Extrapituitary Action of Gonadotropin-Releasing Hormone: Direct Inhibition of Ovarian Steroidogenesis

Abstract. *Gonadotropin-releasing hormone (GnRH) and its agonistic analogs inhibited the follicle-stimulating hormone (FSH)-induced increase of estrogen and progesterone production in vitro by rat ovarian granulosa cells. Likewise, GnRH analogs inhibited FSH-induced changes in ovarian function in hypophysectomized rats in vivo. These results indicate that GnRH, in addition to its well-known gonadotropin-releasing action in the pituitary, exerts a direct inhibition of ovarian steroidogenesis.*

Gonadotropin-releasing hormone (GnRH) stimulates pituitary gonadotropin production (*1*). However, pharmacological doses of GnRH and its agonists have been shown to inhibit a variety of female reproductive functions, such as ovarian steroidogenesis, ovulation, ovum transport, ovum implantation, pregnancy, and ovarian-dependent mammary tumorigenesis (*2*). The mechanism by which GnRH inhibits reproductive function is unknown. Although it is generally believed that the inhibitory effects of GnRH are mediated at the pituitary level, the possibility that GnRH may act at the level of the gonads is not excluded. We now report that GnRH and its agonistic analogs act independently of the pituitary to inhibit gonadal function: blockage of the gonadotropin-induced increases in ovarian steroidogenesis in vitro and antagonism of the follicle-stimulating hormone (FSH)-induced changes in ovarian function in hypophysectomized rats in vivo.

Follicle-stimulating hormone stimulates production of estrogen and progestin in primary cultures of ovarian granulosa cells (*3*). The effect of GnRH on these FSH actions was investigated. Granulosa cells from immature intact (23 to 25 days of age) and hypophysectomized diethylstilbestrol-treated (27 to 30 days of age, 5 days after operation) rats were cultured for 2 days (*4*). In the presence of estrogen synthetase (also called aromatase) substrate ($10^{-7}M$ androstenedione), purified FSH (100 ng/ml; Papkoff G4-150C, FSH potency equal to 50 NIH-FSH-S1 standard units per milligram) stimulated estrogen production by 40- to 60-fold. Concomitant treatment with GnRH or GnRH agonists ([D-Leu^6-(N$^\alpha$Me)Leu^7]GnRH, analog 1; des-Gly^{10}-[D-Try^6, Pro^9-NHEt]GnRH, analog 2) (*5*, *6*) inhibited FSH-stimulated increase in estrogen production (Fig. 1A). Similarly, GnRH and its agonists inhibited the FSH-induced increase in progesterone production by the granulosa cells (Fig. 1B). These results indicate that GnRH exerts a direct inhibition on ovarian steroidogenesis.

The in vivo effects of GnRH agonists on ovarian function were also examined in hypophysectomized estrogen-treated rats. Four days after hypophysectomy, immature estrogen-treated female rats were injected subcutaneously, twice daily, for 2 days with 100 μg of ovine FSH (NIH-FSH-S12) or the same amount of FSH plus GnRH analogs (50 μg of analog 1 per 0.1 ml of saline; 10 μg of analog 2 per 0.1 ml of saline). The ovarian weight and steroidogenic potential were determined 12 hours after the last injection. The FSH induced a significant increase in ovarian weight (control, 12.2 ± 1.1 mg, $N = 11$; FSH treated, 29.0 ± 2.0 mg, $N = 8$). In contrast, concomitant treatment with GnRH agonists inhibited the FSH-induced increase in ovarian weight (FSH plus analog 1, 15.6 ± 2.0 mg, $N = 8$; FSH plus analog 2, 14.7 ± 1.3 mg, $N = 8$). In vivo FSH treatment for 2 days induced a substantial increase in aromatase activity in ovarian granulosa cells. Granulosa cells were harvested from control and treated animals and incubated for 3 hours in the presence of a saturating dose of estrogen synthetase substrate ($10^{-7}M$ Δ^4-androstenedione). The estrogen production was stimulated from an undetectable level in granulosa cells of control animals to 1.65 ng per 10^5 granulosa cells in FSH-treated animals. Concomitant treatment with GnRH agonists inhibited the FSH-induced increase in estrogen production (FSH plus analog 1, 0.29 ng per 10^5 cells; FSH plus analog 2, 0.25 ng per 10^5 cells). The results indicate that treatment in vivo with GnRH agonists in hypophysectomized animals interferes with the FSH action at the ovarian level.

Our results demonstrate that GnRH and its agonists exert extrapituitary action as shown by their ability to inhibit ovarian function directly. This finding may serve as the basis for explaining the inhibitory effect of GnRH on reproductive functions. Thus, in addition to their well-known releasing action on the pituitary gonadotrophs, pharmacological doses of GnRH and its agonists could decrease ovarian estrogen production by directly interfering with normal ovarian function. A GnRH-induced decrease in estrogen production offers an expla-

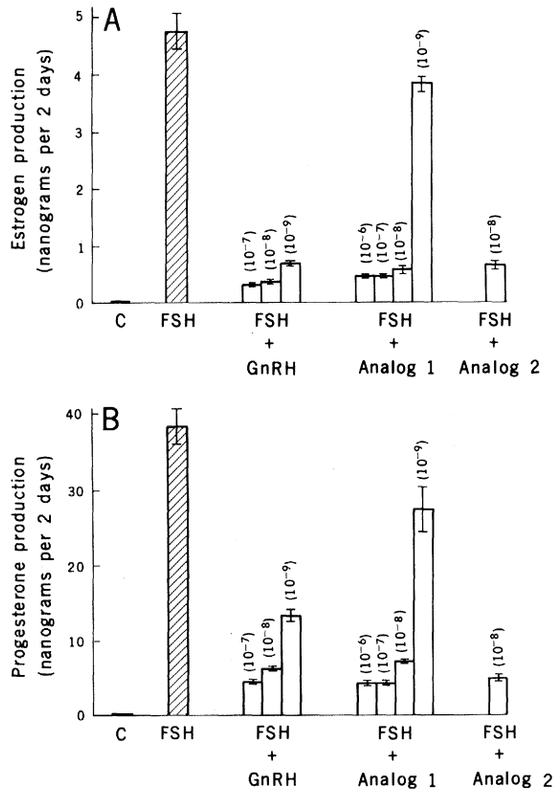

Fig. 1. Effect of GnRH and its agonistic analogs on FSH-induced increase in steroid production by ovarian granulosa cells. (A) Estrogen production; (B) progesterone production. Ovarian granulosa cells were obtained from hypophysectomized estrogen-treated immature female rats and cultured for 2 days in the presence of $10^{-7}M$ androstenedione. Ovine FSH (100 ng/ml), with or without GnRH or its agonists, was added to the media at the beginning of the culture. Estrogen and progesterone content in the media after 2 days culture was determined by radioimmunoassay. Similar results were obtained with granulosa cells from intact immature rats. Each data point represents mean ± standard deviation of four to six separate cultures. The numbers inside the parentheses represent the final concentration of GnRH or analogs in culture medium.

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nation for the reported blocking effect of GnRH on various estrogen-dependent processes, including ovulation, ovum transport, ovum implantation, and mammary tumorigenesis. Moreover, the inhibitory effect of GnRH and agonists on FSH-induced progesterone production in cultured granulosa cells could offer a possible explanation for the interference by GnRH and its agonists in such a progesterone-dependent process as pregnancy. Our results, however, do not exclude the possibility that administration in vivo of pharmacological doses of GnRH in intact animals may also cause imbalances in pituitary gonadotropin production that also result in the inhibition of various reproductive functions (*7*).

The mechanisms by which GnRH exerts inhibition on ovarian granulosa cells are not known. Although GnRH receptors have been identified in the anterior pituitary by radiotracer binding technique and by immunocytochemical methods (*8*), their tissue specificity has not been demonstrated. Bernardo *et al.* (*9*) have reported putative GnRH binding sites in the mouse adrenal cells. It is possible that ovarian granulosa cells may have GnRH receptors and that the inhibitory effect of GnRH that we observed is mediated through hormone-receptor interactions.

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5. Abbreviations are Leu, leucine; Gly, glycine; Try, tryptophan; Pro, proline; NHEt, ethylamide; and Me, methyl.
6. The potencies of the GnRH analog 1 and analog 2 relative to native GnRH, as determined by their ability to cause luteinizing hormone release in cultured pituitary cells, are approximately 6- and 144-fold, respectively.
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10. We thank L. Tucker and C. Fabics for their technical assistance; Dr. S. S. C. Yen for his interest; Drs. N. Ling and J. Rivier for providing the GnRH analogs; and Dr. H. R. Papkoff and the NIAMDD Pituitary Hormone Distribution Program for providing FSH preparations. Supported by NIH grant 1-R01-CA 21867 and Rockefeller Foundation grant RF-75029.

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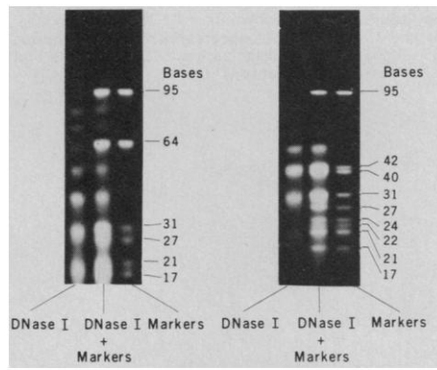
Periodicity of Deoxyribonuclease I Digestion of Chromatin

Abstract. *Two methods have been used to measure the single-strand lengths of the DNA fragments produced by deoxyribonuclease I digestion of chromatin. The average lengths obtained are multiples of about 10.4 bases, significantly different from the value of 10 previously reported. This periodicity in fragment lengths is closely related to the periodicity of the DNA double helix in chromatin, but the two values need not be exactly the same.*

The first level of condensation of DNA in chromatin is brought about by its interaction with histones to form nucleosomes, the elementary subunits of the structure. For a detailed understanding of how the DNA is folded, it is necessary to know both the path of the double helix and its periodicity, or the number of base pairs per turn, in the nucleosome. X-ray crystallographic studies indicate a path in which the DNA is wrapped twice around the histones (*1*). This appears to conflict with measurements on closed, circular DNA extracted from SV 40 chromatin showing nearer one superhelical turn (*2*). The conflict can be resolved by postulating a change in the periodicity of the DNA as it is folded into a nucleosome (*1*). A decrease of only about 5 percent is required, and so before firm conclusions can be drawn it is necessary to know the relevant parameters as accurately as possible.

Noll (*3*) has suggested that the perio-



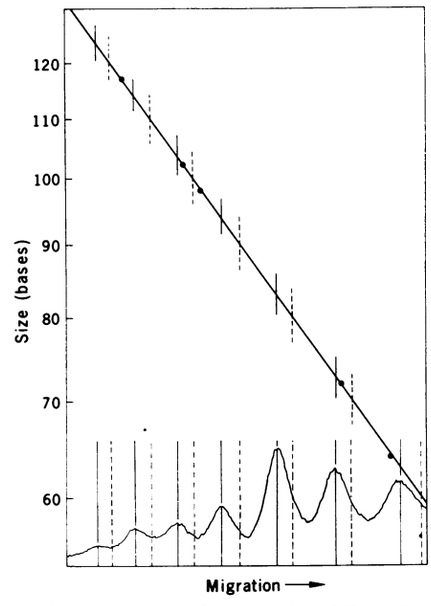


Fig. 1 (left). Comparison of DNase I and marker fragments in polyacrylamide gels. DNase I fragments were prepared by DNase I digestion of rat liver nuclei and extraction of the DNA (*3*). Marker fragments were prepared as described in Table 1. All fragments were labeled with ^{32}P as follows. DNA (40 μg/ml) in 50 m*M* sodium acetate (*p*H 4.6) and 1 m*M* EDTA was boiled for 1 minute and treated with spleen acid phosphomonoesterase B (*16*) (0.1 unit per milliliter) for 2 hours at 37°C. The *p*H was then raised to 8.0 by the addition of tris base to 85 m*M* and the mixture was boiled for 1 minute, supplemented with $MgCl_2$ (10 m*M*), 2-mercaptoethanol (15 m*M*), and γ-^{32}P-labeled ATP (tenfold excess over 5′-OH termini), and treated with polynucleotide kinase (20 unit/ml) (New England Biolabs) for 1 hour at 37°C. The labeled DNA was purified by filtration through Sephadex G-25 in a mixture of 0.1*M* NaCl, 10 m*M* tris-HCl (*p*H 7.5), and 1 m*M* EDTA and subjected to electrophoresis in 12 percent (left panel) and 20 percent (right panel) polyacrylamide–98 percent formamide gels (*17*). Negatives of autoradiograms of the gels are shown. Fig. 2 (right). Size determination of DNase I bands. Densitometer traces of lanes in left panel of Fig. 1 containing DNase I (bottom) and marker fragments were aligned. The sizes and distances of migration of the marker fragments (filled circles) were used to construct a calibration curve from which the sizes of the DNase I fragments were derived. The distances of migration of the DNase I fragments (solid vertical lines) are contrasted with those expected if the fragments were multiples of 10.0 bases (dashed vertical lines).

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