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DIRECT INHIBITORY ACTIONS OF GnRH ON ACCESSORY REPRODUCTIVE ORGANS OF RAT

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Summary—Recently gonadotropin releasing hormone (GnRH) and its agonistic analogs were demonstrated to have some direct actions in accessory reproductive organs. In our study the effects of GnRH and its analogs on some steroid hormone induced responses were investigated. GnRH and its analogs inhibited estradiol induced ornithine decarboxylase (ODC) and glucosamine-6-phosphate synthase activities in the uterus of rat. These enzymes which are markers for cell proliferation are regulatory enzymes in the biosynthetic pathways of polyamines and glycoproteins, respectively. Similarly, GnRH and its analogs also inhibited testosterone stimulated ODC activity in ventral prostate of rat. In addition, GnRH analog inhibited incorporation of radioactive precursors into RNA and protein induced by estradiol in uterus or dihydrotestosterone (DHT) in ventral prostate. In an effort to elucidate the mechanism of action of GnRH in uterus, it was found that GnRH analog treatment does not alter the estradiol receptor content *in vivo*. Also, GnRH does not show any effect on radioactive estradiol binding to its receptor *in vitro*. Hence, the inhibitory actions of GnRH in uterus may not involve estradiol receptors. However, GnRH analogs were found to have post-transcriptional effects. It was observed that DHT induced poly(A) polymerase activity in ventral prostate and estradiol induced poly(A) polymerase activity in uterus were inhibited by GnRH analog treatment. It was further observed that GnRH inhibited incorporation of [^{3}H]uridine into poly(A)$^+$ RNA of ventral prostate. This indicates that the inhibitory effects of GnRH involve post-transcriptional mechanisms.

INTRODUCTION

Though the primary function of gonadotropin releasing hormone was shown to be to stimulate pituitary gland, recent work has demonstrated that it causes direct effects on extrapituitary tissues [1, 2]. GnRH was shown to cause inhibition of hormone induced growth of uterus and ventral prostate in rat [3]. In our laboratory we have observed that GnRH and its analogs cause inhibition of androgen stimulated poly(A) polymerase in the ventral prostate [4] and estrogen induced ornithine decarboxylase (ODC) in the uterus [5]. This paper reviews our work on the effects of extra pituitary actions of GnRH and its analogs on ventral prostate and uterus of rat.

EFFECTS OF GnRH AND ITS ANALOGS ON INCORPORATION OF RADIOACTIVE URIDINE AND AMINO ACIDS INTO VENTRAL PROSTATE AND UTERUS

Adult male or female rats were gonadectomised and 3 days after that the experiments were performed. Castrated male rats were injected with 200 μg of dihydrotestosterone (DHT) or oil alone. Simultaneously 20 μg of GnRH analog (GnRHa, D-Ala^6-des Gly^{10}-ethylamide-GnRH) and 10 μCi of [^{3}H]uridine (3.5 Ci/mmol) was injected into the ventral prostate directly and the animals were sacrificed 4 h later. Figure 1 shows that DHT causes significant increase in the incorporation of uridine into TCA precipitable macromolecules of ventral prostate and simultaneous injection of GnRHa inhibits this DHT induced incorporation. For studies involving the incorporation of amino acids, animals castrated 72 h previously were injected with 200 μg of DHT and simultaneously 100 μg of GnRHa was injected subcutaneously. A second injection of GnRHa at the same dose was given 12 h later. The animals were treated similarly on the second day. Four hours before killing the animals 10 μCi of 1:1 mixture of [^{3}H]phenylalanine (6.3 Ci/mmol) and [^{3}H]leucine (6.8 Ci/mmol) were injected into the two lobes of prostate directly. Figure 2 shows that DHT induced increase in the incorporation of radioactive aminoacids into TCA precipitable material was significantly inhibited by simultaneous treatment with GnRH.

On day 4 of ovariectomy adult female rats were injected with 1 μg of estradiol subcutaneously. GnRHa at a dose of 50 μg per rat was injected subcutaneously in saline at the same time. Twenty hours thereafter 1 μCi of [^{3}H]uridine was instilled into each of the 2 horns of the uterus and 4 h later the animals were killed and the radioactivity incorporated into TCA precipitable material was monitored in the controls, estradiol treated and in estradiol and GnRHa treated animals. In 1 group of animals injection of GnRHa was delayed by 30 min after the injection of estradiol. The results presented in Fig. 3 show that simultaneous injection of GnRHa to estradiol treated animals caused significant inhibition of incorporation of [^{3}H]uridine. However, in the group treated with GnRHa 30 min after estradiol treatment there was no difference in incorporation of

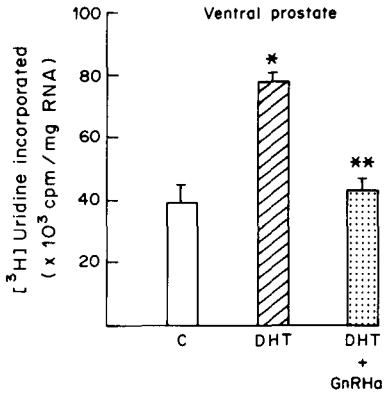

Fig. 1. Effect of GnRHa on DHT induced incorporation of [^{3}H]Uridine into ventral prostate. *P < 0.001 compared to control (C) and $P < 0.001$ compared to DHT treated group.

[^{3}H]uridine when compared to estradiol alone treated group. Figure 4 shows the effect of GnRHa on estradiol induced incorporation of [^{3}H]leucine into TCA precipitable proteins. In this experiment 0.1 μg of estradiol was injected along with 100 μg of GnRHa. A second injection of GnRHa was given 12 h later and the animals were killed 24 h after the injection of estradiol. Four hours before killing the animals 1 μCi of [^{3}H]leucine was instilled into each uterine horn. The TCA precipitable radioactivity was measured in control, estradiol treated and in estradiol and GnRHa treated groups. The results (Fig. 4) show that GnRHa injection abolished estradiol induced increase in incorporation of [^{3}H]leucine into uterine proteins.

EFFECT ON ODC ACTIVITY

Ornithine decarboxylase (EC 4.1.1.17) is a rate limiting enzyme in the biosynthesis of polyamines and this enzyme is stimulated by androgens in the ventral prostate [6] and by estrogens in the uterus [7] of rat. The effect of testosterone and simultaneous injection of GnRHa on ODC is given in Fig. 5. In this experiment testosterone at a dose of 200 μg was subcutaneously injected to 5 day castrated adult rats.

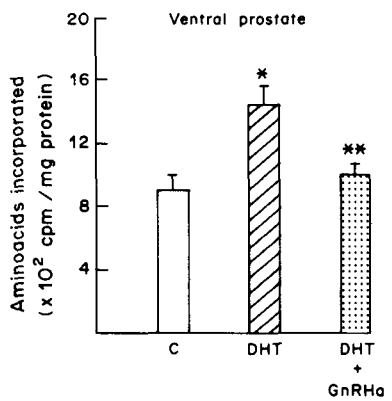

Fig. 2. Effect of GnRHa on DHT induced incorporation of tritiated amino acids into ventral prostate. *$P < 0.02$ vs control (C) and **$P < 0.05$ vs DHT treated group.

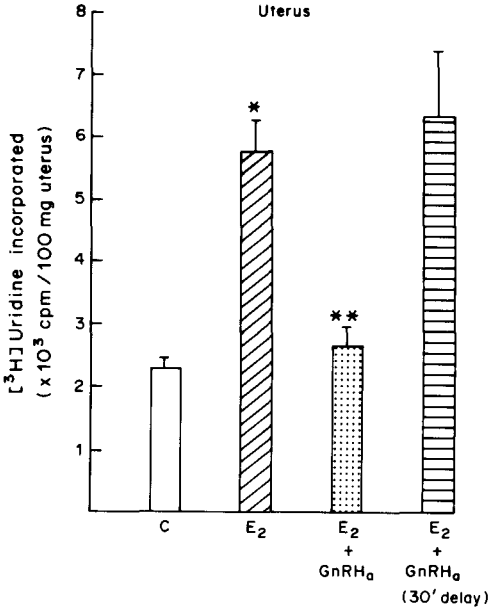

Fig. 3. [^{3}H]Uridine incorporation into TCA precipitable material in uterus following GnRHa treatment. C-Control and E_2-estradiol treated. *$P < 0.001$ and **$P < 0.01$.

Simultaneously 200 μg of GnRH or 25 μg of GnRHa was administered followed by a second injection of GnRH or GnRHa 12 h later. The animals were sacrificed at 24 h after the injection of testosterone and ODC from the ventral prostate was measured [8]. The results show that testosterone induced ODC activity was inhibited by GnRH and also by its analogue. The effect of GnRHa was more profound when compared to GnRH (Fig. 5). The effect of GnRHa on estradiol induced ODC activity was also measured [5]. It was observed in this study that estradiol induced ODC is inhibited by GnRHa in a dose dependent manner in the uterus.

EFFECT ON GLUCOSAMINE 6-PHOSPHATE SYNTHASE

Glucosamine 6-phosphate synthase (EC 5.3.1.19) is a rate limiting enzyme, which is involved in

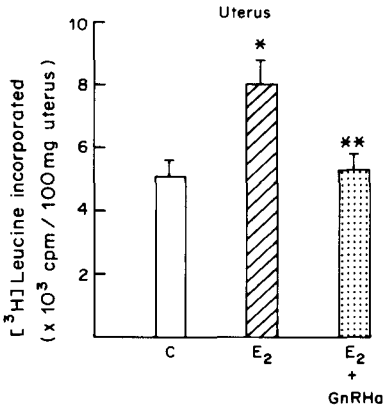

Fig. 4. [^{3}H]Leucine incorporation into uterus following GnRHa treatment. *$P < 0.01$ compared to control (C) and **$P < 0.05$ compared to estradiol (E_2) treated group.

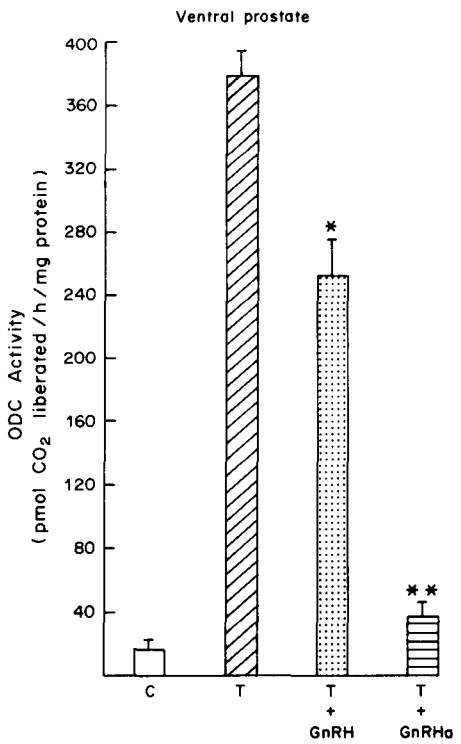

Fig. 5. Effect of testosterone (T) and GnRHa on ODC activity in ventral prostate. $*P < 0.01$ and $P < 0.001$ compared to testosterone treated group.

the biosynthesis of UDP-*N*-acetyl glucosamine and plays an important role in the biosynthesis of glycoproteins, glycolipids and mucopolysaccharides [9]. We have observed that this enzyme is under the regulation of estradiol in the uterus [10]. Figure 6 shows that injection of 0.1 μg of estradiol per rat to 3 day ovariectomised rats causes significant increase in the levels of glucosamine 6-phosphate synthase in the uterus at 24 h. However, injection of 200 μg of GnRHa along with estradiol inhibited estradiol induced enzyme activity.

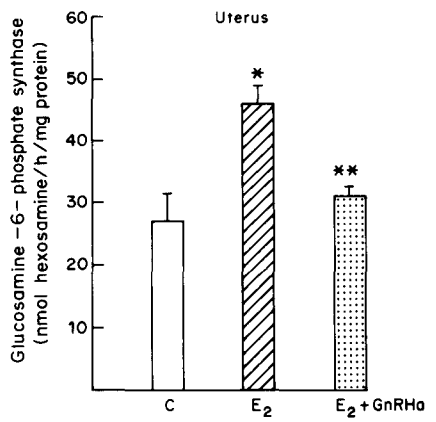

Fig. 6. Effect of estradiol (E_2) and GnRHa treatment on glucosamine 6-phosphate synthase in uterus. $*P < 0.01$ as compared to control (C) and $**P < 0.01$ as compared to E_2 treated group.

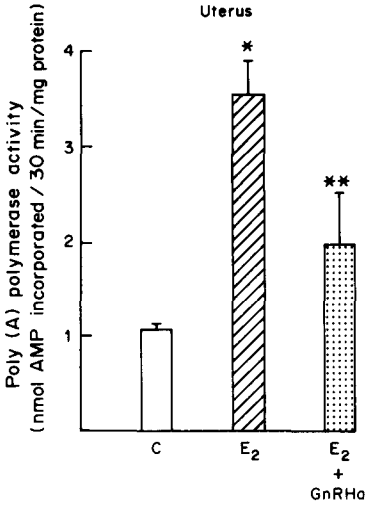

Fig. 7. Levels of Poly(A) polymerase following treatment with estradiol (E_2) and GnRHa. $*P < 0.01$ compared to control (C) and $**P < 0.05$ compared to E_2 treated group.

EFFECT ON POLY(A) POLYMERASE ACTIVITY

In a recent study we have reported that dihydrotestosterone induced poly(A) polymerase (EC 2.7.7.19) is inhibited by treatment with GnRH in the ventral prostate [4]. It was further observed that [^{3}H]uridine incorporation into poly(A)$^+$ mRNA was inhibited by GnRH [4]. This study was extended in females. We have observed that injection of 1 μg of estradiol for 3 days in the ovariectomised animals increased the levels of poly(A) polymerase in the uterus of rat when compared to controls. Two doses of 100 μg of GnRHa inhibited this increase in the enzyme activity of the uterus (Fig. 7).

DISCUSSION

Our studies show that GnRH and its analog causes direct inhibitory effects on the ventral prostate and uterus of rat. However, the mechanism of this inhibitory effect is not clear. Our studies show that general protein synthesis and RNA synthesis in addition to poly(A) mRNA synthesis is inhibited by GnRH. This may indicate that the inhibitory effect of GnRH involves transcription and translational phenomena.

The results also show that the enzymes ODC and glucosamine 6-phosphate synthase are inhibited by GnRH. ODC which is the rate limiting enzyme in the biosynthesis of polyamines is associated with cell proliferation [11]. Similarly glucosamine 6-phosphate synthase is also associated with multiplying and secretory cells [9]. Since GnRHa inhibited these enzymes, the inhibitory action of GnRH may be at the growth promoting mechanisms. The effect of GnRHa on estradiol induced responses appear to be immediate since a delay of even 30 min of treatment with GnRHa following estradiol injection did not inhibit estradiol induced ODC activity [5] or [^{3}H]uridine incorporation into uterus.

Steroid hormones are known to act in the respective accessory reproductive organs after binding to the specific intracellular receptors [12]. While GnRH was shown to exert its effects on gonadal tissue by increasing phosphodiesterase activity, thus decreasing the levels of cAMP [13]. Since steroid hormones may not exert their effect through cAMP levels in the accessory reproductive organs, the inhibitory effects of GnRH still remain enigmatic. It was observed that GnRH does not cause any changes in the binding properties of androgen to cytosol receptor in the mouse kidney [14] and that of estradiol in the uterus [15]. This indicates that the inhibitory action of GnRH is not similar to that of an anti-androgen or an anti-estrogen. Poly(A) segment at the 3'-end of mRNA increases its half life and helps in its stability [16, 17]. Since GnRH inhibited poly(A) polymerase and polyadenylation of RNA, one of the inhibitory effects of GnRH on hormone induced changes may also involve this mechanism. The physiological significance of GnRH action on accessory reproductive organs is not clear. Since GnRH has been localised in several tissues [2], it is possible that GnRH like peptide might be present in the accessory reproductive organs, exerting a local modulatory effect.

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