COMPARISON BETWEEN THE EFFECTS OF LH-RH AND RAISED K^+ CONCENTRATION ON RELEASE OF LH FROM PITUITARY GLANDS OF FEMALE RATS IN VITRO

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1. Introduction

Luteinizing hormone-releasing hormone (LH-RH)-induced release of LH from intact female rats is biphasic: an initial low rate of release of LH is followed by a marked increase dependent upon synthesis of RNA and of protein [1–4]. From these and additional results [5,6] two different pathways in the mechanism of action of LH-RH were distinguished: one is the formation of factors related to the synthesis of protein, the so-called protein factor; the other the releasing action itself. Release of LH is the ultimate result of interaction between the protein factor and the releasing action of LH-RH. In this process the amount of the protein factor can be rate-limiting. However, the resulting release of LH is independent of de novo synthesis of protein.

It is well known that, under in vitro conditions, raising the K^+ levels of the medium also induces release of LH (e.g., [7,9]). Therefore, the aim of this study was to investigate whether elevated K^+ might mimic one or both actions of LH-RH. We therefore studied to what extent release of LH induced by raised K^+ content of the media can be suppressed by inhibition of protein synthesis, when low or elevated levels of the protein factor may be assumed to be present in the pituitary glands of female rats [3,5,6].

2. Experimental

Adult female rats from the Wistar-derived colony kept in this laboratory were decapitated on the second day of dioestrus (intact rats) or 14 days after ovariectomy (OVX rats). Two pituitary halves from different animals were placed in flasks each containing 1 ml medium TC 199 (Difco Labs.). The preincubation of 0,5 h in the same media and the successive incubation(s) with fresh media were carried out at 37°C under continuous shaking and gassing with O_2 and CO_2 (95%:5%). Additions to the media were LH-RH (Beckman; 10 or 1000 ng/ml), KCl (Baker; to final conc. 50 mM K^+) and cycloheximide (Boehringer; 25 μg/ml). The secretagogues were present at maximally effective concentrations; cycloheximide inhibited synthesis of protein for ~95% [3]. Samples of 50 μl medium were withdrawn during and at the end of each incubation period. The LH contents of the media were estimated by radioimmunoassay [3,10] and were expressed as μg LH-RP-1/mg pituitary tissue. The reference and the iodination (LH-I-4) preparations were generous gifts from Dr A. F. Parlow and the NIAMDD. Specific rabbit antiovine LH was a generous gift from Drs J. Dullaart and J. Th. J. Uilenbroek (Erasmus University, Rotterdam). Statistical comparisons were made by analysis of variance followed by Duncan's multiple comparison test [11].

3. Results

3.1. *Release of LH induced by LH-RH and elevated potassium concentration*

As depicted in fig.1 and mentioned in section 1, LH-RH (1000 ng/ml)-induced release from pituitary glands of intact rats is biphasic during 4 h incubation. Only the second phase of increased release of LH was blocked by inhibiting synthesis of protein by cycloheximide, the rate of release of LH in this series remaining the same as observed during the initial phase. Cycloheximide did not affect raised K^+-induced release of LH which equals the protein synthesis independent action of LH-RH. Basal release was not affected significantly by cycloheximide (fig.1; [3]).

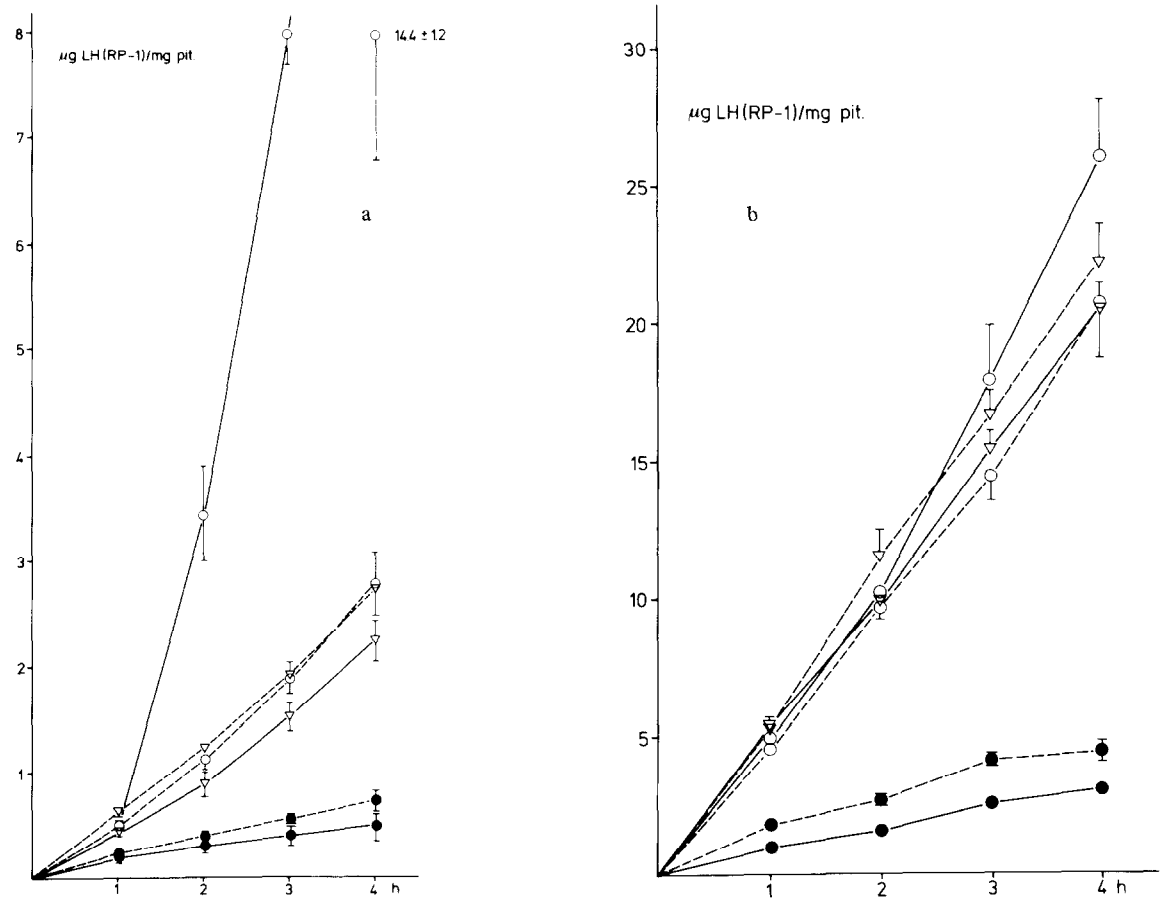

Fig.1. Incubation of pituitary glands from intact (a) or OVX (b) female rats for 4 h in medium with the following additions: (—•—) none; (- - • - -) cycloheximide; (—○—) LH-RH (1000 ng/ml); (- - ○ - -) LH-RH + cycloheximide; (—▽—) K^+ (50 mM) or (- - ▽ - -) K^+ + cycloheximide. The results are expressed as means ± SEM, $n = 4$. Fig.1a and 1b represent separate experiments (note the difference in the scales for LH).

Fig.1 also demonstrates similar high rates of release of LH from pituitary glands of OVX rats stimulated by LH-RH or raised K^+. These releases were maximal from the beginning onwards. Neither stimulated nor basal releases of LH were significantly affected by cycloheximide (fig.1; [3]).

In addition table 1 shows that the concomitant presence of LH-RH and raised K^+ in medium containing cycloheximide did not further increase release of LH.

3.2. *Effect of pretreatment with LH-RH or raised K^+ on the protein-synthesis-independent responsiveness of pituitary glands from intact rats*

In this series of experiments (see table 2) pituitary glands of intact rats were first incubated in medium

Table 1
Concomitant addition of LH-RH (1000 ng/ml) and raising the K^+ level (50 mM) on release of LH from intact and OVX rats during 4 h incubation

Additions to the media	Content of the media in µg LH-RP-1/mg pituitary tissue (means ± SEM, $n = 4$)	
	Intact rats	OVX rats
LH-RH + cycloheximide	2.43 ± 0.41	17.04 ± 0.92
K^+ + cycloheximide	2.87 ± 0.23	20.34 ± 0.91
LH-RH + K^+ + cycloheximide	2.40 ± 0.14	20.01 ± 0.67
Analysis of variance	$p > 0.05$	$p > 0.05$

Table 2
Effect of preincubation of pituitary glands from intact rats with LH-RH (10 ng/ml) for 1.5 h on the protein-synthesis-independent release of LH stimulated by LH-RH (1000 ng/ml) and/or potassium (50 mM) during a further 4 h incubation period

Treatment of the media		LH released during the experimental period (mean ± SEM, $n = 4$)	
Pretreatment period	Experimental period	Expt A	Expt B
LH-RH	LH-RH + cycloheximide	4.01 ± 0.38[a]	5.74 ± 0.40[a]
LH-RH	K^+ + cycloheximide	1.75 ± 0.21[b]	5.33 ± 0.50[a]
LH-RH	Cycloheximide	0.35 ± 0.11[c]	2.09 ± 0.10[b]
LH-RH	LH-RH + K^+ + cycloheximide	4.15 ± 0.32[a]	6.57 ± 0.41[a]
none	LH-RH + cycloheximide	1.37 ± 0.19[b]	2.36 ± 0.31[b,c]
none	K^+ + cycloheximide	1.40 ± 0.25[b]	1.87 ± 0.41[c]

[a–c] In each experiment means without common superscript differ significantly from each other ($p < 0.05$)

The incubations were separated by rinse periods lasting 6-times 20 min (expt A) or 2 min (expt B)

only or in medium containing 10 ng LH-RH/ml for 1.5 h in order to increase the protein factor content of the glands [3,5]. Then the glands were rinsed by 6 consecutive incubations of 20 min each with fresh medium; in the last rinse period cycloheximide was added to the medium. During the subsequent 4 h incubation with cycloheximide the protein-synthesis-independent responses of LH were tested for LH-RH (1000 ng/ml) or raised K^+ separately or in combination. Under these circumstances the secretagogues do not limit the responses of LH; increases in the responsiveness can be ascribed to the formation of protein factor during the pretreatment period [3,5,6]. It can be seen (table 2, expt A) that after pretreatment of the glands with LH-RH during the subsequent experimental incubation the responses of LH for LH-RH with or without K^+ were increased above those of the non-pretreated controls. However, this was not the case for raised K^+ if present alone with cycloheximide during the experimental period of incubation: now, release of LH stimulated by K^+ was not significantly elevated above that of the non-pretreated controls. Furthermore, these results show that after incubation with LH-RH during the first 1.5 h period, subsequent incubation with cycloheximide only (after the rinse-procedure) LH release had nearly returned to basal levels (compare basal release of LH from glands of intact rats in fig.1).

If the same experiment was repeated (table 2, expt B) but now with 6 quick changes of the media (the whole rinse procedure took ~12 min), it was found that the enhanced protein-synthesis-independent responsivenesses were similar for LH-RH and raised K^+ either separately or in combination. However, after pretreatment with LH-RH, release of LH during the experimental period in the presence of cycloheximide only had declined much less than in the previous experiment.

Pre-exposure of pituitary glands from intact rats to raised K^+ for 1.5 h (table 3) followed by the 6-times 20 min rinse procedure did not enhance their protein synthesis-independent-responses during subsequent exposure to either LH-RH (1000 ng/ml) or raised K^+, indicating that raised K^+ did not induce the formation of the protein factor, which is in agreement with the failure of this secretagogue to increase the rate of release of LH with time (fig.1). These results confirm the data shown in table 2, expt A.

Table 3
Effect of preincubation of pituitary glands from intact rats with raised potassium or LH-RH (10 ng/ml) on the protein-synthesis-independent release of LH stimulated by LH-RH (1000 ng/ml) or potassium (50 mM) for a further 4 h incubation period

Additions to the media		LH released during the experimental period (mean ± SEM, $n = 4$)
Pretreatment period	Experimental period	
LH-RH	LH-RH + cycloheximide	5.69 ± 0.27[a]
K^+	LH-RH + cycloheximide	1.96 ± 0.30[b]
LH-RH	K^+ + cycloheximide	2.94 ± 0.07[c]
K^+	K^+ + cycloheximide	1.91 ± 0.14[b]
LH-RH	Cycloheximide	0.26 ± 0.04[d]
K^+	Cycloheximide	0.13 ± 0.02[d]

[a–d] Means without common superscript differ significantly from each other ($p < 0.05$)

The incubations were separated by a rinse procedure lasting 6 times 20 min

4. Discussion

The characteristic differences between the patterns of release of LH from intact and OVX female rats stimulated by LH-RH are most likely the result of their, respectively, low and high endogenous amounts of protein factor [3,5,6]. With glands from intact rats the initial low protein synthesis independent response was followed by a marked increase in the rate of LH release allowed by the formation of protein factor. In the case of pituitary glands from OVX rats an immediate high protein-synthesis-independent response of LH was noted. As can be deduced from tables 2 and 3, elevated amounts of the protein factor alone do not cause release of LH in the absence of LH-RH.

Raising the K^+ levels of the media resulted in a release of LH which was very similar to that caused by the protein synthesis independent action or releasing action of LH-RH in both incubation systems. Hence the endogenous content of protein factor limited the responses of LH for raised K^+ and LH-RH to the same extent. The actions of raised K^+ were independent of protein synthesis ([9]; fig.1).

Pretreatment of pituitary glands from intact rats with LH-RH yielded glands with enhanced responses of LH for LH-RH or raised K^+ alone [8] or in combination. These responses had become independent of protein synthesis and were equal for all secretagogues (table 2, expt B). After an extensive rinse procedure, however, the enhanced responsiveness of the glands which was due to the increased protein factor content disappeared for raised K^+ but not for LH-RH (table 2, expt A). Also, in the latter incubation more rapid decay in release of LH was noted by omitting LH-RH after the pretreatment period. A similar discrepancy was noted between the effects of LH-RH and raised K^+ after preincubation of pituitary glands from OVX rats in medium only (unpublished).

These results tend to the conclusion that raised K^+ might mimic the releasing action of LH-RH. When enhanced responses of LH are concerned, however, the action of raised K^+, at least partly, may consist of an LH-RH-dependent component that quenches in time after withdrawal of LH-RH, leading to the inability of raised K^+ to induce enhanced rates of LH release allowed by the elevated concentration of the protein factor. This permissive action of LH-RH might be mediated by cyclic AMP [12,13].

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