EFFECT OF LH ON TESTOSTERONE PRODUCTION BY FOETAL RAT TESTES IN VITRO

R. PICON and A. KTORZA

Laboratoire de Physiologie Animale, Université Paris 7, 2 Place Jussieu, 75005 Paris, France

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1. Introduction

Testosterone production from foetal rat testes explanted in a synthetic medium has been demonstrated [1]. It was shown that testosterone secretion by 13½-day-old foetal testes which was not detectable on the first day of explantation appeared on the following days and that the initiation of the secretion of the testes could probably occur in the absence of gonadotrophic hormones; dibutyryl cyclic adenosine-3′-5′-monophosphate (dibutyryl cyclic AMP) was found to stimulate significantly testosterone secretion from 14½ days onward. It is well established that LH can stimulate testosterone biosynthesis in the prepubertal [2] and adult [3] testes and this stimulatory effect seems to be mediated through an increase in cyclic AMP cell content [4]. Such an effect would occur in foetal testes if adequate receptors to LH were already present on the target cell membranes. Testosterone secretion of 18-day-old foetal mice testes cultured in medium supplemented with chick embryo serum has been reported to be stimulated by LH, 24 h following explantation [5]. However the capacity of the foetal testes for responding to LH in the first stages of differentiation was not determined nor was the time course characterized.

The present study was undertaken to examine the effect of gonadotrophins in vitro (a) on the onset of testosterone secretion by the foetal rat testis and (b) on testosterone production during the male differentiation of the urogenital system since it was established that the development of the male phenotype depends upon androgen secretion by the foetal testis [6]. All the experiments were done in a synthetic medium where cholesterol was the only exogenous steroid available as substrate for testosterone biosynthesis. It was found that LH stimulates testosterone production by foetal testes as early as 14½ days and the response increased with age and size of the testes, but LH was unable to initiate testosterone production; FSH had some effects probably due to LH contamination.

2. Materials and methods

Ovine LH (NIH-LH-S19, 1.01 units/mg) and ovine FSH (NIH-FSH-S11, 1.15 units/mg) were a gift from the National Institute of Arthritis, Metabolism and Digestive Diseases, Bethesda, Maryland. ACTH was purchased from Endopancrine (France). Testis tissue was obtained from 14–21½-day-old rat foetuses (Sherman strain). Gestational age was based on the timing of coitus [7]. Organ culture technique and radioimmunoassay of testosterone have been described [1,7]. The culture medium, M. 199, purchased from Institut Pasteur (Paris, France) contained 0.2 mg/l cholesterol. The antiserum (A-4210) obtained from rabbits immunized with testosterone 3-carboxy-methyl-oxime-bovine serum albumin was a gift from INRA (France). At a dilution of 1:27 000 it bound 55% of the [^{3}H]testosterone tracer. Standard curves with the representation based upon Rodbard et al. [8] had a regression coefficient $r = -0.9944 \pm 0.0008$ and a slope $b = -0.94 \pm 0.04$. The antiserum displayed cross reaction with 17β-hydroxy-5α-androstan-3-one (64%) and to a lesser degree with 5α-androstane-3β, 17β-diol (4%); androstenedione, dehydroepiandrosterone, 17β-estradiol and progesterone were found to have no effect on the assay in the concentrations studied (100–800 pg). Results reported were the sum of testosterone and 17β-hydroxy-5α-androstan-3-one

concentrations. At the working dilution the blanks were undectable. The displacement curve obtained with increasing concentrations of testosterone added to the culture medium was similar to that of the testosterone standards (correlation coefficient r = 0.990).

3. Results and discussion

LH was found to stimulate the secretion of testosterone by foetal rat testes from 14½ days onward. Addition of increasing amounts of LH was accompanied by a progressive increase of testosterone secretion; maximal response was elicited with 100 ng/ml LH for all stages of development studied (fig.1). Specificity of the response was tested on 20-day-old foetal testes with other trophic hormones. As seen from the results in table 1, effect of LH was detectable with 0.01 ng/ml and statistically significant from 0.1 ng/ml onward; FSH (1 ng/ml) and ACTH failed to stimulate testosterone secretion; secretion was enhanced with 100 ng/ml FSH, but the FSH preparation was known to contain some LH contamination (<0.01). Similar results were obtained with adult unteased testes [3,9,10] and isolated interstitial cells [10,11].

When the time course relationship for testosterone production was studied for short periods, it was observed that the effect of LH was noticeable 20 min after explantation (table 2) and a peak of secretion could be detected during the 2nd hour of explantation in LH stimulated 16 and 20-day old testes whereas testosterone secretion was decreasing in controls (table 3). The response was not different from that of adult tissue [9,11]. The time course relationship was also studied during 3 days of culture (fig.2a,b). As previously observed [1] during the 2nd and 3rd day of explantation, production increased in 14½-day-old testes and decreased in the older stages; with LH (100 ng/ml), testosterone production was enhanced

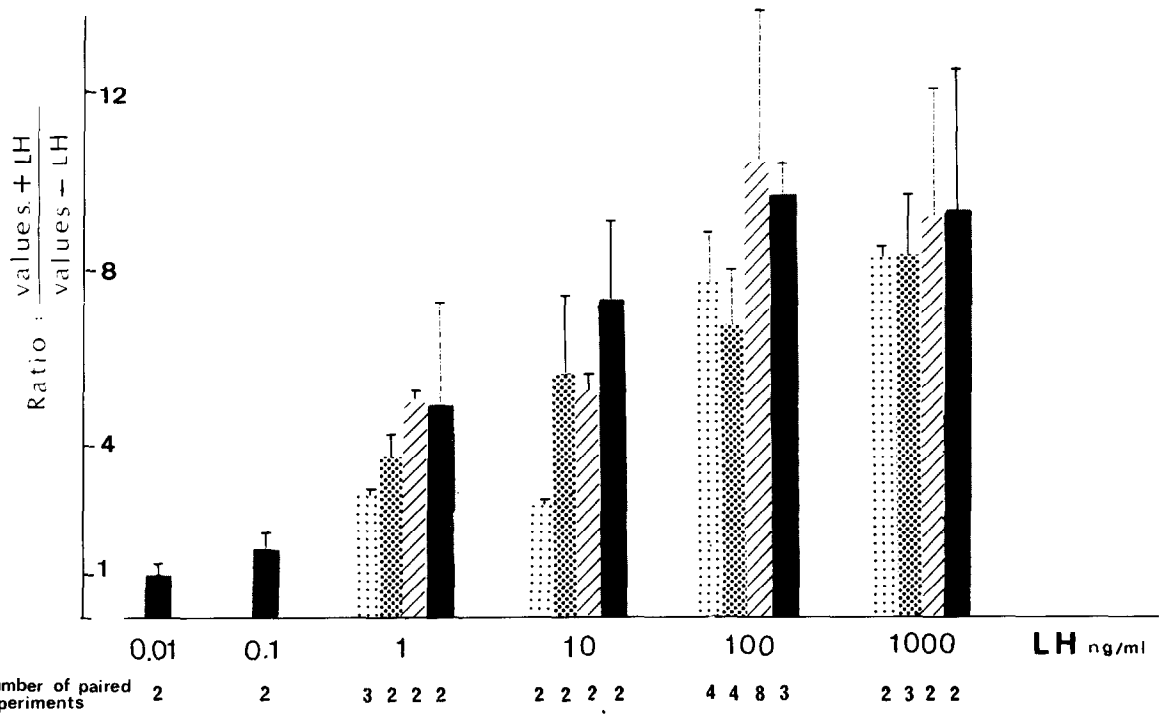

Fig.1. Effect of various amounts of LH on testosterone production from foetal testes aged 21½ days (black), 18½ days (striped), 16½ days (thickly dotted) and 14½ days (lightly dotted). Testes from littermates were cultured with and without LH for 24 h. The ratio of production with LH to production without LH represents means ± S.E.M. (– – – –) for n ⩾ 3 paired experiments or means and ranges (——) for n = 2 paired experiments.

Table 1
Effects of LH, FSH and ACTH on testosterone production from 20-day-old foetal testes

$$\text{Ratio} = \frac{\text{values + hormone}}{\text{values - hormone}}$$

LH (ng/ml)			FSH (ng/ml)			ACTH (ng/ml)
0.01	0.1	1	1	10	100	500
1.33 ± 0.26 n.s.	2.73 ± 0.71 $p < 0.05$	4.90 ± 2.20 $p < 0.05$	< 1 n.s.	1.60 ± 0.17 n.s.	2.08 ± 0.26 n.s.	< 1 n.s.

Each value is a mean from 4 paired experiments. For each foetus, one testis was cultured without hormone and the other one with hormone.

Table 2
Effect of LH on testosterone secretion from 20-day-old foetal testes during the first hour of explantation

	Testosterone pg/testis/min culture		
Culture time	No addition	LH (100 ng/ml)	
0– 5 min	52 ± 4	58 ± 5	n.s.
5–13 min	41 ± 4	39 ± 5	n.s.
13–20 min	33 ± 3	68 ± 9	$p < 0.05$
20–30 min	34 ± 4	116 ± 17	$p < 0.01$
30–60 min	24 ± 3	170 ± 29	$p < 0.01$

The values presented are means ± S.E.M. from 6 different cultures. For each foetus, one testis was cultured without LH and the other one with LH.

and maintained during the 3 days of culture. Testosterone accumulation in the testicular tissue was also stimulated: for example, testosterone content in a 16½-day-old testis = 216 pg; after 1 day of culture, control = 109 pg, + LH = 255 pg; after 2 days, control = 72 pg, + LH = 1017 pg; after 3 days, control = 72 pg, + LH = 577 pg. The experiments mentioned on adult testicular tissue lasted no longer than 6 h.

To determine the moment testosterone secretion starts and to test the effect of LH at this time, explantation of 14-day-old testes was performed (table 3). Testosterone production was undetectable in the medium during the 12 h following explantation and started when the testes were actually 14½ days old;

Table 3
Effect of LH on testosterone secretion from foetal testes during the first day of explantation

	Testosterone pg/testis/60 min culture					
Age of testis	14 days		16 days		20 days	
Culture time	No addition	LH (100 ng/ml)	No addition	LH (100 ng/ml)	No addition	LH (100 ng/ml)
0 – 1 h	undet.	undet.	1120	3783	1770	9497
1 – 2 h	undet.	undet.	1337	6489	1689	20 689
2 – 4 h	undet.	undet.	1188	4524	702	11 491
4 – 6 h	undet.	undet.	1033	3841	636	11 925
6 – 8 h	undet.	undet.	775	4948	544	9755
8 – 12 h	undet.	undet.	580	4195	671	5026
12 – 24 h	9	30	346	1295	371	1954
0 – 24 h	4	24	366	2365	991	8658

Each value is a mean from 2 experiments. For each foetus, one testis was cultured without LH and the other one with LH.
undet.: undetectable.

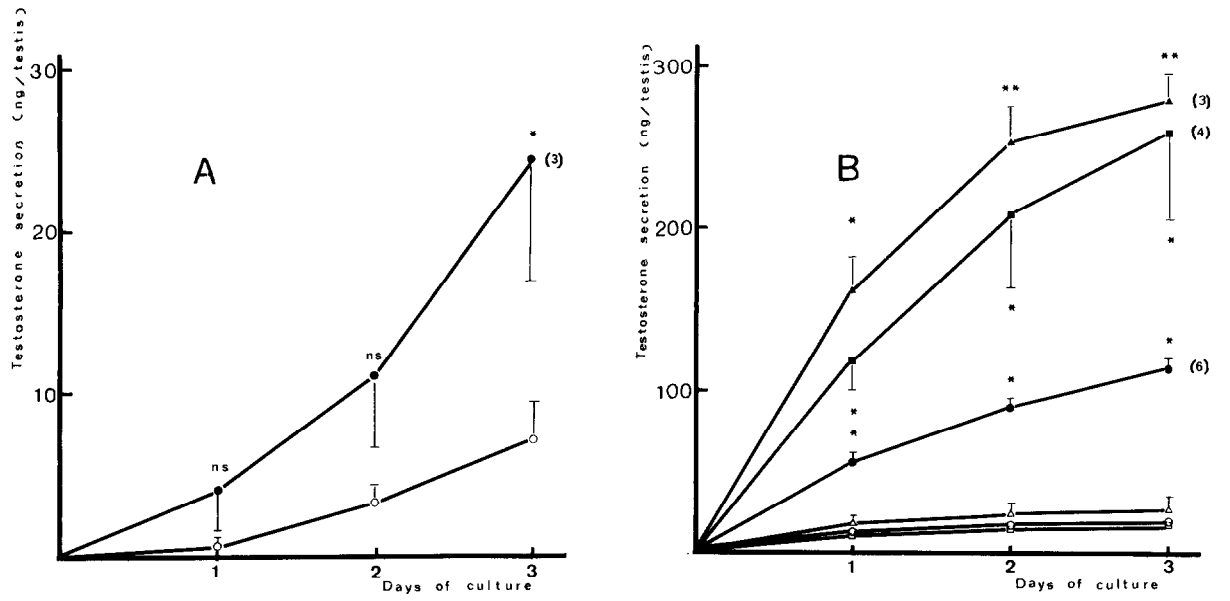

Fig.2. Time course relationship of testosterone production from foetal testes with (full symbols) and without (blank symbols) 100 ng/ml LH. A = 14½ days; B = 16½ days (circles), 18½ days (squares) and 20½ days (triangles). The values presented are means ± S.E.M. In parentheses, number of paired experiments. n.s. = $p > 0.05$; * = $0.05 > p > 0.001$; ** = $p < 0.001$ (paired test).

the effect of LH was noticeable only at the time testosterone secretion started in controls.

In conclusion, the results of the present study demonstrate that testosterone production by foetal testes in vitro can be stimulated by LH. The characteristic of the responses was very similar to that obtained previously with dibutyryl cyclic AMP. A study on the effect of LH on foetal testicular adenylate cyclase activity is now being undertaken to specify the role of cyclic AMP as second messenger of testosterone production in the foetal testes.

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