSF, phenyl methyl sulfonyl fluoride; BSA,Bovine Serum Albumin; ABP Androgen Binding Protein.

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diesterase might play an important role in the maintenance of this refractoriness (1). More recently it has been shown that the Sertoli cell, after exposure to FSH in vitro, becomes insensitive to further hormonal stimulation (11). On the other hand, studies in other cell culture systems have demonstrated that any agent that increases intracellular cyclic AMP levels also induces synthesis of phosphodiesterase (12 - 14) and that the increased phosphodiesterase activity is in turn responsible for the refractory state of the cell (12).

We have therefore investigated the possibility that a similar regulatory mechanism of phosphodiesterase activity operates also in Sertoli cells. In this report we present evidence that both FSH and dibutyryl cAMP regulate phosphodiesterase activity in the immature Sertoli cell in culture.

MATERIALS AND METHODS

Sertoli cell enriched cultures were prepared from 15 day old immature Wistar rats following a procedure previously reported (2) with minor modifications(15).The seminiferous tubule explants were maintained for 3 days in defined medium (Minimum Essential Medium, Eagle) at 32°C in an atmosphere of 95% air and 5% CO_2. On the third day of culture the exhausted medium was removed and the culture briefly exposed to hypotonic buffer (Tris-HCl 20 mM) for 3 min. This treatment removes all the remaining germ cells adhering to the Sertoli cell monolayer (16,17). All treatments were started at least 24 hr after the exposure to hypotonic solution. FSH, dibutiryl-cyclic AMP,MIX were added to the culture by removing the old medium and adding fresh medium supplemented with the various compounds. In the experiments in which cycloheximide (10 μg/ml) was used, the inhibitor of protein synthesis was added 30 min before the addition of hormone.

At the end of incubation the medium was removed by aspiration, cell monolayers rinsed 3 times with phosphate buffered saline (PBS) and the culture plates transferred to 4°C. Cells were scraped with a rubber policeman and transferred to an all glass homogenizer; the homogenization buffer was Tris-HCl 25 mM, pH 8.0, 10 mM β-mercaptoethanol and 2 mM PMSF. The cells were disrupted by 30 strokes of the pestle and the effectiveness of the homogenization was monitored by phase contrast microscopy. The cell homogenate was directly used in the phosphodiesterase assay.

The phosphodiesterase activity was measured according to the method of Thompson and Appleman (18). The incubation mixture contained Tris-HCl buffer, 20 mM, pH 8.0, β-mercaptoethanol 10 mM, cyclic AMP (12.5 μM and approximately 100,000 cpm [^{3}H]cAMP), $CaCl_2$ 1mM, $MgCl_2$ 5mM in a volume of 200μl. The assay was performed by measuring the rate of cAMP hydrolysis by sequential withdrawal of an aliquot of the incubation mixture from a master incubation at 7,14,21 and 28 min. The reaction was stopped by addition of 10 mM EDTA and the sample boiled for 1 min. The reaction products were separated as described elsewhere (18). Under these experimental conditions the rate of the reaction was linear for at least 30 min, and proportional to the amount of protein added. Protein was measured by a modification of the standard Lowry procedure (19), using BSA as standard.

[^{3}H]cAMP was from NEN; dibutiryl cyclic AMP, PMSF and adenine from Sigma; BSA was from Miles; MEM from Gibco; MIX from Aldrich. Rat FSH (NIAMDD-rat-FSH-B1) was a generous gift of the NIAMDD rat pituitary hormone distribution program.

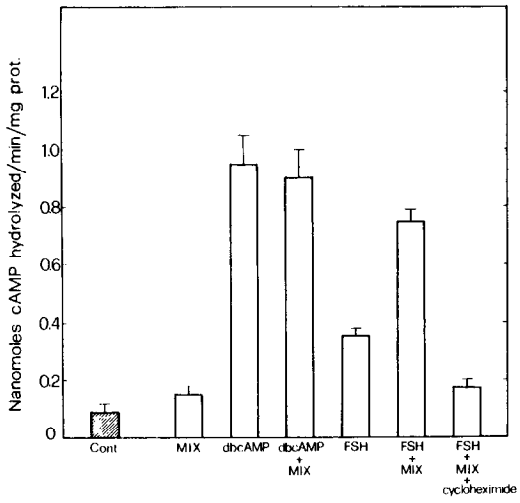

FIGURE 1. Effect of different treatments on total phosphodiesterase activity in immature Sertoli cell in culture. Cell monolayers were exposed for 24 hr to fresh medium with the following additions: MIX (0.1 mM), db-cAMP (0.5 mM), rat FSH (10 μg/ml) MIX (0.1 mM) + db-cAMP (0.5 mM), FSH (10 μg/ml) + MIX (0.1 mM), cycloheximide (10 μg/ml). At the end of the incubation cell monolayers were processed and phosphodiesterase activity assayed as described in the Methods. Each bar represents the Mean ± 1 SDM on three determinations on three different culture plates. Data are expressed as nanomoles of cAMP hydrolyzed/ min/mg protein of cell homogenate.

RESULTS AND DISCUSSION

The effect of various treatments on Sertoli cell total phosphodiesterase activity is presented in Fig. 1. The addition of MIX to the culture medium for 24 hr produced an increase in phosphodiesterase activity of approximately 2 fold. A much larger effect was observed when db-cAMP (0.5 mM) was used; the combination of MIX and db-cAMP did not show additive effects at the concentration used, the phosphodiesterase activity being already maximally stimulated. FSH at a concentration of 10 μg/ml was also effective in raising the total enzyme activity, but unlike db-cAMP, the effect of the gonadotropin was potentiated by the phosphodiesterase inhibitor MIX (Fig.1). The combined effect of FSH + MIX was slightly but consistently lower than that obtained with db-cAMP. Data collected from 4 different experiments showed that treatment of the culture with FSH + MIX for 24 hr enhanced the phosphodiesterase activity from a basal value of 66 ± 16 pmoles/min/mg protein (Mean ± SDM) to 597 ± 72 pmoles/min/mg protein.

In order to test whether the observed stimulation of phosphodiesterase activity required protein synthesis, Sertoli cell cultures were treated, 30 min before and throughout hormone stimulation with cycloheximide (10 μg/ml). Under these experimental condition the effect of FSH + MIX was almost completely abolished (Fig. 1).

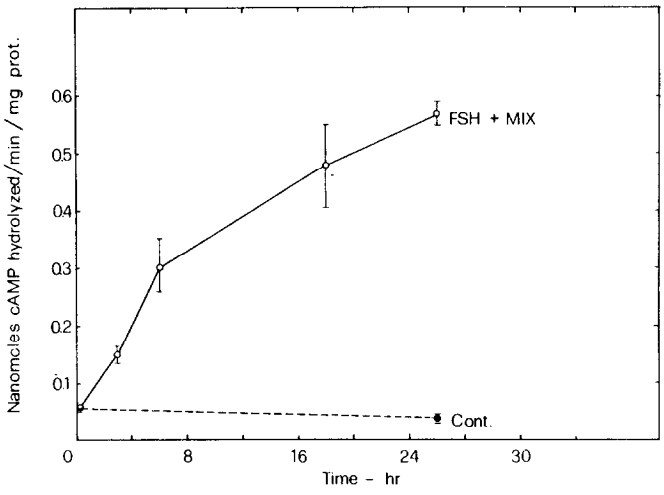

FIGURE 2. Time course of induction of phosphodiesterase activity in Sertoli cell in culture : effect of FSH + MIX. Immature rat Sertoli cell cultures were exposed to FSH (10 μg/ml) and MIX (0.1 mM) for the indicated time intervals. At the end of the culture cell monolayers were processed and phosphodiesterase activity assayed as described in the Methods. Each point represents the Mean ± 1 SDM of three determinations on three different plates. Data were corrected for protein content.

Total phosphodiesterase activity, studied during a 26 hr treatment with FSH + MIX, showed a fast increase during the first 8 hr of incubation (Fig. 2), followed by a progressively slower increase up to 26 hr. Longer stimulation times were not studied.

The stimulation of phosphodiesterase activity was dependent on the FSH concentration in the incubation medium (Fig. 3). FSH alone maximally stimulated the enzyme activity (4 fold) at a dose of 5 - 10 μg/ml with a ED50 of 0.2 μg/ml. The presence of the phosphodiesterase inhibitor MIX, besides the mentioned increase in maximal response, also rendered the cell more sensitive to FSH stimulation, the half maximal response being produced at a dose of 0.07 μg/ml.

The data reported suggest that phosphodiesterase activity of cultured immature Sertoli cells can be regulated by FSH and db-cAMP treatment. The maximal stimulation obtained by db-cAMP and the synergistic effect of MIX and FSH are both consistent with the hypothesis that the induction of phosphodiesterase is primarily regulated by cAMP levels. It is therefore expected that any other agent that would raise Sertoli cell cAMP concentration, for an appropriate period of time, would also produce an increase of phosphodiesterase activity.

Fakunding et al. (20) have reported that exposure of Sertoli cell enriched seminiferous tubules to FSH for 20 min produces a 50% decrease in phosphodiesterase activity. This

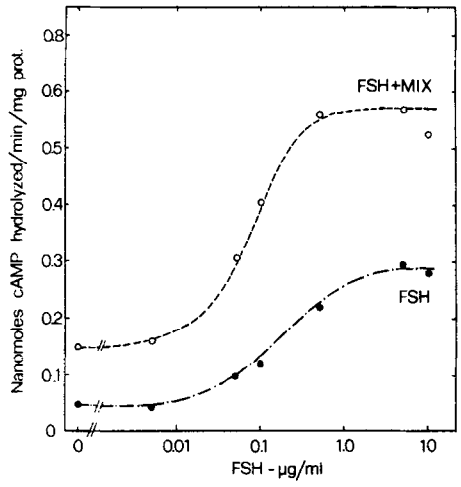

FIGURE 3. Dose response study of the effect of FSH and FSH + MIX on phosphodiesterase activity in immature Sertoli cell in culture. Cell cultures were exposed for 24 hr to increasing FSH concentrations in absence and presence of 0.1 mM MIX. At the end of the incubation cells were processed and phosphodiesterase activity measured as described in the Methods. Each point represents the Mean of triplicate enzyme determinations on two different culture plates. The deviation from the mean was within 10% for all observations.

finding is not in contrast with our data that describe a delayed effect of FSH: the two events could both occur in the same cell at different times.

The relatively slow induction of phosphodiesterase activity and the inhibitory effect of cycloheximide suggest that the enhancement of enzyme activity requires de novo protein synthesis. It will be necessary to determine whether actual phosphodiesterase de novo synthesis is involved or more complex events are at the base of the increased phosphodiesterase activity.

It is worth mentioning in this regard, that the synthesis of calmodulin, a factor known to stimulate phosphodiesterase activity (21) is not under FSH control (22): it is unlike, therefore, that the observed increase in phosphodiesterase activity depends on increase in calmodulin levels. On the contrary FSH has been shown to increase the synthesis of a protein kinase inhibitor (9) which also inhibits phosphodiesterase activity at least in a cell free system (23). The effect of this regulation on the induction of phosphodiesterase activity remains to be defined.

Stimulation of phosphodiesterase activity by db-cAMP and xanthine has been reported for fibroblasts (12-13) and myoblasts (14) in culture, however the amplitude of the stimulation experienced in our study (up to 12 fold) is higher than that observed in those

systems. It is worth noticing that, unlike fibroblasts and myoblasts, Sertoli cells were cultured for 4 days in a chemically defined medium lacking serum or FSH, prior to hormone or db-cAMP stimulation.

Finally, the increased cAMP catabolism consequent to increased phosphodiesterase activity might be a relevant phenomenon to the induction of a short term refractory state described in Sertoli cells (11). Whether this mechanism is also involved in the age dependent Sertoli cell unresponsiveness remains to be determined.

In view of the finding that the Sertoli cell contains multiple phosphodiesterase iso forms (1) it will also be interesting to verify whether the induction of phosphodiesterase is limited to a particular isoenzyme.

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