

## Sensitivity of self-potentiating effect of luteinising hormone-releasing hormone to cycloheximide

THE hypophysiotropic luteinising hormone-releasing hormone (LHRH) has a number of distinct actions on the anterior pituitary gland. In addition to stimulating the release and increased synthesis of gonadotrophins<sup>1</sup>, this peptide also has a priming or self-potentiating effect so that intermittent stimulation of the pituitary with identical doses of LHRH causes a progressively increasing series of responses of luteinising-hormone (LH) secretion. Self-potentiating by LHRH has been observed *in vivo* in rats<sup>2</sup> and man<sup>3</sup> and may play an important role in the generation of the LH surge which precedes ovulation. The effect can be obtained<sup>4</sup> following repeated, brief exposures of the isolated, perfused rat pituitary gland to LHRH and the time course of development and decay of the potentiated state in these conditions is similar to that observed in the intact rat<sup>2</sup>. We have investigated the effects of an inhibitor of protein synthesis on this phenomenon and report here that cycloheximide suppresses the priming effect of LHRH without impairing the acute response of LH release.

Pituitary glands from four dioestrous female rats were halved and divided into two groups of paired halves. Each group was placed in an identical Millipore Swinnex filter chamber and perfused simultaneously at 37 °C with Krebs-Ringer bicarbonate medium (pH 7.4) containing bovine serum albumin (2.5 g l<sup>-1</sup>), glucose (2 g l<sup>-1</sup>) and gassed with 95% O<sub>2</sub>-5% CO<sub>2</sub> (v/v). The flow rate was maintained at 0.2 ml min<sup>-1</sup> by means of a peristaltic pump and the effluent medium was collected every 10 min (2 ml fractions) and assayed for LH using an NIH radioimmunoassay kit.

Cycloheximide (5 μM) was added to the medium used to perfuse one group of halved pituitaries whereas the other group received only the control medium. After an initial period of 100 min in which the secretion of LH was allowed to decline towards basal levels, both groups of halved glands were subjected to 5 mins perfusion with 10 ng of synthetic LHRH dissolved in 1 ml of perfusion medium, introduced by transferring the inlet tube of the system to the test sample. Identical challenges with LHRH were administered at intervals of 1 h for a further period of 4 h and the effects on LH secretion in both groups are shown in Fig. 1.

Control and cycloheximide treated groups both showed similar patterns of LH secretion during the preliminary period of perfusion and also in response to the first stimulation with LHRH, which was given before LH secretion had reached basal levels. With subsequent exposures to LHRH, however, only the hemipituitaries perfused with control medium showed the typical pattern of increasing responsiveness to LHRH and after five such challenges the rate of secretion of LH had risen to about five times that of the glands treated with cycloheximide. The latter did not exhibit a potentiated response and a series of equal peaks of LH secretion were obtained in the presence of 5 μM cycloheximide.

Inhibition of the self-potentiating effect of LHRH has been consistently obtained in similar experiments in which concentrations of cycloheximide ranging from 5 μM to 250 μM were used. In no case was the acute release of LH in response to LHRH abolished. Dissociation between the acute release of LH and the development of enhanced responsiveness to a series of LH-releasing stimuli has also been obtained in preliminary experiments in which isolated perfused pituitary glands were exposed for 5 min to medium containing 50 mM K<sup>+</sup>. Osmolarity was maintained by adjusting the Na<sup>+</sup> concentration and the stimulus was administered at 1-h intervals. The high potassium medium caused an acute release of LH similar to that observed with LHRH but, as in the presence of cycloheximide,

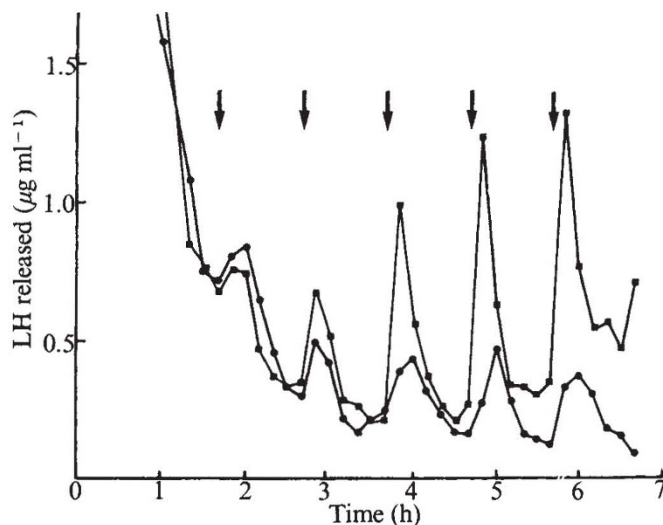


Fig. 1 Effect of cycloheximide on stimulation of LH release by LHRH. Two groups of paired, halved rat adenohypophyses were simultaneously perfused with Krebs-Ringer bicarbonate medium alone (■) and containing 5 μM cycloheximide (●). Glands were exposed to synthetic LHRH (10 ng ml<sup>-1</sup>) for 5 min at the times indicated by arrows. LH released to the perfusion medium in 10 min intervals was measured by radioimmunoassay and results are expressed in terms of the NIAMD-rat LH-RP-1 reference preparation. Cycloheximide abolished the self-potentiating produced by LHRH without reducing the acute release of LH.

the series of LH peaks were of equal magnitude and did not exhibit the increased responsiveness shown by glands primed with LHRH.

These results show clearly that the augmented release of LH which occurs from the primed pituitary gland represents a specific response to the hypophysiotropic hormone rather than a nonspecific sequel to the discharge of LH. Release of LH evoked by LHRH in the presence of cycloheximide or induced by depolarising levels of K<sup>+</sup> does not cause a potentiated response to further stimulation. The priming effect would seem to involve the synthesis of new proteins and it has been suggested that increased synthesis of LH may be important<sup>3</sup>. Only a small percentage of the LH content of the gland is, however, released in response to acute stimulation with a supramaximal dose of LHRH. An intermittent exposure to LHRH in the presence of cycloheximide does not cause a diminution of the acute secretory response. It is likely that the availability of LH is not a limiting factor and that the synthesis of some other component of the release mechanism may be essential for self-potentiating to occur. The effects of LHRH and cycloheximide on the incorporation of labelled amino acids into LH and various subcellular components are being studied and should resolve this issue.

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