THE MECHANISM OF THE STIMULATION OF LH PRODUCTION BY SYNTHETIC LH-RELEASING HORMONE(LH-RH) IN TISSUE CULTURE

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Summary: Synthetic LH-RH was found to stimulate production of LH by human female adenohypophysis in monolayer culture. This effect occurs at 0.30 μg/2 ml LH-RH. New messenger-RNA synthesis does not have to occur to stimulate the production of LH by the action of synthetic LH-RH in cultures of under 4 days. In cultures of over 4 days, this synthesis must occur in order for LH to be produced by the action of LH-RH. However, new DNA synthesis does not have to occur to stimulate the production of LH by the action of synthetic LH-RH.

Introduction: Hypothalamic control of the secretion of the luteinizing hormone(LH) of the pituitary gland is well understood (1,2,3). In addition, it has been postulated that the hypothalamus influences the synthesis of LH as well as its release(4,5). Evans and Nikitovitch-Winer(6) found by observing pituitary cytology and ovarian morphology that continuous infusion of median eminence extracts reactivated pituitaries autografted under the kidney capsule. Evidence indicating stimulation of synthesis of bioassayable LH by the hypothalamic hormones has been reported(7,8,9). Recently Mittler, et al.(10) suggested that LH-RH stimulates release and synthesis of LH in rat pituitary cultures.

In order to study the mechanism of the stimulation of LH production by synthetic LH-RH in the absence of variable neural and vascular influences, the effects of cycloheximide, actinomycin

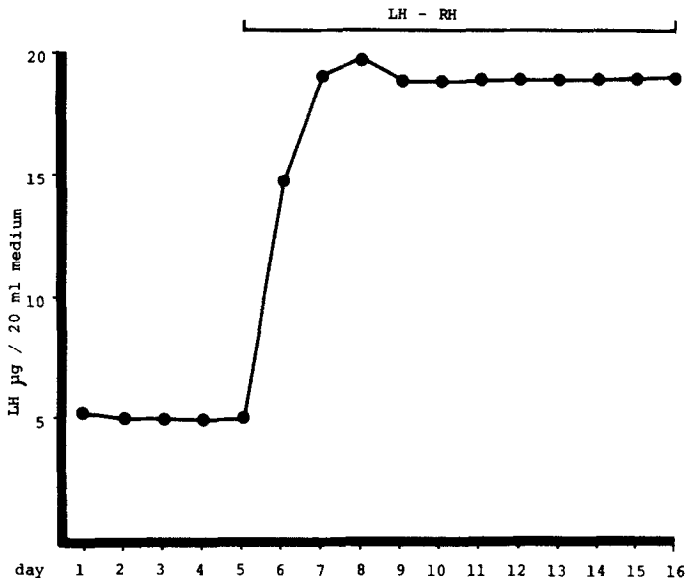

Fig. 1 Effect of Synthetic LH-RH on Daily LH Production in Tissue Culture

D and hydroxyurea on the stimulation of LH production by synthetic LH-RH in tissue culture were examined.

Materials and Methods: Synthetic LH-RH was supplied by the Tanabe Seiyaku Company.

The anterior pituitary from a 25-year-old woman killed in a car accident was finely minced into explants approximately 1 mm in diameter. Confluent monolayers developed after the pituitary explants had been in culture for 30 days, and studies were then performed.

As the control medium, four of the explants were cultured together on a coverslip in a test tube containing 1.8 ml of NCTC-109 medium to which about 0.2 ml of horse fetal serum, 100 units of penicillin, and 100 µg streptomycin were added, making altogether 2 ml.

After 4 control days, 1) 0.30 µg of synthetic LH-RH was added to one medium(2 ml per test tube) for 11 days(Fig. 1). Other

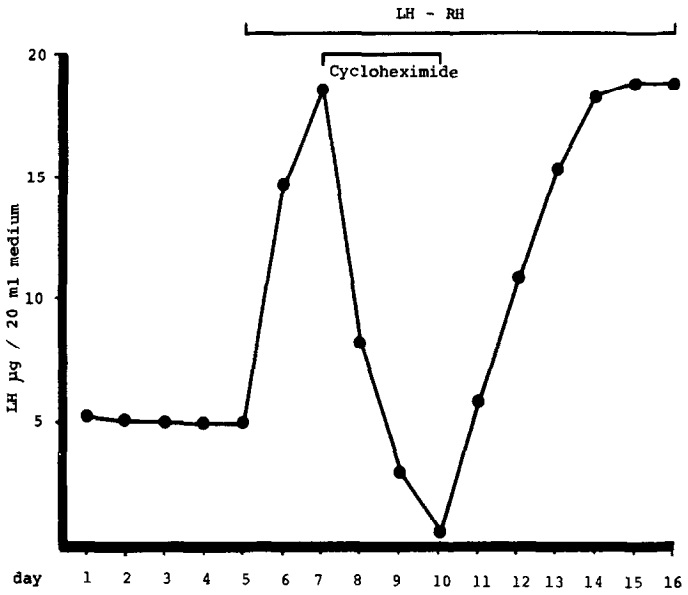

Fig. 2 Effect of Cycloheximide on Daily LH Production by LH-RH in Tissue Culture

cultures were treated as follows: 2) Synthetic LH-RH, 11 days, with cycloheximide(2.0 µg/2 ml medium) present on days 7~10(Fig.2). This dose of cycloheximide blocked ^{3}H-leucine incorporation into protein by more than 95 %, but did not block new RNA synthesis. 3) Synthetic LH-RH, 11 days, with actinomycin D(0.2 µg/2 ml medium) present days 7~13(Fig. 3). This dose of actinomycin D blocked new messenger-RNA synthesis by more than 95 %. 4) Synthetic LH-RH, 11 days, with hydroxyurea(10^{-3}M) present days 7~13(Fig. 4). The medium was changed every day during the test periods and frozen for subsequent LH determination. LH concentrations in the media were measured with the double antibody radioimmunoassay using a human LH standard(0.9 x NIH-LH-S1). LH concentrations in the media below 2.7 ng/ml were undetectable. All results were expressed in units equivalent to NIH-LH-S1.

Results: After 30 days culture, the pituitary cells turned into confluent monolayers, but mitotic figures were still observed

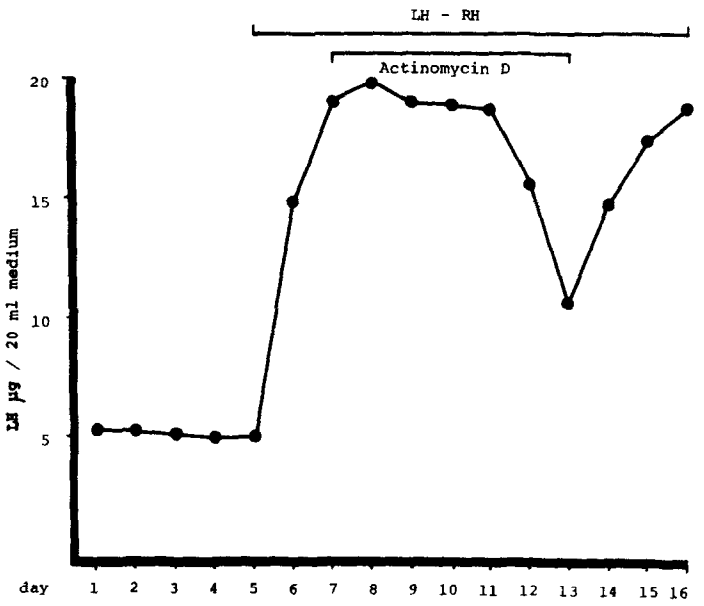

Fig. 3 Effect of Actinomycin D on Daily LH Production by LH-RH in Tissue Culture

frequently. These cells were stained weakly with PAS-iron hematoxylin. LH concentrations in the control media showed slow decline(from 5.2 to 5.0 μg LH/20 ml medium).

On the other hand, the addition of synthetic LH-RH(0.30 μg LH-RH/2 ml medium) caused a marked increase in LH content in the media(Fig. 1). Maximal LH concentrations, which were about 390 % of mean control levels, were achieved 3 days after the addition of synthetic LH-RH. After that, LH level was slightly down, but LH concentrations sustained higher than baseline levels during the 8-day period.

Cycloheximide(2.0 μg/2 ml medium) markedly inhibited the LH production normally stimulated by synthetic LH-RH(Fig. 2). However, when cycloheximide was removed from the medium, the increase of LH production occured promptly with synthetic LH-RH present.

The quantity of LH produced by synthetic LH-RH was not altered

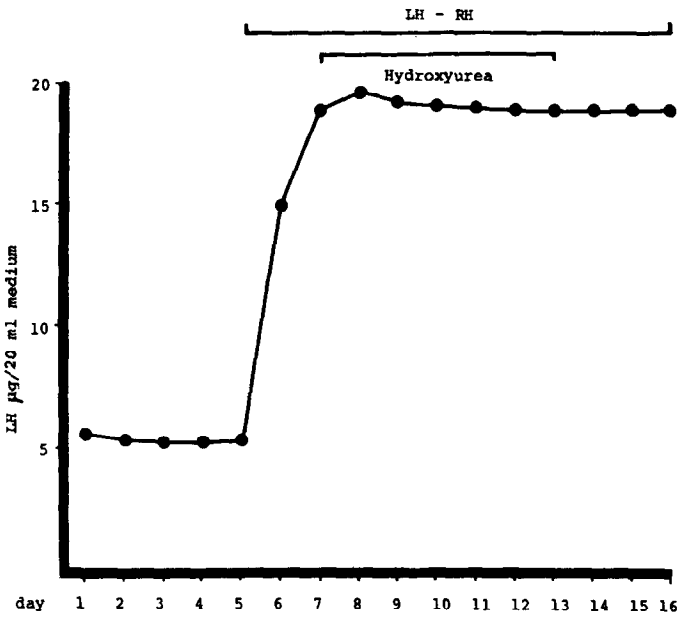

Fig. 4 Effect of Hydroxyurea on Daily LH Production by LH-RH in Tissue Culture

by the action of an actinomycin D for the 4-day period extending from days 7~10. After day 7, LH concentrations in the media showed marked decline. However, when actinomycin D was removed from the medium, the increase of LH production occured promptly with synthetic LH-RH present(Fig. 3). Hydroxyurea at 10^{-3}M blocked ^{3}H-thymidine incorporation into DNA by more than 60 %, but did not block the effect of LH-RH on LH production during experimental period(Fig. 4).

In the concentrations used in this study, the inhibitors caused no histologic evidence of toxicity.

Discussion: As demonstrated above, the addition of synthetic LH-RH to monolayer cultures of human female adenohypophysis causes marked stimulation in LH production and in cell proliferation. The increase in LH level in the media after the addition of synthetic LH-RH may be the result of (1) decreased degradation of

LH in the media, (2) release of LH from the cells, or (3) synthesis of new protein. The first possibility, that of decreased LH degradation, is inadequate to explain the speed of ^{3}H-LH degradation, which did not change after the addition of synthetic LH-RH in vitro. Although synthetic LH-RH may apparently alter membrane structure, it was found that the cytoplasmic membranes of LH-cells did not change ultrastructually after it was added. This evidence may exclude the possibility of a simple change in membrane permeability resulting in the passive release of LH as the mechanism of action for synthetic LH-RH. The final hypothesis, that synthetic LH-RH acts through the synthesis of new protein, is supported by the blocking effect of cycloheximide on LH synthesis. Synthetic LH-RH does cause an increase in LH production after the removal of cycloheximide, indicating that production occurs with the resumption of protein synthesis. Actinomycin D failed to inhibit LH elevation for the first 4 days after it was added. This failure indicates that new messenger-RNA synthesis is probably not required for LH production, suggesting that when actinomycin D is present, the following two possibilities may occur either singly or jointly: (1) Long-acting messenger-RNA can survive a maximum of 4 days; (2) the template of ribosomal RNA made by messenger-RNA can remain intact for as long as 4 days.

Hydroxyurea failed to inhibit LH elevation for the entire experimental period after it was added. This result suggests that new DNA synthesis does not have to occur to stimulate the production of LH by the action of synthetic LH-RH.

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