

## Effect of luteinizing hormone releasing hormone analogues on testosterone metabolism *in vitro*—A study with mature rat ventral prostates

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MS received 14 August 1987; revised 15 February 1988

**Abstract.** The effects of two luteinizing hormone releasing hormone analogues (a superagonist and an antagonist) on the conversion of testosterone to dihydrotestosterone in homogenates prepared from adult rat ventral prostates were studied. At higher doses, the superagonist showed a significant dose-dependent inhibition of the conversion of testosterone to dihydrotestosterone. In comparison, the antagonist showed only a marginally inhibitory trend. The implications of these observed effects *vis-a-vis* the use of the analogues in the endocrine management of prostatic cancer have been discussed.

**Keywords.** Prostate; LHRH analogues; testosterone metabolism; 5  $\alpha$ -reductase activity.

### Introduction

Since 1971, when Schally and associates elucidated the structure of naturally occurring luteinizing hormone releasing hormone (LHRH), numerous synthetic analogues have been developed, many of which have been found to have greatly increased potency compared to naturally occurring LHRH (Joseph and Smith, 1987). Chronic administration of pharmacologic doses of LHRH and its analogues has been demonstrated to inhibit steroidogenesis in a variety of species (Trachtenberg, 1982). LHRH compounds, in combination with pure antiandrogen flutamide have been used in bringing about a hypoandrogenic state in patients with advanced stages of prostate cancer. However, a clear understanding of the effect of these compounds on the prostate still needs to be determined in order to confirm its use as a pharmacologic agent of castration. In this context steroidogenic conversions in the prostate, mainly conversion of testosterone to dihydrotestosterone, would be of importance since DHT has been found to be a useful marker for antiandrogen therapy in prostate cancer (Geller *et al.*, 1984). No studies have so far been available on this aspect. A preliminary study carried out by us using a synthetic LHRH demonstrated some increase in the *in vitro* conversion of testosterone to DHT in immature rat ventral prostates. This change was however not significant (Sheth *et al.*, 1987b). It was interesting therefore to extend the study to LHRH analogues in order to gain insight into the interaction of these compounds with steroidogenic

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Abbreviations used: LHRH, Luteinizing hormone releasing hormone; DHT, dihydrotestosterone.

conversions in the prostate. This paper describes studies with two such LHRH analogues.

### Materials and methods

Seventy five day old male rats of Holtzman strain were used. The ventral prostates of these rats were excised under ether anaesthesia and processed as described by Sheth *et al.* (1987a). However, instead of using minced prostate tissue, the prostates were pooled and homogenized such that 1 ml of the homogenate contained 40 mg of the prostate tissue. Conversion of testosterone *in vitro* in the presence of NADPH was carried out using 1 ml of homogenate in each tube. The procedures involving *in vitro* conversion, extraction, separation and quantitation of 5  $\alpha$ -reductase activity have been described in detail earlier (Joseph *et al.*, 1987; Sheth *et al.*, 1987a).

Using this method we determined the effect of Ovurelin<sup>R</sup> a gonadotropin releasing hormone superagonist, and HB235, an LHRH inhibitor, on the 5  $\alpha$ -reductase activity from mature rat prostates. As before, the results are described in terms of the amount of testosterone reduced to its major metabolite, DHT.

The validity criteria employed to validate the method have been fully described earlier (Sheth *et al.*, 1987a).

### Results

Table 1 presents data indicating 5  $\alpha$ -reductase activity obtained in the presence of 4 different doses of the superagonist Ovurelin and in the absence of this compound (control). At the lower doses used (25 and 50 ng) no significant changes were observed compared to the control either in the amount of testosterone reduced or in percentage conversion. However, at higher doses (75 and 250 ng), both parameters showed a clear declining trend. At 250 ng, the decrease was significant with respect to the control as well as the 25 and 50 ng doses. At 75 ng the amount of testosterone

**Table 1.** Effect of LHRH superagonist (Ovurelin) on testosterone metabolism in mature rat ventral prostate *in vitro*.

Ovurelin concentration (ng/10 mg tissue)	Testosterone reduced (p mol/10 mg tissue)	Percentage of testosterone reduced/10 mg tissue	DHT cpm/T cpm
0 (4)	286 ± 19.84	39.5 ± 1.32	0.14 ± 0.012
25 ng (3)	290 ± 19.51	42.67 ± 2.85	0.115 ± 0.02
50 ng (4)	272 ± 14.47	40.25 ± 2.01	0.094 ± 0.009 <sup>a</sup>
75 ng (4)	232 ± 12.46 <sup>b</sup>	34.0 ± 1.78 <sup>c</sup>	0.110 ± 0.008
250 ng (3)	219 ± 7.75 <sup>d</sup>	28.67 ± 1.20 <sup>e</sup>	0.073 ± 0.004 <sup>f</sup>

All values are mean ± S E of mean.

Figures in parentheses indicate the number of determinations.

<sup>a</sup> Significantly lower than control ( $P < 0.05$ ).

<sup>b</sup> Significantly lower than 25 ng ( $P < 0.05$ ).

<sup>c</sup> Significantly lower than control ( $P < 0.05$ ); 25 ng ( $P < 0.05$ ).

<sup>d</sup> Significantly lower than control ( $P < 0.05$ ); 25 ng ( $P < 0.05$ ); 50 ng ( $P < 0.05$ ).

<sup>e</sup> Significantly lower than control ( $P < 0.01$ ); 25 ng ( $P < 0.05$ ); 50 ng ( $P < 0.001$ ).

<sup>f</sup> Significantly lower than, control ( $P < 0.01$ ); 75 ng ( $P < 0.005$ ).

reduced was significantly lower compared to that at 25 ng whereas the percentage reduction was significantly lower with respect to the control as well as the 25 ng dose. Thus the results clearly establish a dose-dependent inhibitory effect of Ovurelin on reduction of testosterone. The DHT/testosterone ratios also confirm this inhibitory effect. The maximum decrease in this ratio was observed at the highest dose (250 ng). At 50 ng also the decrease was significant with respect to the control; at other doses it was less significant.

The LHRH antagonist HB235 did not cause any significant change with respect to the control either in the amount of testosterone reduced or in the percentage conversion (table 2). However, the DHT/testosterone ratios do indicate an inhibition of the conversion of testosterone to DHT at higher doses (75 and 250 ng).

**Table 2.** Effect of LHRH antagonist (HB235) on testosterone metabolism in mature rat ventral prostate *in vitro*.

HB235 concentration (ng/10 mg tissue)	Testosterone reduced (p mol/10 mg tissue)	Percentage of testosterone reduced/10 mg tissue	DHT cpm/T cpm
0 (7)	141 ± 12.56	38.71 ± 3.99	0.147 ± 0.026
25 (3)	139 ± 10.40	39.0 ± 3.51	0.137 ± 0.008
50 (7)	158 ± 5.06	41.57 ± 2.03	0.179 ± 0.027
75 (4)	151 ± 13.66	40.25 ± 4.13	0.086 ± 0.017 <sup>a</sup>
250	119 ± 12.75 <sup>b</sup>	31.67 ± 4.37 <sup>c</sup>	0.056 ± 0.001 <sup>d</sup>

All values are mean ± S E of mean.

Figures in parentheses indicate the number of determinations.

<sup>a</sup> Significantly lower than 25 ng ( $P < 0.05$ ); 50 ng ( $P < 0.05$ ).

<sup>b,c</sup> Significantly lower than 50 ng ( $P < 0.05$ ).

<sup>d</sup> Significantly lower than control ( $P < 0.05$ ); 25 ng ( $P < 0.001$ ); 50 ng ( $P < 0.001$ ).

Thus a comparison of the two analogues tested shows that the superagonist has a significant dose-dependent inhibitory effect on the 5  $\alpha$ -reductase activity at higher doses; the effect of the antagonist is relatively marginal.

## Discussion

The methodology adopted in the present study is based on the major assumption that in rat prostate tissue more than 90% of testosterone is converted to DHT. This has been previously demonstrated under different experimental conditions by Massa and Martini (1974) and later confirmed by Purvis *et al.* (1986). The results obtained in our previous study with LHRH using immature rat prostates did not show any significant changes although a trend towards augmentation of the 5  $\alpha$ -reductase activity was perceptible. In this context, it is interesting to observe that both the LHRH analogues tested in the present study showed an inhibitory effect on the *in vitro* conversion of testosterone to DHT in mature rat prostate tissue homogenate, although the extent of inhibition varied greatly. The agonist showed a predominantly greater inhibition of the 5  $\alpha$ -reductase activity than the antagonist under similar conditions.

Levels of DHT, which is the major androgen in prostate tissue, have been used as an indicator of the adequacy of antiandrogen therapy in prostate cancer (Geller *et al.*, 1979). As stated by these authors, the potential usefulness of any antiandrogenic agent would be determined by the DHT levels in prostate tissue. The level of DHT would depend upon the following factors—plasma testosterone substrate, 5  $\alpha$ -reductase, 3-oxidoreductase and receptor binding, all essential biochemical steps for the mediation of androgen action. Therefore inhibition of the 5  $\alpha$ -reductase activity observed in our study is interesting, since it would lead to a decline in the tissue DHT store. However, to obtain any conclusive evidence, the other factors mentioned above also need to be investigated. Studies on the effect of LHRH analogues on steroidogenic enzymes in the testis have been extensively carried out but such studies (for the prostate) are few. Using the agonistic analogue Buserelin, Trachtenberg (1982) demonstrated that in spite of 90% reduction in serum androgen concentration, prostatic weight and prostatic androgen receptor content remained largely unchanged. Detailed studies on similar lines need to be carried out using Ovurelin and HB 235 in order to determine their potential in prostate cancer therapy.

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