Inhibition of epinephrine and gonadotropic hormone induced ornithine decarboxylase activity by phenoxybenzamine in the testis of immature rat

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The effect of α and β adrenergic receptor blockers on epinephrine and gonadotropic hormone induced ornithine decarboxylase (ODC) activity in the testis of immature rats was studied. Intratesticular injection with phenoxybenzamine at 15 min before treatment with epinephrine or gonadotropic hormones blocked ODC activity. Similar injection with propranolol or practolol had no effect on ODC activity. These results show that α adrenergic receptors are involved in the action of epinephrine and gonadotropic hormones in the testis.

Epinephrine *Gonadotropic hormone* *Phenoxybenzamine* *Inhibition* *Ornithine decarboxylase* *Testis*

1. INTRODUCTION

In our earlier studies [1–5] we have shown that the gonadotropic hormones, prostaglandins, catecholamines and luteinizing hormone releasing hormone (LHRH) stimulate the activity of ornithine decarboxylase (ODC, EC 4.1.1.17) in the testis of immature rat. This effect of gonadotropic hormones and prostaglandins appears to be mediated through cAMP [1–2]. However, the mechanism of stimulation of ODC activity by catecholamines and LHRH is not clear. β-Adrenergic antagonists were shown to inhibit catecholamine induced cAMP stimulation in the Sertoli cells of rat [6]. In addition, catecholamines were also shown to have a role in the mediation of luteinizing hormone action as α-adrenergic antagonist phenoxybenzamine blocked the LH induced ovulation in hen ovarian follicles in vitro [7]. In view of these observations the effect of α- and β-adrenergic blockers on epinephrine and gonadotropic hormone induced ODC activity in the testis was investigated. The results presented in this study show that the α-adrenergic blocker phenoxybenzamine inhibits epinephrine, LH and FSH induced ODC activity in the testis.

2. MATERIALS AND METHODS

Ornithine, pyridoxal phosphate, DL-propranolol, dithiothreitol and epinephrine bitartrate were purchased from Sigma Chemical Co., USA. Ovine luteinizing hormone (NIH-LH-S-20), ovine follicle stimulating hormone (NIH-FSH-S-12) and LHRH were generously provided by the National Pituitary Agency, NIAMDD, USA. Prostaglandin $F_{2\alpha}$ was a gift from the Upjohn Co., USA. D,L-[1-^{14}C]Ornithine monochloride (58 mCi/mmol) was purchased from the Radiochemical Centre, Amersham, England. Phenoxybenzamine and practolol were obtained from Smith Kline and French (India) Ltd. All other chemicals were obtained locally and were of analytical grade.

Epinephrine, LH, FSH and LHRH were dissolved in saline. $PGF_{2\alpha}$, practolol, propranolol and phenoxybenzamine were made up in 1:9 ethanol–saline mixture.

21–22-day-old immature rats were used in all experiments. Hormones were injected intratesticularly in 5 μl of vehicle [1]. Propranolol, practolol and phenoxybenzamine were similarly injected 15 min before the injection of the hormones. Control ani-

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mals received 10 μl of 1:9 ethanol–saline mixture. At 2 h after the hormone injection the animals were killed by cervical dislocation and decapsulated testes from 2 animals were pooled and homogenized in 4 vol. of 25 mM Tris, 0.1 mM EDTA and 1 mM DTT (pH 7.4). The homogenate was centrifuged at 25000 × g for 30 min in a MSE refrigerated centrifuge and the supernatant was used for the assay of ODC activity as described [8]. Protein content of the supernatant was measured as per the method of Lowry et al. [9]. ODC activity is expressed as pmoles $^{14}CO_2$ liberated.h^{-1}.mg protein^{-1}.

3. RESULTS

The effect of treatment with propranolol, practolol or phenoxybenzamine at 15 min before the treatment with epinephrine on ODC activity is given in table 1. The results show that β-adrenergic blockers propranolol at a dose of 10 or 50 μg and practolol at a dose of 10 μg per testis had no effect on the epinephrine stimulated ODC activity at 2 h. However, phenoxybenzamine at both doses of 10 and 20 μg per testis caused significant inhibition of ODC activity.

Table 1

Effect of α- and β-adrenergic antagonists on epinephrine-stimulated ODC activity

Group No.	Treatment	ODC activity (pmol·h^{-1}·mg protein^{-1})
1.	Control	709 ± 64
2.	Epinephrine	1716 ± 114
3.	Epinephrine + propranolol (10 μg)	1872 ± 60
4.	Epinephrine + propranolol (50 μg)	1651 ± 141
5.	Epinephrine + practolol (10 μg)	1530 ± 54
6.	Epinephrine + phenoxybenzamine (10 μg)	948 ± 53[a]
7.	Epinephrine + phenoxybenzamine (20 μg)	1108 ± 119[a]

[a] $P < 0.01$ as compared to group 2

1 μg of epinephrine per testis was injected in all groups. Data are mean ± SEM of 3–5 determinations from 6–10 animals per group

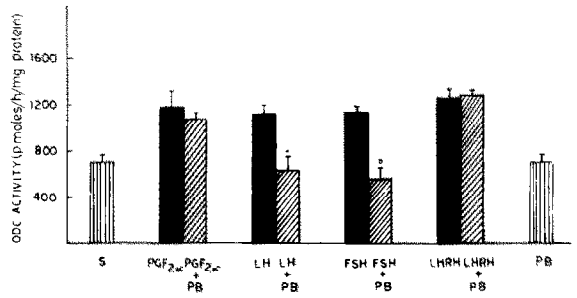

Fig.1. Effect of phenoxybenzamine on LH, FSH, LHRH and $PGF_{2\alpha}$ induced ODC activity. 10 μg of phenoxybenzamine (PB) per testis was injected 15 min before the injection of 40 μg of LH, 40 μg of FSH, 1 μg of LHRH or 10 μg of $PGF_{2\alpha}$. All animals were killed at 2 h after the injection of hormones. Each bar represents mean ± SEM of 3–5 determinations from 6–10 animals. (a,b) $P < 0.001$ when compared to respective controls treated with hormone alone. S represents saline (10 μl) treated group.

Figure 1 shows the effect of phenoxybenzamine on $PGF_{2\alpha}$, LH, FSH and LHRH induced ODC activity. Prior treatment with 10 μg of phenoxybenzamine per testis caused inhibition of ODC activity in animals treated with LH and FSH. However, such inhibition was not observed in $PGF_{2\alpha}$ and LHRH treated groups.

4. DISCUSSION

These results show that the stimulatory activity of ODC by epinephrine is mediated through α-adrenergic receptors since only the α-adrenergic blocker phenoxybenzamine inhibits ODC activity. This finding is in contrast to the results reported earlier [6] in which the β-adrenergic blockers were shown to cause inhibition of catecholamine induced cAMP response. It is possible that catecholamine induction of ODC activity and cAMP are mediated through two independent receptor systems in the testis.

It is interesting to note that LH and FSH induced ODC activity is inhibited by prior treatment with phenoxybenzamine. This implicates α-adrenergic receptors in the mediation of LH and FSH action in the testis. It was suggested that probably LH interacts with epinephrine or norepinephrine at the target site in the ovary and causes ovulation [7]; inhibition of this interaction with

phenoxybenzamine was shown to cause inhibition of ovulation. The inhibitory effect of phenoxybenzamine on LH and FSH induced ODC activity in the testis is probably similar to the inhibition of ovulation. Since $PGF_{2\alpha}$ and LHRH induced ODC activity is not inhibited by phenoxybenzamine, it is possible that these two stimulatory agents act through a different mechanism. This work shows that the α-adrenergic receptors participate in the action of epinephrine and are also involved in the action of gonadotropic hormones in the testis of rat.

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