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Inhibitory effect of gonadotropin releasing hormone in the uterus of rat

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Abstract. Estradiol-induced incorporation of radioactive uridine and leucine into the uterus of rat was inhibited by simultaneous treatment with an analogue of gonadotropin releasing hormone. The inhibitory action of gonadotropin releasing hormone was not mediated through modulation of estradiol receptor content. However, estradiol-induced increase in the level of poly(A) polymerase was inhibited by gonadotropin releasing hormone analogue, indicating that the inhibitory effect might involve post-transcriptional phenomenon.

Keywords. Gonadotropin releasing hormone; RNA and protein synthesis; estradiol receptors; poly(A) polymerase.

Introduction

Gonadotropin releasing hormone (GnRH) stimulates the production and release of follicle stimulating hormone (FSH) and luteinizing hormone (LH) from pituitary, (Schally, 1978; Guillemin, 1978). However, high doses of GnRH and its potent agonists were shown to inhibit a variety of reproductive functions in females and males (Hsueh and Jones, 1981; Sharpe, 1982). Chronic treatment with a GnRH agonist was shown to inhibit ovulation in women (Nillius *et al.,* 1978). Ovarian growth induced by human chorionic gonadotropin (hCG) and pregnant mare serum gonadotropin and uterine growth induced by hCG were found to be inhibited by GnRH (Ying and Guillemin, 1979; Rippel and Johnson, 1976). GnRH and its agonists were shown to inhibit FSH induced estrogen and progesterone production in cultured granulosa cells, thus demonstrating its direct extra-pituitary effects (Hsueh and Erickson, 1979a). A decrease in testicular LH/hCG receptors and inhibition of testicular steroidogenesis was caused by GnRH (Hsueh and Erickson, 1979b). Similar inhibition of LH receptor content and steroidogenesis in testis by GnRH agonists was shown in immature and adult hypophysectomized rats (Bambino *et al.,* 1980). However, during short-term treatment GnRH and its agonists caused stimulation of Leydig cell steroidogenesis (Hunter *et al.,* 1982) and ornithine decarboxylase activity (Madhubala and Reddy, 1982). In addition to the effect on gonads, GnRH and its agonists were shown to have direct effects on accessory reproductive organs. Sundaram *et al.* (1981) showed that GnRH and its

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Abbreviations used: GnRH, Gonadotropin releasing hormone; FSH, follicle stimulating hormone; LH, luteinizing hormone; hCG, human chorionic gonadotropin; ODC, ornithine decarboxylase; GnRHa, GnRH analogue; TCA, trichloroacetic acid.

agonists inhibit testosterone induced growth of ventral prostate and seminal vesicles and estrogen stimulated utering growth in immature, hypophysectomized and castrated rat. However, the biochemical basis for such effects and the mechanism of action of GnRH in accessory reproductive organs is not clear. We have recently demonstrated that GnRH and its agonistic analogs inhibit estradiol-induced ornithine decarboxylase (ODC, EC 4.1.1.17) and glucosamine-6-phosphate synthase (EC 5.3.1.19) activities in adult ovariectomized rat uterus (Rao and Reddy, 1984).

Poly(A) polymerase (EC 2.7.7.19) catalyses the addition of AMP residues at the 3′ end of mRNA (Darnell *et al.,* 1971) and it was shown by us that GnRH causes inhibition of poly (A) polymerase and polyadenylation in ventral prostate (Raju and Reddy, 1983). The present study extends these observations in the uterus. In addition, the effect of GnRH on uterine cytosol receptors for estradiol and on the incorporation of radioactive precursors into RNA and protein was also studied.

Materials and methods

GnRH was a gift from the National Pituitary Agency, NIAMDD, USA. GnRH analogue (GnRHa, D-Ala^{6}, des Gly^{10}-ethylamide-GnRH) was purchased from Sigma Chemical Co., St. Louis, Missouri, USA. Tris, sucrose, mercaptoethanol, 17-β-estradiol, poly(A), creatine phosphate, creatine Phosphokinase and ATP were also obtained from Sigma Chemical Co., St. Louis, Missouri, USA. All other chemicals were of analytical grade and were procured locally.

[^{3}H]-Uridine (12·7 Ci/mmol), [^{3}H]-leucine (6·8 Ci/mmol)and 8-[^{3}H]-adenosine-5′-triphosphate (1·5 Ci/mmol) were purchased from Bhabha Atomic Research Centre, Bombay. 6,7-[^{3}H]-Estradiol (40 Ci/mmol) was purchased from Radio Chemical Centre, Amersham, England.

Two to four months old female rats of Wistar strain weighing around 150 g were used in these experiments. They were housed at 25°C with 14 h light and 10 h dark schedule with free access to water and pelleted food supplied from Hindustan Lever, Bombay.

Incorporation studies

On the morning of day 4 of ovariectomy, rats were subcutaneously injected with estradiol in 0·2 ml of sesame oil at a dose of 0·1 μg per rat for [^{3}H]-leucine incorporation studies and with l·0 μg per rat for [^{3}H]-uridine incorporation studies. The above two doses of estradiol were chosen for studies on [^{3}H]-leucine and [^{3}H]-uridine incorporation respectively, as it required these doses to cause significant incorporation of precursors. Various doses of GnRHa were subcutaneously injected in 0·1 or 0·2 ml of saline either simultaneously with estradiol or at various intervals after estradiol treatment. When given in two doses, the second dose of GnRHa was given 10 h after the first injection. All the animals were killed 24h after estradiol injection. [^{3}H]-Leucine or [^{3}H]-uridine at a dose of 1 μCi. per uterine horn was injected through intrauterine route, 4 h before killing the animals. The incorporation of [^{3}H] -uridine or [^{3}H]-leucine into trichloroacetic acid (TCA) precipitable materials was studied and the amount of incorporation of radioactive precursors is expressed as C.P.M. per 100mg uterine weight.

Estradiol receptor measurement

Three days after ovariectomy, rats were given subcutaneous injection of 0·1 μg of estradiol in 0·2 ml of sesame oil. GnRHa was injected subcutaneously at a dose of 40 μg per rat in 0·2 ml of saline along with estradiol. All animals were killed 6 h thereafter and the uteri were removed, washed with cold saline for further processing. Uteri from 2 rats were pooled and homogenized in 4 ml of TED buffer, pH 7·4 (Tris 10 mM, EDTA 1·5 mM, DTT 0·5 mM and 30% glycerol, v/v). Separation of nuclear myofibrillar fraction and cytosol was done according to Traish *et al.* (1977). Nuclear estrogen receptor was extracted with KCl according to the procedure of Okulicz *et al.* (1983).

Estradiol receptor content in cytosol and nuclear fractions was assayed using the exchange assay as detailed by Muller and Wotiz (1977). The radioactivity was counted in a Beckman liquid scintillation spectrometer. The pellet obtained after KCl extraction was dissolved in 0·3 M KOH to estimate DNA content (Burton, 1956). The protein content of cytosol was estimated according to Lowry *et al.* (1951).

Assay of poly (A) polymerase activity

One day after the ovariectomy, rats were subcutaneously injected with 1 μg of estradiol in 0·2 ml of sesame oil for three consecutive days. GnRHa was injected on all these days at a dose of 100 μg per rat along with estradiol A second dose of GnRHa was given 10 h after the first injection. All the animals were killed 24 h after the last injection of estradiol and the uteri from two animals were pooled and processed for the assay of poly(A) polymerase activity according to the method of Orava *et al.* (1980) as described (Raju and Reddy, 1983). Protein content was measured according to Lowry *et al.* (1951). Poly(A) polymerase activity is expressed as nmol AMP incorporated per 30 min per mg protein. Statistical analysis was done using Student's '*t*' test.

Results

Effect of GnRHa on [^{3}H]-leucine incorporation

Effect of GnRHa on estradiol, induced total protein synthesis was studied by monitoring [^{3}H]-leucine incorporation into TCA precipitable material (table 1). The results show that estradiol at a dose of 0·1 μg per rat causes significant increase (*P*

Table 1. Effect of GnRHa on estradiol induced [^{3}H]-leucine incorporation into uterus.

Treatment	[^{3}H]-Leucine incorporated (C. P. M./100 mg uterus)
Control	5049 ± 1366 (7)
Estradiol (0·1 μg)	7990 ± 2023 (7)*
Estradiol + GnRHa (100 μg + 100 μg)	5304 ± 1186 (5)**

Values are mean ± S.D. of number of animals given in parentheses. *$P < 0·01$ as compared to control and **$P < 0·05$ as compared to estradiol treated group.

< 0·01) in the incorporation of [^{3}H]-leucine into the uterus. Injection of GnRHa along with estradiol causes inhibition ($P < 0·05$) of estrogen induced increase in leucine incorporation.

Effect of GnRHa on [^{3}H]-uridine incorporation

Estradiol injection induced significant increase ($P < 0·001$) in the incorporation of [^{3}H]-uridine into the uterus (table 2). Simultaneous treatment with a single dose of 50 or 100 μg of GnRHa caused significant reduction ($P < 0·01$) in uridine incorporation. Two more injections of GnRHa caused further reduction ($P < 0·001$) in incorporation of uridine. The effect of delayed treatment with GnRHa on estradiol induced [^{3}H]-uridine incorporation is also given in table 2. It is interesting to note that the inhibitory dose of GnRHa did not elicit the same response, when it was administered 30 min after estradiol treatment. Similarly when given 6 or 18 h after estradiol injection, GnRHa did not cause any change in [^{3}H]-uridine incorporation into the uterus.

Table 2. Effect of GnRHa on estradiol-induced [^{3}H]-uridine incorporation into uterus.

Group No	Treatment	[^{3}H]-Uridine incorporated (C. P. M./100 μg uterus)
1.	Control	2320 ± 408 (7)
2.	Estradiol (1 μg)	5767 ± 1087 (5)†
3.	Estradiol + GnRHa (25 μg)	4573 ± 1624 (4)
4.	Estradiol + GnRHa (50 μg)	2631 ± 540 (3)*
5.	Estradiol + GnRHa (100 μg)	2478 ± 984 (4)*
6.	Estradiol + GnRHa (100 μg + 100 μg)	2689 ± 636 (5)**
7.	Estradiol + GnRHa (50 μg) (30 min delay)	6318 ± 1861 (3)
8.	Estradiol + GnRHa (100 μg) (6 h delay)	5007 ± 2226 (4)
9.	Estradiol + GnRHa (100 μg) (18 h delay)	4522 ± 591 (3)

Values are mean ± S.D. of number of animals given in parentheses. In groups 7,8 and 9 GnRHa was given in a single dose at various times after estradiol injection, as indicated in parentheses. †$P < 0·001$ as compared to group 1; *$P < 0·01$ and **$P < 0·001$ as compared to group 2.

Effect of GnRHa on estradiol receptor content in vivo

With a view to understand the mechanism of inhibition of estradiol-induced response in the uterus by GnRH, the effect of GnRHa on cytosolic and nuclear estrogen receptor of estrogen treated rats was studied. Simultaneous treatment with GnRHa at a dose of 40 μg per rat did not cause any change in the receptor content. The cytosolic receptor content of both estradiol treated and that of estradiol and GnRHa treated groups was similar (160 fmol/mg protein). Similarly the nuclear estradiol receptor content of both of these groups was around l·5 pmol/mg DNA.

Poly(A) polymerase activity

The design of this experiment for monitoring poly(A) polymerase activity was different since it was observed that the response of induction of this enzyme on estradiol treatment was rather slow. Hence, the level of poly(A) polymerase was maintained by injecting with 1 μg of estradiol per day for 3 days, starting from one day after ovariectomy. This caused a significant increase in the enzyme activity ($P < 0{\cdot}01$) in the uterus. The data given in table 3 shows that the treatment with GnRHa along with estradiol inhibited this induction ($P < 0{\cdot}05$).

Table 3. Effect of GnRHa on estradiol-induced poly(A) polymerase activity in the utertus of rat.

Treatment	Poly(A) polymerase activity (nmol AMP incorporated/ 30 min/mg protein)
Control	1·08 ± 0·12(3)
Estradiol	3·54 ± 1·02 (8)*
Estradiol + GnRHa	1·98 ± 0·93 (3)**

Values are mean ± S.D. of number of animals given in parentheses. *$P < 0{\cdot}01$ as compared to control and **$P < 0{\cdot}05$ as compared to estradiol treated group.

Discussion

These results are consistent with our earlier study in which it was shown that enzymes associated with growth of the tissue are inhibited by GnRHa (Rao and Reddy, 1984). Since enhanced incorporation of [^{3}H]-leucine and [^{3}H]-uridine indicate general growth promotion, inhibition by GnRHa indicates that the growth of the uterus caused by estradiol is inhibited. An interesting feature of the present results is that the inhibition of uridine incorporation by GnRHa is observed only if it is given simultaneously along with estradiol. When GnRHa injection was delayed by even 30 min, the inhibitory effect was not observed. This suggests that the inhibition of early biochemical events, induced by estradiol, is required to observe the inhibitory effect of GnRH.

Magoffin *et al.* (1981) observed that the inhibitory effect of GnRH on androgen secretion in ovarian interstitial cells is rapid (t 1/2 = 10 min) and persists, even after washing the cells, for about two days. It was suggested that GnRH possibly enters the cytoplasm to cause prolonged inhibition. By immunochemical techniques it was observed that GnRH is internalized in the pituitary cells and binds to gonadotroph lysosomes and secretory granules (Bauer *et al.,* 1981). It is not known whether GnRH enters the uterine cells also and binds to any specific loci to cause the inhibitory effects. It is well known that estrogens enter cytoplasm and bind to specific receptors (Jensen and Jacobson, 1962). The classical anti-estrogens are known to bind competetively to the estrogen receptors and block estrogen-induced response (Sankaran and Prasad, 1972; Emmens *et al.,* 1962). Our results show that GnRHa, at a dose which caused

inhibition of estradiol-induced ODC stimulation (Rao and Reddy, 1984) did not cause any change in the level of estradiol receptor *in vivo.* From this study it can be deduced that the inhibitory action of GnRH and its analogue on estradiol-induced responses does not involve estradiol receptors. Lecomte *et al.* (1982) have also observed that though the agonists of GnRH cause inhibition of androgen induced β-glucorinidase activity in the kidney of mouse, the cytosolic androgen receptors were not affected.

Since an exposure with GnRH was shown to cause prolonged effects (Magoffin *et al.,* 1981), it is possible that the treatment with GnRH may cause post-transcriptional Effects. In this study, it was observed that estradiol-induced poly(A) polymerase is inhibited by treatment with GnRHa. It is well known that poly(A) segment stabilises the newly synthesized mRNA molecules and increases its half-life and efficiency of translation (Wilson *et al.,* 1978; Hruby, 1978). Inhibition of poly(A) polymerase by GnRH or its agonists may result in short lived mRNA molecules, thus affecting the efficiency of their translation. It was observed that dihydrotesterone-induced poly(A) polymerase activity and polyadenylation of mRNA are inhibited by GnRH in the ventral prostate of rat (Raju and Reddy, 1983); the results presented in this study support this observation.

The physiological implications of the present results are interesting. Since GnRH is produced in small amounts from hypothalamus (Eskay *et al.,* 1977) it may not be sufficient to reach the various peripheral target organs to exert its actions. It was proposed that GnRH-like peptides may be produced locally in some reproductive tissues and modulate their own functions (Sharpe, 1982). Though the presence of GnRH-like substances has not yet been demonstrated in the uterus, the present data suggest the physiological functions of GnRH in the uterus.

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