Vol. 109, No. 1, 1982 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
November 16, 1982 Pages 269-274

STIMULATION OF ORNITHINE DECARBOXYLASE ACTIVITY BY LUTEINIZING HORMONE RELEASING HORMONE IN THE TESTIS OF IMMATURE RAT

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Received October 4, 1982

The activity of ornithine decarboxylase (ODC) was found to increase in the testis of immature rats following intratesticular injection with luteinizing hormone releasing hormone (LHRH). Maximal stimulation of ODC activity occurred with 1 μg of the hormone at 2 h. The enzyme activity returned to control levels at 4 h. The minimal effective dose was found to be 0.1 μg per testis. The stimulating effect of LHRH was confined to Leydig cells alone. The seminiferous tubules did not show any change in ODC activity following LHRH treatment. These results show that LHRH acts directly on the testis and influences the levels of ODC in the Leydig cells of rat.

LHRH and its analogues stimulate gonadotropic hormone production in the pituitary (1,2). However, it was shown that these agents also act directly on extra pituitary organs (3). The analogues of LHRH were shown to cause inhibition of steroidogenesis and decrease the binding of hCG in Leydig cells *in vitro* (4). More recent work has shown that exposure of Leydig cells to LHRH for short periods of 4-6 h caused stimulation of testosterone production while inhibition occurred following exposure to a period of 2-3 days *in vitro* (5,6). Ornithine decarboxylase (E.C.4.1.1.17) is a rate limiting enzyme in the biosynthesis of polyamines (7). The levels of ODC were shown to increase following treatment with gonadotropic hormones, prostaglandins and catecholamines in the testis of rat (8-11). The purpose of this study was to see if LHRH causes any changes in ODC activity of testis.

0006-291X/82/210269-06$1.00/0

MATERIALS AND METHODS

Wistar strain immature male rats, aged 21-22 days, were used in this study. LHRH was generously provided by the National Pituitary Agency, NIAMDD, U.S.A. Ornithine, pyridoxal phosphate, dithiothreitol, glutathione and Tris were purchased from Sigma Chemical Company. D,L-(1-^{14}C) Ornithine monochloride (58mCi/mmol) was obtained from Radiochemical Centre, Amersham, England. All other chemicals were of analytical grade and were procured locally.

LHRH was injected intratesticularly in 0.15 M sodium chloride under mild ether anesthesia as described previously (8). The animals were killed by cervical dislocation and the testes were homogenized in 4 vol of TED buffer (Tris 25 mM, EDTA 0.1 mM, DTT 1.0 mM, pH 7.4). The homogenate was centrifuged in a MSE refrigerated centrifuge for 30 min at 25,000 x g. The supernatant was used for the assay of ODC activity (12) with some modifications (9). ODC activity of Leydig cell and seminiferous tubule fractions was estimated as described by us (10). Protein content was measured by the method of Lowry *et al* (13) and statistical analysis was done using Student's 't' test.

RESULTS

Fig. 1 shows the effect of injection with 1 µg of LHRH per testis on ODC activity at various time intervals. The

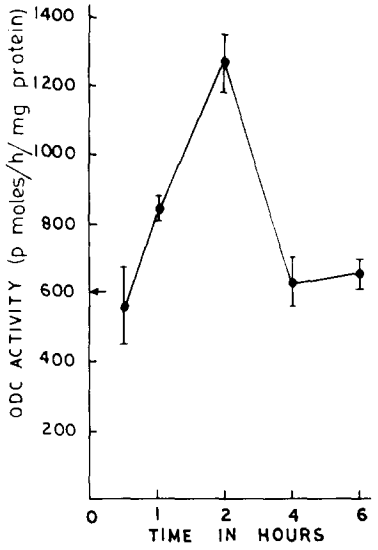

Fig. 1 Effect of LHRH on ODC activity at various time intervals. LHRH at a dose of 1 µg/testis was injected intratesticularly and the animals were killed at various time intervals. The arrow indicates saline treated control value at 2 h. Each point represents Mean ± S.E.M. of 3-5 determinations from 6-10 animals.

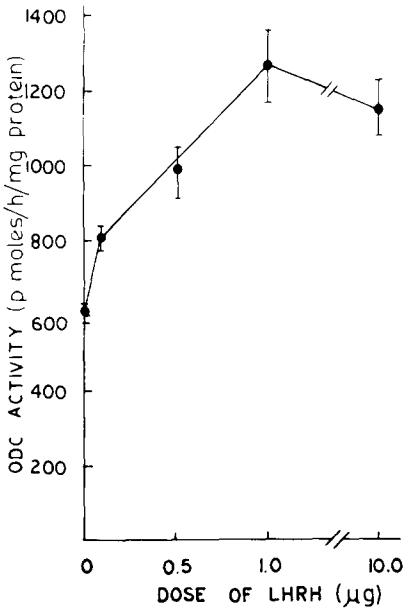

Fig. 2 Effect of various doses of LHRH on ODC activity of testis. LHRH was injected intratesticularly and the enzyme activity estimated at 2 h. Mean ± S.E.M. of 3-5 determinations from 6-10 animals.

results show that ODC activity was significantly stimulated ($P<0.05$) at 1 h after the injection, reaching to maximal levels at 2 h ($P<0.001$). The levels of ODC returned to saline treated control levels at 4 h. The effect of various doses of LHRH on ODC activity at 2 h after the injection are shown in Fig. 2. A dose as small as 0.1 μg per testis caused significant stimulation ($P<0.05$) of ODC activity and maximum effect was observed with 1 μg of LHRH per testis. Increasing the dose of LHRH to 10 μg per testis did not cause additional stimulation of ODC activity.

Table I shows the effect of 1 μg LHRH on Leydig cell and seminiferous tubule fractions at 2 h. Injection of LHRH caused stimulation of ODC activity in the Leydig cells alone ($P<0.01$) while there was no change in the seminiferous tubules.

TABLE I

EFFECT OF LHRH ON ODC ACTIVITY IN THE LEYDIG CELLS AND SEMINIFEROUS TUBULES

Treatment	ODC activity (p mol/h/mg protein)	
	Leydig cells	Seminiferous tubules
Saline	293 ± 9	559 ± 32
LHRH, 1 µg	435 ± 37*	628 ± 30

The results are Mean ± S.E.M. of 3-4 determinations from 15-20 animals per group. ODC activity was measured at 2 h after the injection of LHRH. *$p<0.01$ as compared to saline treated control group.

DISCUSSION

This study, for the first time, shows that LHRH causes stimulation of ODC activity in the Leydig cells. The *in vivo* effect of LHRH observed in this study by intratesticular injection is direct on the testis and is not mediated through pituitary as we could not observe any stimulation of ODC activity in the testis at 2h following intraperitoneal injection with 1 or 10 µg of LHRH (results not shown). Secondly, optimal stimulation of ODC activity occurs at 4 h in response to gonadotropic hormones (8), while LHRH caused maximal stimulation at 2 h, returning to control levels at 4 h. Since LHRH is rapidly inactivated *in vivo* (14) it is possible that the intraperitoneally injected LHRH was broken down before reaching the testis, while local injection caused stimulation of ODC activity in the testis.

The stimulatory effect of LHRH on testicular ODC activity was confined to Leydig cells alone. This could be due to the presence of LHRH receptors on the Leydig cells only (15-18).

Gonadotropic hormones, prostaglandins and catecholamines appear to stimulate the levels of ODC through cAMP (8-11). However, the involvement of cAMP in the action of LHRH is not clear. While varying levels of cAMP were monitered in Leydig cell cultures in the presence of LHRH (5), it was observed that LHRH caused inhibition of adenyl cyclase and stimulated phosphodiesterase activity (19). Hence it is possible that the action of LHRH may be mediated through some other second messenger like calcium (5).

The physiological function of LHRH in the testis is not clear. It was proposed that a 'LHRH-like' peptide is secreted by the Sertoli cells which acts locally on the Leydig cells (20). It is not known if the exogenous effect of LHRH observed in this study and in the literature are similar to the actions of endogenous testicular 'LHRH-like' peptide. Further work is necessary to elucidate the role of LHRH and its mechanism of action in the Leydig cells of testis.

ACKNOWLEDGEMENT

This work was supported by the research funds of the University of Hyderabad. The authors thank Prof. P.S.Ramamurty for his interest in this study. One of us (RMB) is a receipient of a fellowship from the University Grants Commission. Mr. P. Omprakash typed the manuscript.

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