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INHIBITION OF SEX-STEROID HORMONE INDUCED ORNITHINE DECARBOXYLASE AND POLY(A)-POLYMERASE ACTIVITIES BY A GnRH AGONIST IN THE RAT KIDNEY

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Summary—Recently GnRH and its synthetic agonists were shown to exert extrapituitary actions and inhibit the growth-promoting effects of testosterone and estradiol. In the present study, the long-term effect of GnRH agonist [des-Gly^{10}, D-Ala^{6}-GnRH ethylamide GnRHa] on the steroid-induced renotropic effect was investigated. Gonadectomy of 30-day old male and female rats resulted in the drastic reduction of kidney ornithine decarboxylase (ODC) activity. Injection of testosterone (75 μg/rat) or estradiol (1 μg/rat) to castrated rats significantly increased the enzyme activity at 24 h. Treatment with the GnRH agonist, two doses of 100 μg each, caused significant inhibition of the hormone induced ODC activity. Similarly injection of dihydrotestosterone to castrated adult male rats significantly increased poly(A)-polymerase activity at 24 h. Two doses of 100 μg of GnRHa caused significant inhibition of DHT induced poly(A)-polymerase activity. These results show that chronic exposure to potent GnRH analogs may have anti-steroidal activity in the kidney.

INTRODUCTION

Gonadotropin releasing hormone (GnRH) and its synthetic analogs have been shown to exert multiple extrapituitary actions at various target tissues [1]. Long-term exposure of GnRH analogs cause inhibition of androgen induced growth of male accessory sex organs and the uterotrophic activity of estradiol [2]. In addition it was shown that androgen responsive mouse kidney β-glucuronidase induction could be inhibited by GnRH analogs [3]. Recent studies in our laboratory demonstrated that GnRH analogs inhibit estrogen induced ornithine decarboxylase (ODC, EC 4.1.1.17) activity in uterus [4] as well as androgen induced poly(A) polymerase (EC 2.7.7.19) and ODC activities in ventral prostate [5, 6]. In the present study, we have examined the direct long-term effect of a GnRH analog on renal ODC and poly(A) polymerase activities induced by testosterone (T), dihydrotestosterone (DHT) or estradiol (E_2).

EXPERIMENTAL

GnRH analog (D-Ala^{6}, des-Gly^{10}-ethylamide (GnRHa) was purchased from Sigma Chemical Co. U.S.A. DL [1-^{14}C]Ornithine monochloride (58 mCi/mmol) was purchased from Radiochemical Centre, Amersham, England. For experiments involving ODC as a parameter Wistar strain rats of 30–35 days age and weighing around 60 g were gonadectomized and seven days later testosterone (75 μg/rat) or 17β-oestradiol (1 μg/rat) was given subcutaneously in 0.2 ml saline and a second injection of GnRH at the same dose was given 12 h later. Animals were sacrificed 24 h after steroid hormone administration. Renal ODC activity was assayed according to the procedure followed for uterine ODC assay as described [4]. Adult male rats were used for the experiments involving poly(A) polymerase. In one group of animals, 72 h after castration, DHT at a dose of 500 μg was injected in 0.2 ml of sesame oil subcutaneously. A second group of animals received same dose of DHT followed by simultaneous injection of 100 μg of GnRHa. At 12 h after the first injection a second injection of 100 μg of GnRH was given. All animals were sacrificed at 24 h after the injection of DHT. Control animals received 0.2 ml of saline and were killed along with experimental animals. The assay of the enzyme poly(A) polymerase from the kidney was done as described [5]. Protein content was estimated by Folin phenol method [7] Student's t-test was used for statistical analysis.

RESULTS AND DISCUSSION

Gonadectomy of male rats caused a drastic reduction in renal ODC activity. Administration of testosterone to these rats resulted in several fold increase in ODC activity. Treatment with GnRHa inhibited testosterone induced increase in renal ODC activity (Table 1). Similarly, injection of 1 μg of estradiol 17-β to ovariectomized rats resulted in significant increase in the enzyme activity at 24 h. Administration of two doses of GnRHa drastically reduced estrogen induced ODC activity (Table 1).

Table 2 shows that injection of DHT to orchidectomised rats caused significant stimulation of

Table 1. Effect of GnRHa on steroid hormone induced renal ODC activity

Group	Treatment	ODC Activity (pmol CO_2 liberated/h/mg protein)
I.	Orchidectomized	700 ± 132 (3)
	Orchidectomized + T	6339 ± 1681 (3)
	Orchidectomized + T + GnRHa	907 ± 328* (5)
II.	Ovariectomized	239 ± 12 (3)
	Ovariectomized + E_2	876 ± 65 (6)
	Ovariectomized + E_2 + GnRHa	370 ± 63* (7)

Values are mean ± SE of number of animals in each group as indicated in parentheses. *$P < 0.001$ as compared to hormone treated group.

Table 2. Effect of GnRHa on DHT induced renal poly(A) polymerase activity

Treatment	Poly(A) polymerase activity (nmol AMP incorporated/30 min/mg protein)
Castrated	14.7 ± 0.9 (12)
DHT	21.1 ± 1.8 (5)*
DHT + GnRHa	15.9 ± 1.9 (4)**

Values are mean ± SEM of number of animals in each group in parentheses. *$P < 0.01$ compared to castrated control and **$P < 0.05$ compared to DHT treated group.

poly(A) polymerase over that of castrated controls. However, simultaneous injection of GnRHa to DHT injected animals resulted in drastic inhibition ($P < 0.05$) of the enzyme activity. The values of the enzyme in this group were comparable to the untreated castrated controls. The results presented in this study establish the direct inhibitory effect of GnRHa on steroid hormone induced ODC and poly(A) polymerase activity in gonadectomized rat kidney. Ornithine decarboxylase is the rate limiting enzyme in the biosynthesis of polyamines, to which a variety of functions have been attributed and ODC activity correlates well with cell growth [8]. Hence inhibition of ODC activity by GnRHa may affect the growth promoting action of the steroid hormones. Polyadenylation of RNA is a post transcriptional phenomenon [9] and is catalysed by the enzyme poly(A) polymerase. Poly(A) segment at the 3′ end is known to increase the stability of mRNA [10, 11]. GnRHa caused inhibition of DHT induced poly(A) polymerase confirming our earlier report for rat ventral prostate [5].

This study thus lends credence to the earlier demonstrations that chronic administration of GnRHa results in inhibition of hormone induced growth of various reproductive tissues [2] as well as the regression of hormone dependent carcinomas [12]. The exact mode of anti-steroidal action of GnRH is unclear. A possible mode of action could be the post-transcriptional restriction of polyadenylation of messenger RNA [5]. The presence of GnRH like substances in different tissues suggests some physiological function for these peptides, probably protecting the cells from over stimulation [1]. Even though GnRH like peptides have not yet been demonstrated in renal tissue, the present and earlier studies by Lecomte *et al.*[3] support the view that these peptides may exert a local modulatory effect in tissue growth and function.

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