STIMULATION OF ORNITHINE DECARBOXYLASE ACTIVITY BY PROSTAGLANDINS IN THE ISOLATED CELLS OF IMMATURE RAT TESTIS

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1. Introduction

In [1] evidence was presented showing hyperstimulation of ornithine decarboxylase (ODC) activity following combined treatment with saturating levels of prostaglandin E2 (PGE_2) and follicle-stimulating hormone (FSH) or PGE_2 and luteinizing hormone (LH) in the testis of immature rat [1]. It was presumed that PGE_2, FSH and LH were probably acting on different cell types and were causing a cumulative effect on ODC. Hence experiments were designed to study the effect of prostaglandins, FSH and LH on ODC activity in the isolated Leydig cells and seminiferous tubules of immature rat testis. The evidence presented in this study shows that PGE_2 and $PGF_{2\alpha}$ stimulate ODC levels in both Leydig cells and seminiferous tubules.

2. Materials and methods

Ovine luteinizing hormone (NIH-LH-S-20), ovine follicle stimulating hormone (NIH-FSH-S-12) and prostaglandins were obtained from the National Institutes of Health and the Upjohn Company, respectively. Ornithine, pyridoxal phosphate, dithiothreitol, reduced glutathione, collagenase and bovine serum albumin were purchased from Sigma, St Louis. D,L-[1-^{14}C] ornithine monochloride (58 mCi/mmol) was purchased from the Radiochemical Centre, Amersham. All other chemicals were of analytical grade and were obtained locally.

Stock solutions of prostaglandins were prepared in ethanol at 10 mg/ml. They were diluted with saline to the desired concentrations before injecting the animals. Hormones were made in saline.

Immature male rats (21–22 days, 25–30 g) were used in all experiments. Rats were injected intratesticularly with 10 μg PGE_2 alone or in combination with 40 μg FSH or LH in 10 μl total vol. as in [2]. Control animals received 10 μl saline. At appropriate times rats were killed by spinal dislocation and testes were dissected out. The decapsulated testes from 6–8 animals were pooled and Leydig cells and seminiferous tubules were separated by incubation in Krebs-Ringer bicarbonate buffer (pH 7.4) containing collagenase (1 mg/ml) as in [3]. Addition of 1 mM reduced glutathione increased the stability of ODC; hence it was added to the buffer during incubation.

The Leydig cells and seminiferous tubules, following their separation, were homogenized in 4 vol. 25 mM Tris, 0.1 mM EDTA and 1 mM DTT buffer (pH 7.4) in a glass homogenizer, and centrifuged at 25 000 × g for 30 min. The supernatant was used for the assay of ODC activity as detailed in [4]. The assay mixture contained 0.25 μmol unlabelled ornithine, 2.5 μmol DTT, 0.1 μmol pyridoxal phosphate, 0.2 μCi radioactive ornithine and 200 μl enzyme in 0.5 ml total vol. Following incubation at 37°C for 1 h the reaction was stopped by injecting 0.5 ml 10% trichloroacetic acid and the tubes were re-incubated for an additional 30 min to trap all liberated $^{14}CO_2$ in the centre wells which contained 0.1 ml hyamine hydroxide. The radioactivity was counted in a toluene-based scintillant using a Beckman Liquid Scintillation Spectrometer (Model LS 3133 P). Protein was determined by the Lowry method [5]. ODC activity is expressed as pmol $^{14}CO_2$ liberated . h^{-1} . mg $protein^{-1}$.

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3. Results and discussion

The effects of PGE_2 and $PGF_{2\alpha}$ alone and PGE_2 in combination with FSH or LH are shown in table 1. It was observed that PGE_2 and $PGF_{2\alpha}$ significantly stimulated the activity of ODC in both Leydig cells and seminiferous tubules. LH caused significant stimulation of the enzyme activity in Leydig cells while FSH increased the levels in seminiferous tubules.

The above results show that PGE_2 and $PGF_{2\alpha}$ act on both Leydig cells and seminiferous tubules and stimulate ODC activity. Treatment with FSH and PGE_2 did not increase the levels of ODC in the seminiferous tubules over the levels seen in animals treated with PGE_2 alone. Similarly injection of LH and PGE_2 did not cause hyperstimulation in the Leydig cell fraction. This may be due to a common rate-limiting intermediate in the action of both these compounds. Cyclic AMP stimulated the levels of ODC in [1,2]. Prostaglandins [6] and gonadotropic hormones [7] are known to stimulate cAMP levels in the testis of rat. Stimulation of ODC levels observed here following treatment with prostaglandins and gonadotropic hormones may be through the stimulation of cAMP levels in the Leydig cells and seminiferous tubules of rat. Hyper-stimulation of ODC levels following treatment with PGE_2 and gonadotropic hormones observed earlier in the whole testis [1] appears to be due to the combined effect of these compounds on different cell types in the testis of rat.

Table 1
Effect of PGE_2, $PGF_{2\alpha}$, FSH and LH on ODC activity in the isolated leydig cells and seminiferous tubules of testis

Group no.	Treatment	ODC (pmol . h^{-1} . mg protein^{-1})	
		Leydig cells	Seminiferous tubules
1.	Saline	322 ± 18	549 ± 40
2.	PGE_2	970 ± 133[d]	1852 ± 228[d]
3.	LH	540 ± 75[a]	643 ± 69
4.	LH + PGE_2	859 ± 79[d]	1116 ± 176[b]
5.	FSH	360 ± 46	1006 ± 176[a]
6.	FSH + PGE_2	753 ± 292[a]	1033 ± 144[c]
7.	$PGF_{2\alpha}$	1012 ± 110[d]	1277 ± 338[c]

[a] $P < 0.05$; [b] $P < 0.02$; [c] $P < 0.01$; [d] $P < 0.001$ as compared to group 1

All animals were killed at 2 h after the injection of prostaglandins or at 4 h after the injection of LH or FSH. The animals in groups 4 and 6 were treated with PGE_2 2 h after the administration of LH or FSH. The values are mean ± SEM of 4–5 different observations

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