Ovulation Induced by Synthetic Luteinizing Hormone–Releasing Hormone in the Hamster

Abstract. *A synthetic decapeptide, corresponding to the chemical structure of luteinizing hormone–releasing hormone from porcine hypothalami, was tested for the induction of ovulation in golden hamsters that had previously been treated with phenobarbital to prevent spontaneous ovulation. Subcutaneous injection of 0.089 to 0.357 nanomole of this synthetic luteinizing hormone–releasing hormone stimulated release of luteinizing hormone and induced ovulation.*

Ovulation is controlled by a complex interplay between certain hypothalamic structures, pituitary gonadotropins [follicle-stimulating hormone (FSH) and luteinizing hormone (LH)], and ovarian hormones (estrogens and progestins). It has been well established that the hypothalamus elaborates LH- and FSH-releasing hormones (LH-RH and FSH-RH) that are released into the hypophyseal portal system and transported to the anterior pituitary where they stimulate the secretion of LH and FSH (*1*). Intensive efforts have been made by several groups of investigators to purify LH-RH and FSH-RH and to elucidate their chemical structures. We have recently accomplished these tasks and established the structure of LH-RH of porcine origin as the decapeptide (*2*) (pyro)Glu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH_2.

The decapeptide was synthesized by Merrifield's solid phase method (*3*); this method may have resulted in oxidation of tryptophan to some extent, because of its lability to acid. The synthetic peptide was then purified by counter current distribution and electrophoresis and used in the present experiments. The synthetic LH-RH showed the same behavior as natural porcine LH-RH in both counter current distribution and electrophoresis. As in the case of natural LH-RH, the synthetic LH-RH possesses FSH-RH activity (*4*). The dose of the synthetic LH-RH was expressed in moles based on its tryptophan content.

Previously we demonstrated the induction of ovulation in rats by administration of a purified porcine LH-RH preparation; spontaneous ovulation was prevented by an injection of pentobarbital at the critical time of the afternoon of proestrus (*5*). We wished to ascertain whether administration of synthetic LH-RH would also induce ovulation by releasing LH in animals in which physiological preovulatory surge of secretion of these pituitary hormones is prevented. Female golden hamsters (Con Olson Co., Inc.) were used in the present study because the estrous cycle in this species is less irregular than it is in rats. All animals were housed in the animal quarters equipped to provide controlled light (5 a.m. to 7 p.m., light; 7 p.m. to 5 a.m., dark) and temperature, and they had access to Purina Chow biscuits and water. The estrous cycle was checked by inspecting vaginal discharge, which occurred every 4 days in most of the animals (*6*). Experiments were started after the animals showed two regular 4-day cycles. At 1:00 p.m. of the day of proestrus, which precedes the day when thick vaginal discharge is observed, all hamsters were injected intraperitoneally with 13 mg of phenobarbital per 100 g

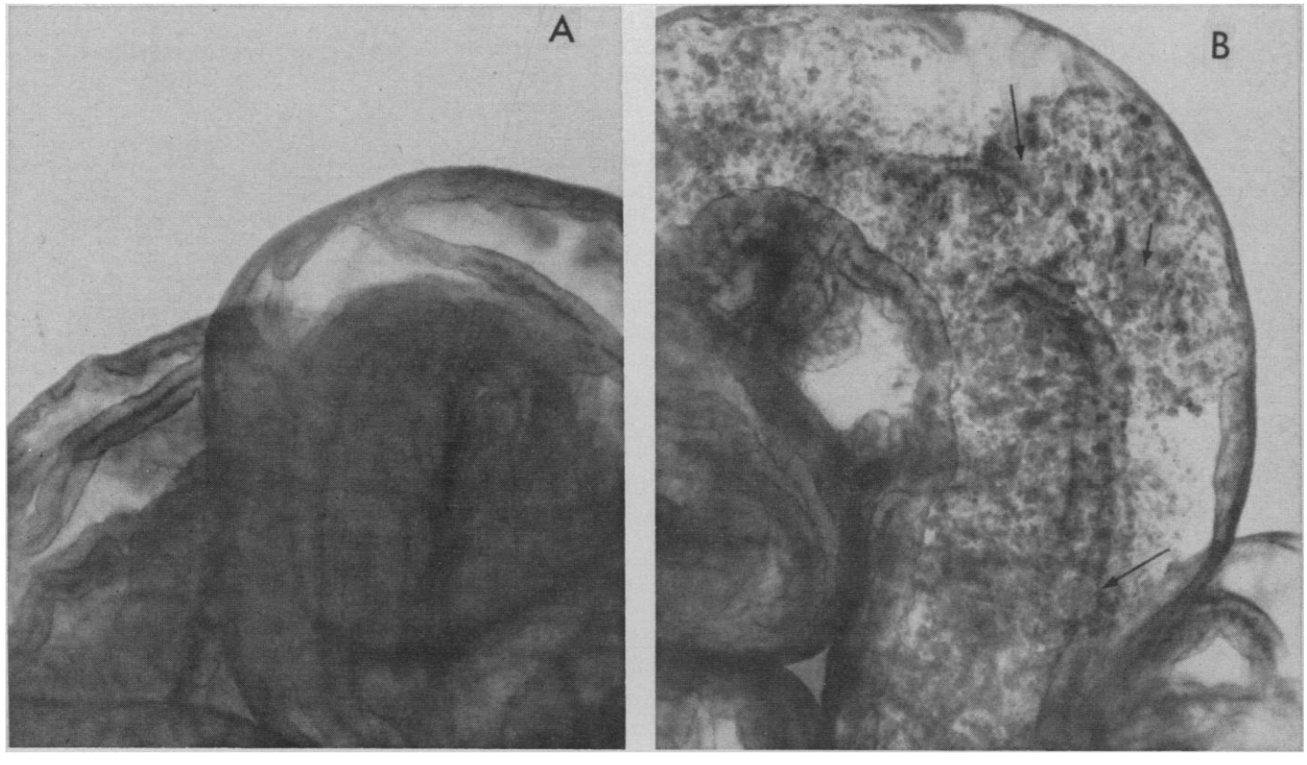

Fig. 1. Low-power micrographs of the oviduct of golden hamsters (× 40). They were injected with 13 mg of phenobarbital at 1:00 p.m. on the day of proestrus. (A) The oviduct from a control hamster that received 0.5 ml of acidified saline subcutaneously at 3:00 and 4:00 p.m. of the same day. The transparent part represents the ampulla of the oviduct. (B) The oviduct from a hamster injected with 0.179 nmole of synthetic LH-RH at 3:00 and 4:00 p.m. of the day of proestrus, with a total of 0.357 nmole. The ampulla is considerably dilated and contains cumulative mass in which several ova (indicated by arrow) are present. Fifteen ova were found in both oviducts in this animal.

Table 1. Induction of ovulation by synthetic LH-RH in golden hamsters pretreated with phenobarbital. Synthetic LH-RH was administered subcutaneously; LH, intraperitoneally.

Group	Additional pretreatment	Sample injected	Dose	Animals tested (No.)	Animals ovulated (No.)	Mean No. of ova found in the oviducts (± S.E.)
1		Saline		12	0	0
2		Synthetic LH-RH	0.357 nmole	5	5	13 ± 0.9
3		Synthetic LH-RH	0.179 nmole	4	4	9 ± 0.7
4		Synthetic LH-RH	0.089 nmole	5	3	4 ± 1.9
5	Hypophysectomy	Synthetic LH-RH	0.357 nmole	8	0	0
6	Hypophysectomy	LH	10 μg	6	6	11 ± 0.8

of body weight to block spontaneous ovulation (*7*). Experimental animals received various doses of synthetic LH-RH dissolved in 0.5 ml of acidified saline (0.01*M* acetic acid in 0.9 percent saline) in two subcutaneous injections at 3:00 and 4:00 p.m. to insure prolonged elevation of circulating LH. Control animals received 0.5 ml of acidified saline according to the same schedule. Some of the hamsters were hypophysectomized through the auditory canal soon after they were anesthetized. Some of these hypophysectomized animals received 0.357 nmole of synthetic LH-RH or 10 μg of ovine LH (NIH-LH-S 17) in 0.5 ml saline intravenously at 4:00 p.m.

Some of the experimental and control animals were bled from the jugular vein 20 minutes after the second injection of synthetic LH-RH or saline for determination of circulating LH levels. Luteinizing hormone was measured by radioimmunoassay as described by Goldman and Porter (*8*) and expressed as nanograms per milliliter of serum in terms of NIH-LH-S-18 standard.

The following morning the hamsters were killed and their oviducts were inspected under a microscope for the presence of ova. The results are shown in Table 1. All control hamsters injected with saline alone failed to ovulate, which indicates 100 percent blockade of spontaneous ovulation by phenobarbital, in agreement with the findings by Greenwald (*7*). On the other hand, subcutaneous injection of 0.357 nmole of synthetic LH-RH induced full ovulation in all hamsters. Figure 1 shows the ampulla of the oviduct from a control hamster and from an experimental hamster in which 15 ova were found in the oviducts. All animals that were given 0.179 nmole of synthetic LH-RH also ovulated, but the mean number of ova found in this group was smaller than the number found in the group that received 0.357 nmole. Administration of 0.089 nmole of LH-RH induced ovulation in three out of five hamsters. Injection of 0.357 nmole of synthetic LH-RH did not induce ovulation in hypophysectomized hamsters, excluding the possibility that synthetic LH-RH stimulates the ovaries directly. Intraperitoneal administration of 10 μg of LH to hypophysectomized hamsters induced ovulation in all the hamsters tested, which indicates that the ovaries of these hypophysectomized hamsters are capable of responding to LH.

Mean serum levels of LH 20 minutes after the second injection of saline and 0.179 nmole of synthetic LH-RH were 2.8 ± 0.72 (S.E.) and 38.4 ± 1.11 ng/ml, respectively. Although we could not determine circulating FSH levels, release of FSH may also have occurred following injection of synthetic LH-RH, as it does after the administration of natural LH-RH (*4*). It is likely that both the LH and the FSH released played a synergistic role in inducing ovulation (*7*).

The amount of peptide was estimated by basing it upon the content of tryptophan. The amount of active decapeptide may have been smaller than was estimated. This indicates that a very small dose of synthetic LH-RH was able to induce ovulation. We also observed that quick intravenous injection of 0.357 nmole of synthetic LH-RH did not affect blood pressure and the electrocardiogram as tested in the anesthetized rat, which indicates lack of undesirable side-effects on the cardiovascular system. Because of the ease of administration (subcutaneous injection), the synthetic LH-RH should become a very useful tool in clinical and veterinary fields.

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Human Red Blood Cells: Prostaglandin E_2, Epinephrine, and Isoproterenol Alter Deformability

Abstract. *The human red blood cell responds to prostaglandin E_2, epinephrine, and isoproterenol with a decrease in deformability. The maximum decrease is brought about by 10^{-10}M prostaglandin E_2, 10^{-9}M epinephrine, or 10^{-7}M isoproterenol. The dose response curve is biphasic. The sensitivity of the red cell to prostaglandin suggests that this cell may be a primary target for prostaglandin action. These changes in response to vasoactive substances indicate that the red cell must be considered an active element in circulatory control.*

Control of blood flow through the microcirculation has been thought to reside somewhere in the vascular bed. Changes, therefore, in the relations between pressure and flow of the circulation in an intact animal or in a perfused organ after various treatments have been interpreted as indications solely of