ACUTE RELEASE OF GONADOTROPINS MEDIATED BY DIBUTYRYL-CYCLIC AMP IN VITRO

H. KERCRET, L. BENOIST and J. DUVAL

Laboratoire de Neurobiologie Moléculaire, Equipe de Recherche associée au CNRS, no. 567, Université de Rennes, 35031 Rennes Cedex, France

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1. Introduction

One of the first reports concerning the role of cyclic AMP in the release of gonadotropins from the pituitary appeared 8 years ago [1]. Since that time, numerous investigations have suggested that this nucleotide could be an intermediate in the action of the gonadoliberin (Gn-RF) (for an extensive bibliography, see [2]). At variance with these results, others have mentioned that it neither promotes any in vitro increase in the basal release of LH and FSH [3] nor raises the serum LH level after injection to a male rat [4]. In addition, the cyclic AMP has been described to stimulate not only the biosynthesis of total adenohypophyseal proteins [5] but more specifically that of FSH [6]. In fact, all the in vitro studies have been performed using conditions in which the glands were incubated for several hours in the presence of the substance to be tested. We thus decided to reinvestigate the possible role of cAMP using the perifusion technique which allows the distinction between 2 phases in the release of gonadotropins (in preparation).

2. Materials and methods

Immature male rats of the Wistar strain were purchased from CERJ (53 Le Genest). They were used when 35–45 days old. The Gn-RF was a generous gift from Farbwerke Hoechst; $N^6,O^{2'}$-dibutyryl adenosine 3',5' monophosphoric acid (dbcAMP) was from Sigma and theophyllin a commercial product from Merck. Preparation of hemi-pituitaries, perifusion system and perifusion medium (Krebs-Ringer–bicarbonate–glucose solution) were as described [7]. Flow-rate was 12 ml/h and 4 ml fractions were collected at 0°C into test tubes containing 0.2 ml dilution buffer used for radioimmunoassays (including 1% bovine serum albumin). The collected fractions were spun at 40 000 × g to eliminate all particulate material. LH and FSH amounts were estimated by radioimmunoassay using the rat LH and rat FSH kits supplied by the NIAMD; the 2nd antibody was replaced by polyethyleneglycol (PEG 6000) as precipitant [7].

Address correspondence to: Professeur Jacques Duval, Laboratoire de Neurobiologie Moléculaire, Avenue du Général Leclerc, 35031 Rennes Cedex, France

3. Results

When hemi-pituitaries (4/cell) are superfused with KRBG alone, a high non-specific release of LH and FSH occurs during at least 90 min. The infusion of Gn-RF or dbcAMP thus starts 2 h after the initiation of the experiment. If the Gn-RF is continuously infused, a 1st peak of LH (fig.1) and FSH (not shown) occurs followed by a sustained high release of both hormones which ends as soon as the hypothalamic factor is withdrawn.

Successive pulses of dbcAMP elicit different responses of the hemi-pituitaries according to the duration of these pulses; using 10 min infusion times, the releases of LH and FSH are not stimulated at

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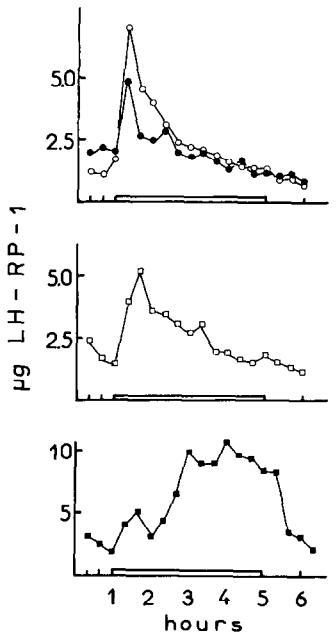

Fig.1. Influence of dbcAMP (○) or theophylline (●) (upper panel), dbcAMP plus theophylline (middle) and Gn-RF (lower) on the release of LH. 4 hemi-pituitaries were placed in each cell and superfused during 2 h with KRBG alone at a flow rate of 12 ml/h. The effluent of the 1st h was discarded (high non-specific release) and fractions were then collected in the cold every 20 min (0 time is the beginning of collection.) Gn-RF (10 ng/ml), dbcAMP (0.5 mM) or theophylline (1 mM) were infused during 4 h (open horizontal bar) followed by KRBG during 80 min.

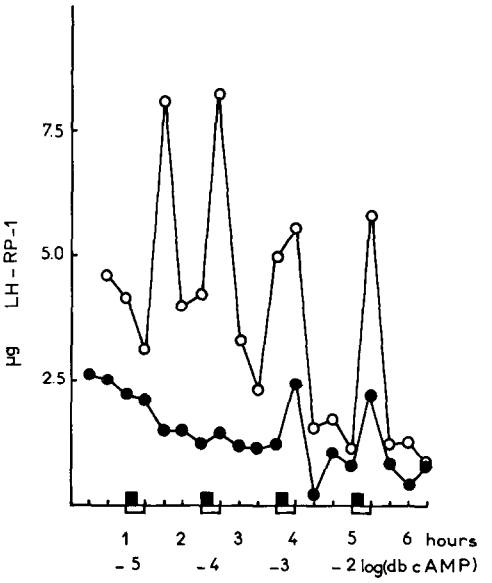

Fig.2. Influence of dbcAMP on the acute release of LH. Same experimental conditions as in fig.1, but dbcAMP was infused during successive 10 min pulses (black symbols) or 20 min pulses (open symbols) every 80 min.

10^{-5} M and 10^{-4} M whereas 10^{-3} M and 10^{-2} M concentrations of the nucleotide are efficient; using 20 min, all the concentrations tested promote high releases of LH (fig.2) and FSH (not shown); the base-values are reached again as soon as the trigger is withdrawn.

When the dbcAMP is continuously injected into the cell at a 0.5×10^{-3} M concentration, the acute release immediately takes place as was observed with the Gn-RF but it progressively declines to the base-line value though the nucleotide is still infused (fig.1). So as to prevent a possible hydrolysis of the cyclic nucleotide by the cytoplasmic phosphodiesterase, theophylline and dbcAMP were simultaneously injected into one cell, theophylline alone into the 2nd cell. One sees (fig.1) that the xanthine derivative exerts a slight transitory stimulating effect on LH (and FSH) release whereas the mixture of both compounds gives roughly the same response as the nucleotide alone.

4. Discussion

We have shown that the gonadotropin release observed during a constant infusion of Gn-RF results from two distinct processes. The 1st one takes place as soon as the Gn-RF reaches the pituitaries and is transitory; it can represent the output of the contents of secretory granules linked to – or in a close vicinity of – the cell membrane. The 2nd step can be described as an intense mobilization of the intracellular LH and FSH pools so as to compensate for the depletion in peripheral granules; it lasts as long as the Gn-RF is present. Such 2 distinct processes have been partly described recently on the basis of their sensitivity to cycloheximide [8–10].

The acute release is elicited by exogenous dbcAMP and, to a certain extent, by theophylline which prevents the hydrolysis of the intracellular cyclic nucleotide

[11]. Thus, cAMP mimics the acute pituitary response to the hypothalamic hormone. One can imagine that as soon as the dbcAMP crosses the membrane, it triggers the release mechanism, possibly through the activation of a protein kinase as previously suggested [12]. The in vitro secretory response of the pituitaries appears to be a function both of the concentration of the exogenous nucleotide and of its diffusion across the membrane. Though protected by theophylline, it cannot promote by itself the second phase of secretion observed in the presence of the Gn-RF. We thus conclude that cAMP plays a key role only at the membrane level as a trigger for the release mechanism and not in the intracellular transfer of the gonadotropins from their respective pools towards the membrane.

Using this interpretation, it becomes easy to explain the opposite results obtained in vitro by the various groups. In fact, during a 4 h incubation in the presence of Gn-RF, as for perifusion, most of the LH and FSH released in the medium comes from the 2nd phase; if one incubates with theophylline or with dbcAMP alone, only the acute release is promoted which, in 4 h, is not very high above the basal release as compared to the one elicited by the hypothalamic factor during the mean time; it thus can be easily missed.

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