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RESPONSE OF THE TESTIS TO HCG STIMULATION AS A FUNCTION OF AGE IN THE IMMATURE RABBIT

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SUMMARY

50, 150, 300 and 500 U.I. of HCG were administered to male rabbits, 20, 40, 60 and 180 days of age. Plasma and testicular testosterone were quantified by radioimmunoassay or gas chromatography 1 h after the injection. At all stages, HCG significantly increased plasma and testicular testosterone compared with saline-injected controls of the same age. Expressed as a per cent of basal level, the plasma testosterone was increased 422% at 20 days, 216% at 40 days, 386% at 60 days and 1122% at 180 days. The content and concentration of testosterone in the testes were increased 485 and 366% at 20 days, 378 and 517% at 40 days, 244 and 107% at 60 days, 1062 and 1106% at 180 days. At all stages studied, the response of the immature testis was much less than that in adults. It did not increase between 20 and 60 days.

INTRODUCTION

With the advent of sensitive methods of estimation of steroids, it has become feasible to show that in numerous species (such as rat [1–3], mouse [4, 5], guinea-pig [6], ram [7, 8], monkey [9], man [10, 11], and bull [12]) puberty in the male, is characterized by an increase in plasma and testicular testosterone. Furthermore, in some species, especially the rat, it seems that the hypothalamo–hypophyseal-testicular axis may be functional from birth onwards and during prepubertal period [13, 14]. The blood level of steroids is determined by at least two factors: the plasma concentration of pituitary gonadotrophins and the testicular responsiveness to these hormones. According to Ramirez and Mc Cann [15], Mc Cann *et al.* [16], in the rat, a change in the hypothalamic sensitivity to steroids would occur at puberty that would induce a rise in the blood level of gonadotrophins and thus testosterone. However, it is not known whether the rise in blood testosterone at puberty is due to an increase in plasma LH, for in some species such as sheep [17] and guinea-pig [18], the circulating LH level is relatively stable during sexual maturation, and in others such as the rat, it increases very little at puberty [19, 20]. Odell *et al.* [21] suggest that in sexual maturation, a change in the FSH-induced testicular sensitivity to LH might occur. This hypothesis was recently confirmed in the rat [22]. We have shown [23] that the testis of 30 day-old immature rabbits was able to respond significantly to HCG stimulation and that the response was identical from 30 to 120 min after the injection. We report the course of this response from 20 to 180 days, assessed by simultaneous measurement of plasma and testicular testosterone in order to determine the effects of HCG on the synthesis and liberation of testosterone.

EXPERIMENTAL

Male rabbits of the New Zealand strain raised in the laboratory were used. The conditions of temperature (20°C ± 1°C), lighting (daylight) and feeding (complete pelleted food and water given freely) were the same for controls and treated animals. Newborn rabbits of both sexes were raised by their mothers; when 30 day old, males were isolated 3 per cage (until 60 days), then one per cage until 180 days. The 180 day old males, sexually mature, were first used as reproducers. Males [83] divided up into 4 groups, were killed at 20, 40, 60 and 180 days. HCG (Human chorionic gonadotropin from ISH Laboratories) was prepared in 0.9% saline solution and 50, 150, 300 and 500 I.U., were injected into an ear vein, respectively at 20, 40, 60 and 180 days. The dose of HCG administered to adult rabbits was chosen so as to obtain a very strong response [24]; for the other stages, identical doses were used, worked out on a weight basis. Controls were injected with identical vol. of saline solution. For each group, the basal level of plasma and testicular testosterone was determined. All rabbits were killed by section of the jugular and carotid vessels, 1 h after injection. The blood plasma and both testes were removed and frozen at −25°C until assayed. Testosterone was measured in the plasma and in the left testis by radioimmunoassay for the 20 day old rabbits and by gas chromatography with an electron detector for the later stages. These two methods, previously described [25, 26] will be only briefly recalled.

Table 1. Responses of plasma and testicular testosterone to HCG injection as a function of age in male rabbits

Age, days	Treatment	Body weight g	Testes weight mg	Testes weight mg/100 g body weight	Plasma testosterone, ng/ml	Testicular testosterone ng/testis	Testicular testosterone ng/100 mg testis
20	Saline solution (8)	302.5 ± 37.0	42.49 ± 4.41	14.79 ± 1.35	0.4 ± 0.2	5.7 ± 2.5	34.2 ± 15.8
	HCG (13)	300.0 ± 21.2	41.49 ± 1.87	14.28 ± 0.66	*2.2 ± 0.2	*33.4 ± 3.8	*159.6 ± 15.8
40	Saline solution (8)	769.4 ± 51.7	116.87 ± 11.70	15.12 ± 0.97	0.8 ± 0.4	29.0 ± 12.2	47.0 ± 15.1
	HCG (12)	676.2 ± 48.1	93.52 ± 9.00	13.79 ± 0.61	*2.4 ± 0.3	*139.2 ± 17.0	*290.3 ± 25.7
60	Saline solution (11)	1324.1 ± 41.1	239.68 ± 20.45	18.07 ± 1.40	1.5 ± 0.3	152.2 ± 40.9	134.8 ± 32.8
	HCG (12)	1477.9 ± 69.1	*392.59 ± 17.61	*26.82 ± 1.04	*7.3 ± 0.6	*529.3 ± 48.5	*279.6 ± 29.3
180	Saline solution (9)	3840 ± 85	5733.00 ± 467.19	149.60 ± 12.51	0.9 ± 0.2	112.0 ± 23.2	4.0 ± 0.8
	HCG (10)	3770 ± 141	5219.10 ± 152.61	139.13 ± 3.36	*11.6 ± 1.1	*1302.6 ± 183.4	*48.4 ± 6.3

Values are means ±SE. Numbers in parentheses indicate number of observations.
* Significantly different from the saline injected controls of the same age.

Gas chromatography. Plasma and testicular androgens were extracted with ether; the testosterone was isolated by t.l.c., converted to testosterone-17β-heptafluorobutyrate, purified and injected into a Packard chromatograph equipped with an electron detector (63 Ni). The estimations were carried out with an average precision of 10%. The lowest amount of testosterone measurable in biological samples was one ng.

Radioimmunoassay. The androgens were extracted with ethyl acetate–isooctane (3:7, v/v). Dihydrotestosterone was eliminated by chromatography on a celite column. Testosterone, tritiated testosterone and the antibody diluted to 1:45000, were incubated overnight at +4°C. Antibody-bound and free hormone were separated using Dextran coated charcoal. The assay was sensitive to 50 pg, with intra and inter-assay coefficients of variation of 5.6% and 9.5% respectively. The antibody was prepared in rabbits against testosterone-3-O-carboxymethyl oxime BA. The major cross-reacting steroid in this system, 5α-dihydrotestosterone (69%), was eliminated by the chromatography. Among the 17 other steroids studied, the cross reaction of 5α-androstane-3β, 17-β diol and 5α-androstane-3α, 17β-diol was 7.8%; for the others, it was less than 2%.

Simultaneous measurements of various samples, by radioimmunoassay and gas chromatography, showed an average variation of 12%, with a highly significant coefficient of correlation ($r = 0.87$) which permits comparison of the results obtained by both methods.

RESULTS

Body and testicular weights of control and treated animals did not differ significantly at any age except for the testicular weight of 60 day old animals which was higher in the treated group (Table 1).

Table 1 also shows the concentration of testosterone in the plasma of control and HCG-treated animals of various ages. Basal levels of plasma testosterone, measured in saline-injected controls were increased from 0.4 ng to 1.5 ng/ml, between 20 and 60, then slightly reduced at 180 days (0.9 ng/ml). In all groups studied, HCG stimulation increased plasma testosterone levels from 216% at 40 days to 1122% at 180 days (Table 2). The concentrations of testosterone, found after HCG stimulation in 20 and 40 day old animals (2.2 and 2.4 ng/ml) were similar to those measured in 10 adult males receiving neither HCG nor saline solution (1.7 ± 0.6 ng/ml). Compared to the same controls, the testosterone level of 60 day old HCG-treated males (7.3 ng/ml) was much higher. However it must be pointed out that in the rabbit strain used, a marked rise in plasma testosterone level occurs at this stage (60 days) (unpublished data).

The per cent increase over basal values, induced by HCG, varies as a function of age (Table 2). The increase obtained in 180 day old males (1122%) was significantly higher than those observed at other stages (from 216 to 422%). In saline injected controls, the testosterone content of the testis closely followed those in the blood: they increased from 5.7 ng/testis at 20 days to 152.2 at 60 days and then decreased

Table 2. Per cent increase in plasma and testicular testosterone after HCG injection

Age, days	Serum testosterone	Testicular testosterone Absolute content	Testicular testosterone Concentration
20	422 ± 52	485 ± 67	366 ± 46
40	216 ± 43	378 ± 58	517 ± 54
60	386 ± 37	244 ± 30	107 ± 21
180	1122 ± 111	1062 ± 163	1106 ± 156

Values are means ±SE.

to 112 at 180 days. In all groups, the injection of HCG significantly raised the testosterone content of the testis from 244% at 60 days to 1062% at 180 days, the testicular response of 180 day old rabbits being significantly higher than that of younger animals. The testosterone content, measured after HCG stimulation in the left testes of 40 (139.2 ng/testis) and 60 (152.2 ng/testis) day old animals was similar to that of uninjected adult controls (255.6 ng/testis) although their weights were 63-fold (40 days) or 15-fold (60 days) lower. Such a result demonstrates the importance of testosterone synthesis induced by HCG in the testis before puberty.

In saline injected-controls, the testicular testosterone concentration increased from 34.2 ng/100 mg at 20 days to 134.8 ng/100 mg at 60 days, then fell to 4.0 ng/100 mg at 180. HCG stimulation increased significantly the testicular testosterone concentration in all groups (from 107% at 60 days to 1106% at 180 days). Expressed as a per cent of the initial concentration, the increase observed at 180 days, was significantly the highest.

DISCUSSION

Our results showed that although HCG significantly stimulates the synthesis and secretion of testosterone by the immature testis, the response obtained, expressed as a per cent of basal levels was always much lower than in adults. After HCG stimulation of the immature testis, plasma testosterone concentration reached values similar to the levels seen in adult controls as observed in the rat [27], in the monkey [28] and in man [10]. Furthermore, we have shown that, not only liberation but also the testicular synthesis of testosterone were increased by HCG in immature animals. It seems that in the rabbit, specific receptors for HCG are present in the testis and functional in the early stages of development, whereas, in the rat, they are present but not functional [29].

In our experiments, from 20 to 60 days, minimal changes were noted in the receptivity of the testis to HCG whereas, in the rat, the testicular response to LH regularly increases from 21 to 40 days [22]. This discrepancy could perhaps be related to the species or to a difference in the chronology of FSH secretion. According to Odell *et al.* [22], continual exposure of the Leydig cells to FSH increases sensitivity to LH.

The fact that the response of immature testis to HCG is always less than that in adults remains difficult to explain. It may be due to the number and maturation of specific HCG receptors, the development of testicular enzymes involved in steroids biosynthesis, or endogenous secretion of gonadostimulins. As far as the latter factor is concerned, it seems that its influence was at least limited in our experiments. If one supposes that basal levels of plasma testosterone reflect LH secretion, the relatively small variations in plasma testosterone observed in controls at various stages, are in favour of comparable endogenous gonadotropin stimulation. For instance, the controls, at 40 and 180 days, had similar plasma testosterone levels (0.8 and 0.9 ng/ml) and a quite different HCG response. Furthermore, in the immature rat, it is known that the disappearance of the first generation of Leydig cells [30, 31] is followed by a regression in testicular enzyme activities [32] and a reduction in the biosynthesis of steroids [33, 34]. It is not known whether the same thing occurs in the rabbit but the presence of a testosterone secretion at the same stages, and its rise following HCG injection do not favour a more or less complete cessation of the enzymatic activities of steroid biosynthesis. The lesser reactivity of the immature testis to gonadotropin stimulation may then be related to a low ability to concentrate HCG, as observed in the rat [35].

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