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INHIBITION OF POLYADENYLATION OF mRNA BY GONADOTROPIN RELEASING HORMONE

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The mechanism of extra pituitary inhibitory action of gonadotropin releasing hormone (GnRH) was investigated. Simultaneous injection of GnRH caused dose dependent inhibition of dihydrotestosterone (DHT) induced poly(A) polymerase in the ventral prostate of rat. In addition injection of GnRH to DHT treated animals caused reduced incorporation of ^{3}H-uridine into poly(A)$^{+}$ mRNA. Since poly(A) segment is known to help in translation of mRNA, it is possible that the inhibitory effect of GnRH is due to the inhibition of polyadenylation of mRNA.

GnRH causes secretion and release of luteinizing hormone and follicle stimulating hormone from the pituitary (1,2). Recently it was also shown to act directly on extra pituitary organs (3). Long term exposure of GnRH causes inhibition of steroidogenisis in the granulosa cells and Leydig cells(4,5), while during short term treatment it causes stimulation of ornithine decarboxylase in the Leydig cells (6) and steroidogenesis in rat follicular cells (7). In addition the agonists of GnRH cause inhibition of testosterone induced growth of ventral prostate and seminal vesicles (8). However, the mechanism of this inhibitory action of GnRH on extra pituitary organs is not clear. Most of the mRNA molecules in eukaryots are polyadenylated at the 3' end (9) and this polyadenylation increases its half life and efficiency of translation (10,11). Polyadenylation of RNA is a post-

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transcriptional phenomenon (12) and is catalysed by the enzyme poly(A) polymerase (E.C. 2.7.7.19). In this study we show that DHT induced poly(A) polymerase and poly(A)$^+$ mRNA are inhibited by a potent analogue of GnRH in the ventral prostate of rat.

MATERIALS AND METHODS

Materials: GnRH(D-Ala^6 des Gly^{10}-N-ethylamide), DHT, tris, dithiothreitol, EDTA, creatine phosphate, creatine phosphokinase, oligo (dT)-cellulose and poly(A) were obtained from Sigma Chemical Co., U.S.A. ^{3}H-Adenosine triphosphate (1.5Ci/mmole) and ^{3}H-uridine (3.5 Ci/mmole) were purchased from Bhabha Atomic Research Centre, Trombay, India. All other chemicals were of analytical grade and were procured locally.

Animals and treatment: Adult male rats weighing about 250g, derived from Wistar strain were used in this study. Rats were castrated through scrotal route under mild ether anesthesia. In the first experiment to monitor the levels of poly(A) polymerase DHT and GnRH treatment was initiated 24h after castration. DHT at a dose of 100µg per animal was given in 0.2ml of sesame oil subcutaneously. GnRH at a dose of 100µg or 200µg per animal was similarly injected simultaneously in 0.1 ml of saline. All animals were treated for 4 days and killed at 24h after the last injection.

Animals castrated 72h previously were used for the second experiment to study the incorporation of ^{3}H-uridine into poly(A)$^+$mRNA. DHT at a dose of 200µg per animal was injected subcutaneously in 0.2ml of sesame oil. Simultaneously 20µg of GnRH and 10µCi of uridine in 10µl of saline was injected into each of the two lobes of the ventral prostate under ether anesthesia. In situ injection of ^{3}H-uridine into ventral prostate was found to increase its incorporation into RNA (13) and we have also observed that in situ treatment of GnRH caused more profound effect on the ventral prostate. Hence this method of injection was used in this study. All animals were killed at 4h after the injection of ^{3}H-uridine and GnRH.

Partial purification and assay of poly(A) polymerase: The animals were killed by stunning and the ventral prostates were removed immediately and homogenised in 4 vol of cold buffer containing 50mM tris, 250mM sucrose, 5mM Mg So_4, 25mM KCl and 5mM EDTA, pH 7.6. The homogenate was centrifuged at 30,000 g for 30 min in a MSE refrigerated centrifuge. The supernatant was precipitated with ammonium sulphate and the precipitate obtained between 30-45 % saturation was suspended in 2ml of 50mM tris-HCl buffer (pH 8.5) containing 0.4mM EDTA, 5mM mercaptoethanol and 20 % glycerol. After dialysis against the same buffer the 30-45 % $(NH_4)_2$ So_4 fraction was used for the assay of poly(A) polymerase as described (14). The reaction mixture

consisted 50mM tris-HCl(pH 7.8), 0.5mM $MnCl_2$, 40mM KCl, 2mM DTT, 10mM creatine phosphate, 5µg creatine phosphokinase, 8mM ATP, 0.5 µCi ^{3}H-ATP (200,000 cpm), 50µg poly(A) as primer and 20µl of enzyme extract (25-50µg protein) in a final volume of 100µl. All incubations were carried out in triplicate for 30 min at 37°C. After incubation the tubes were chilled in ice water and 0.5ml of 5 % TCA containing 1 % $Na_4P_2O_7$ was added to each of the tube. The radioactivity was trapped and washed twice with 2ml of 5 %. TCA on Whatman GF/A filter discs using a Millipore filtration apparatus. After an additional wash with 2ml of ethanol the filters were dried and counted in a Beckman liquid scintillation spectrometer (Model LS 3133p) in Bray's mixture. Protein content of the enzyme extract was estimated according to the procedure of Lowry et al (15).

Isolation of poly(A)$^+$mRNA: Total RNA from ventral prostates was extracted as described by Cox (16) using 8M guanidine-HCl. Poly(A)$^+$mRNA was isolated by passing the total RNA on an oligo (dT) cellulose column (0.9cmx20cm) according to the procedure of Aviv and Leder (17). Poly(A)$^+$ mRNA was monitered at 260nm using a Gilford spectrophotometer. The amount of incorporation of ^{3}H-uridine into total RNA and poly(A)$^+$ mRNA was measured in Brays' mixture in the scintillation counter.

RESULTS AND DISCUSSION

Table 1 gives the data on the effect of DHT and GnRH on poly(A) polymerase activity. The results show that the activity of the enzyme increased significantly in the animals treated with 100µg of DHT per day when compared with the oil treated controls. Simultaneous treatment with 100µg of GnRH caused about 50 % inhibition of enzyme activity and increasing the dose of GnRH to 200µg

Table I. DHT induced poly(A) polymerase inhibition by GnRH

Treatment	Poly(A) polymerase activity (nmol AMP incorporated/mg protein/3o min)
Control	4.5 ± 0.8 (3)$^{++}$
DHT	23.8 ± 6.8 (8)
DHT+GnRH 100 µg	12.3 ± 8.8 (6)$^{+}$
DHT+GnRH 200 µg	4.8 ± 1.6 (7)$^{++}$

Mean + S.D. of the number of observations given in parentheses
+ $P < 0.02$ and ++ $P < 0.001$ compared to control.

TABLE II. Inhibition of ^{3}H-uridine incorporation by GnRH

Treatment	Input of ^{3}H labelled RNA on oligo(dT) column		Retained poly(A)$^{+}$ mRNA by column	
	A_{260} units	CPM	A_{260} units	CPM
Control	4.0	30,888	0.15	8,590
DHT	4.0	20,425	0.17	11,880
DHT+GnRH	4.0	24,200	0.15	6,630

per animal caused further reduction, comparable to the group treated with vehicle alone. The data in Table 2 shows that treatment with DHT causes significantly more incorporation of ^{3}H-uridine into poly(A)$^{+}$ mRNA compared to oil alone treated controls. Simultaneous treatment with GnRH to DHT treated animals resulted in inhibition of incorporation of ^{3}H-uridine into poly(A)$^{+}$ mRNA.

GnRH was shown to cause inhibition of follicle stimulating hormone induced adenylate cyclase and also to stimulate phosphodiesterase activity, thus decreasing the levels of cAMP in the granulosa cells (18). However, since the mechanism of action of peptide hormones and steroid hormones on their respective target organs is different the mechanism of GnRH inhibition in the androgen dependent tissues may be different. Our results show that GnRH causes inhibition of DHT induced poly(A) polymerase activity and inhibits polyadenylation of mRNA in the ventral prostate. Poly(A) segment at the 3' end increases the stability of mRNA(10,11) and the deadenylated globin mRNA failed to induce globin synthesis in vitro (19). Though the stability of all mRNA molecules may not be dependent on polyadenylation (20), it is possible that several molecules of mRNA are efficiently

translated when they are polyadenylated. Inhibition of poly(A) polymerase and polyadenylation of mRNA by GnRH appears to be one of the mechanisms through which it exerts its inhibitory function in the androgen dependent tissues.

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