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INTRACELLULAR MECHANISM OF THE ACTION OF INHIBIN ON THE SECRETION OF FOLLICULAR STIMULATING HORMONE AND OF LUTEINIZING HORMONE INDUCED BY LH-RH IN VITRO

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Translation of "Un mécanisme intracellulaire de l'action de l'inhibine sur la sécrétion de l'hormone folliculo-stimulante et de l'hormone lutéinisante induite par le LH-RH in vitro," Comptes Rendus Des Séances de la Société de Biologie et de Ses Filiales, Vol. 175, No. 3, 1981, pp. 385-388.

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Summary

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The FSH secretion-inhibiting action of inhibin in vitro under basal conditions and also in the presence of LH-RH is suppressed by the addition of MIX, a phosphodiesterase inhibitor. In the presence of LH-RH, inhibin reduces significantly the intracellular level of cAMP in isolated pituitary cells. In contrast, the simultaneous addition of MIX and inhibin raises the cAMP level, and this stimulation is comparable to the increase observed when MIX is added alone. These observations suggest that one mode of action of inhibin could be mediated by a reduction in cAMP within the pituitary gonadotropic cell.

Introduction

Inhibin is a proteinaceous hormone produced by the Sertoli cells of the testicle and by the cells of the granulosa of the ovary. It acts on the pituitary level where it specifically reduces synthesis of follicular stimulating hormone (FSH) under basal conditions, and preferentially reduces liberation of FSH by the hypothalamic releasing factor (LH-RH) [1]. We have undertaken a study of the intracellular mechanisms through which inhibin exercises its action. In this note we will describe the effect of inhibin on the secretion of gona- /386
dotropins by isolated pituitary cells in culture and on their cAMP level in the presence and absence of a phosphodiesterase inhibitor.

Material and Methods

Culture of Pituitary Cells

*Numbers in the margin indicate pagination in the foreign text.

This method has been described in detail in a preceding publication [1]. We succintly restate the stages of it and the particular methods of this study.

The pituitaries are removed from adult Wistar rats and the cells are broken up by the method of Hopkins and Farquhar using trypsin [2]. The broken cells are placed in suspension in "Dulbecco Modified Eagle's Medium" (DMEM) containing 5% horse serum, 2.5% fetal calf serum, 1% glutamine, and 1% nonessential amino acids. The cells are distributed in 60 mm diameter culture boxes at the rate of 0.5×10^6 cells per box and incubated at 37°C for 3 days in a water-saturated atmosphere constituted of 95% air and 5% CO_2. After this period the substances under investigation are added to the culture. The total incubation volume is 1.6 ml. Incubation is continued for 24 hours. At the end of this period the culture media are collected for measuring the secretion of FSH and LH. The cultures are rinsed with fresh medium and set to incubate for another 6 hours in DMEM with the same substances described above, but with the presence of LH-RH at a concentration of 10^{-8} M. At the end of these 6 hours of incubation the culture media are gathered. Water (1.6 ml) is added to the cells that are still congealed. After decongelation the soluble cellular contents are collected by centrifugation and used (after dilution by half) for measuring cyclic AMP. Each substance or group of substances is studied in five different cultures of isolated pituitary cells.

Tested Substances

The preparation of inhibin used was extracted from the liquid of the rete testis by filtration on Sephadex G100 (Pharmacy) (Pic 1A). Its molecular weight is greater than 10,000 daltons [3]. This preparation was investigated at the rate of 10 µg/ml of culture medium.

3-isobutyl-1-methyl-xanthine (MIX). MIX possesses the property of inhibiting phosphodiesterase activity and therefore per-

mits an accumulation of cAMP within the cell. The concentration investigated was 0.2 mM.

Measurements

FSH and LH were measured in the culture media by a radioimmunological method using the material graciously supplied by NIAMDD, whom we thank. The results were expressed in ng of hormone per ml of medium.

cAMP was also measured by a radioimmunological method [4]. Separation of the antigen bound in antibodies from free antigen is carried out by the technique of double precipitation. The results were expressed in pmol/ml.

Results

Under basal conditions, inhibin significantly reduces secretion of FSH. MIX has no effect when it is used alone, but it hinders the /387 inhibitory action of inhibin on FSH (Table 1).

At the time of stimulation by LH-RH, liberation of FSH and LH is more significant in 6 hours than the basal secretion in 24 hours. Liberation of FSH is significantly reduced in the presence of inhibin, but this effect disappeared when inhibin was incubated with MIX (Table 2). Inhibin does not change the secretion of LH under base conditions nor under the influence of LH-RH.

The intracellular level of cAMP (Table 2) was significantly reduced in the presence of inhibin. In contrast, in the presence of MIX the intracellular level of cAMP was significantly increased.

The simultaneous addition of MIX and inhibin to the culture medium brings about an increase of the intracellular level of cAMP in comparison to standard cultures and to those treated with inhibin.

Discussion

Inhibin would seem to operate on the pituitary gonadotropic cell through specific intermediate receptors, different from those of LH-RH [5,6]. This activation leads to a reduction in production of intracellular cAMP, usually induced by LH-RH [7], at the same time as a reduction in liberation of FSH.

MIX, which is an inhibitor of phosphodiesterase, hinders the degradation of cAMP [8]. It accompanies an accumulation of cAMP within the pituitary cells, which persists in the presence of inhibin. One can therefore think that inhibin operates in reducing the amount of cAMP formed under the influence of LH-RH. The concentration of the intracellular level of cAMP under base conditions has not yet been determined. Nevertheless, observation of an inhibitory effect of inhibin on the basal secretion of FSH, and of its disappearance in the presence of MIX, brings to mind the possibility that inhibin operated in reducing the production of cAMP.

With the objective of defining the intracellular mechanism of action of inhibin, studies are underway to demonstrate a possible action of inhibin on the different enzymes implicated in the metabolism of cAMP[1].

[1]Work subsidized by grant number 3.4501.80 of F.N.R.S.

TABLE 1. SPONTANEOUS SECRETION OF FSH AND LH IN CULTURE MEDIUM

Treatment	FSH (*) ng/ml (NIAMDD-FSH-RP$_1$)	LH (*) ng/ml (NIAMDD-LH-RP$_1$)
Standard	524 ± 41	574 ± 172
Inhibin 10 µg	403 ± 25 (**)	551 ± 125
MIX 0,2 mM	493 ± 35	572 ± 105
MIX 0,2 mM + inhibin 10 µg	470 ± 37	672 ± 114

(*) Mean ± 1 standard deviation
(**) $p < 0.05$

TABLE 2. SECRETION OF FSH AND LH IN CULTURE MEDIUM IN THE PRESENCE OF LH-RH (10^{-8} M) AND CORRESPONDING INTRACELLULAR CONCENTRATIONS OF CYCLIC AMP.

Treatment	FSH (*) ng/ml (NIAMD-FSH-RP$_1$)	LH (*) ng/ml (NIAMD-LH-RP$_1$)	cAMP pmol/ml (*)
Standard	1033 ± 65	3334 ± 299	0,129 ± 0,026 (a)
Inhibin 10 µg	832 ± 71 (**)	3055 ± 606	0,096 ± 0,024 (b)
MIX 0,2 mM	1070 ± 54	3559 ± 283	0,214 ± 0,042 (c)
Inhibin + MIX	1073 ± 78	3720 ± 194	0,173 ± 0,028 (d)

(*) Mean ± 1 standard deviation
(**) $p < 0.05$
a versus b and a versus d : $p < 0.025$; a versus c : $p < 0.005$; c versus d : not significant; b versus c : $p < 0.0005$; b versus d : $p < 0.0005$.

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