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ENDOGENOUS STEROID PRODUCTION IN PREPARATIONS OF RAT TESTIS AFTER LONG-TERM TREATMENT WITH HCG

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SUMMARY

The effect of prolonged treatment with Human Chorionic Gonadotrophin (HCG) on endogenous steroid production in homogenates and mitochondrial fractions of rat testis tissues was investigated. Following HCG treatment (5 days, 100 i.u. s.c. per day) testosterone production in homogenates of whole testis tissue and of isolated interstitital tissue was increased by a factor of 2·5 and 2·4 respectively. In the presence of cyanoketone mitochondrial fractions obtained from whole testis tissue and isolated interstitial tissue of HCG treated rats produced respectively 2·4 times and 3·3 times more pregnenolone than comparable mitochondrial preparations obtained from control testes.

The production of steroids from endogenous precursors in homogenates and mitochondrial fractions of isolated seminiferous tubules was very low and contributed very little to steroid production in preparations of whole testis tissue.

After HCG treatment the activity of 3β-hydroxysteroid-dehydrogenase was increased in whole testis tissue (from 140 to 320 μU/mg), in isolated interstitial tissue (from 1030 to 2200 μU/mg) as well as in isolated seminiferous tubules (from 8 to 20 μU/mg).

These findings indicate that the interstitial compartment is the main site of steroid production in rat testis and that enhanced testicular steroid production after treatment with HCG is caused by an activation of the steroid production in the interstitial tissue.

Steroid production by rat testis is maintained and regulated by peptide hormones, released from the pituitary [1]. De Jong *et al.*[2] have previously reported that administration of 100 i.u. HCG daily for 5 days increased the testosterone concentration in testicular venous plasma of rats with intact pituitaries. This indicates an activation of testicular steroid production by exogenous gonadotrophins. However, little information is available on the steroid production by testis tissue preparations *in vitro* following prolonged HCG treatment of adult animals with intact pituitaries. Hall and Eik-Nes[3] reported a qualitative increase of the conversion of [^{14}C]-acetate into [^{14}C]-testosterone by slices of rabbit testes, and Purvis and coworkers[4] observed an increase in mitochondrial and microsomal cytochrome P_{450}, an essential co-enzyme for testicular steroid production. In order to obtain information about the quantitative effects of HCG treatment on testicular steroidogenesis we have investigated the production of steroids in homogenates and mitochondrial fractions obtained from whole testis tissue, isolated interstitial tissue and isolated seminiferous tubules. Moreover, the enzyme activity of 3β-hydroxysteroid dehydrogenase, responsible for the conversion of pregnenolone to progesterone, was studied.

Steroids were measured with radioimmunoassay techniques, essentially as described by Verjans *et al.*[5]. Endogenous steroid production was calculated from the difference in the amount of pregnenolone or testosterone present in the incubation mixture after 1 h and at zero time.

The results in Table 1 show that prolonged treatment with HCG causes an increased testosterone production from endogenous precursors in homogenates of whole testis tissue. This increased androgen production is apparently due to an increased steroid production in interstitial tissue as reflected by the results for homogenates of interstitial tissue. The production of testosterone in homogenates of seminiferous tubules was very low and contributed hardly at all to the total testosterone production. From the present results it cannot be concluded that the tubular testosterone production is caused by some steroidogenic cell types in this tissue compartment, or by contamination with material from the interstitial tissue. We observed (Table 1) a marked enhancement of 3β-hydroxysteroid dehydrogenase activity in homogenates of rat testis tissue. This is in contrast with observations from Shikita and Hall[6], that HCG treatment of intact rats older than 22 days does not increase 3β-hydroxysteroid dehydrogenase activity. The predominant interstitial localization of this hydroxysteroid dehydrogenase has been reported previously [7, 8].

It is generally accepted that the mitochondrial conversion of cholesterol to pregnenolone is a rate-limiting step in steroid biosynthesis in the ovary, adrenal and testis (for a review see P. F. Hall[9]) and that trophic stimulation of steroid production in these organs is caused by an increased conversion of cholesterol to pregnenolone in the mitochondria. The results in Table 2 reflect the effect of prolonged HCG treatment on the amount of protein assayed in mitochondrial fractions isolated from whole testis tissue,

Table 1. Testosterone productions and 3β-hydroxysteroid dehydrogenase activities in homogenates of rat testis tissues (mean values of at least 4 rats). *P* values calculated with Student's *t*-test

	Whole testis			Interstitial tissue*			Seminiferous tubules*		
	Control	HCG		Control	HCG		Control	HCG	
Testosterone production†									
pmoles/mg, h	112	267	($P < 0.05$)	1040	2800	($P < 0.05$)	2·1	6·8	(NS)§
nmoles/2 testes, h	23·7	60·2	($P < 0.05$)	13·6	33·1	($P < 0.05$)	0·20	0·57	(NS)
3β-Hydroxysteroid dehydrogenase‡									
μU/mg	140	322	($P < 0.001$)	1030	2200	($P < 0.05$)	8	20	($P < 0.05$)
μU/2 testes	30·2	70·5	($P < 0.001$)	12·8	33·9	($P < 0.025$)	0·72	1·78	(NS)

* Values are not corrected for losses of tissue during dissection.
† Aliquots of tissue homogenates were incubated in a Tris-HCl buffered medium pH 7·35 containing 0·125 M sucrose, 0·5 mM EDTA, 20 mM sodium succinate, 1·0 mM NADPH, 1·0 mM NAD^+, 10 mM glucose 6-phosphate, 0·3 units glucose 6-phosphate dehydrogenase, 0·7 mM $CaCl_2$ and 5·0 mM $MgCl_2$ at 33°C in $O_2 + CO_2$ (95:5, v/v) atmosphere.
‡ Assay conditions were as previously described [8].
§ Not significant.

Table 2. Pregnenolone production and amount of protein in mitochondrial fractions of rat testis tissues (mean values of at least 4 rats)

	Whole testis			Interstitial tissue*			Seminiferous tubules*		
	Control	HCG		Control	HCG		Control	HCG	
Pregnenolone production†									
pmoles/mg, h	580	1420	($P < 0.025$)	2665	7050	($P < 0.025$)	17	37	(NS)§
nmoles/2 testes, h	16·0	38·3	($P < 0.025$)	6·1	20·7	($P < 0.025$)	0·16	0·32	(NS)
Mitochondrial protein									
mg/2 testes	28·8	27·0	(NS)	2·1	3·0	($P < 0.05$)	9·4	8·5	(NS)

* Values are not corrected for losses of tissue during dissection.
† Incubation conditions were as previously described [8].
§ Not significant.

interstitial tissue and seminiferous tubules and on production of pregnenolone from endogenous precursors in these mitochondrial fractions. Cyanoketone was present in the incubation mixtures in order to prevent further conversion of pregnenolone [8].

Prolonged HCG treatment had no effect on the total amount of protein in mitochondrial fractions from whole testis tissue and isolated seminiferous tubules, but increased the protein content of the mitochondrial fraction of interstitial tissue with a factor 1·4 ($P < 0.05$). Pregnenolone production expressed as pmol/mg, h or as nmol/2 testes equivalents, h was significantly increased in incubations of mitochondrial fractions from whole testis tissue and from interstitial tissue ($P < 0.025$ in all cases). The production of pregnenolone by mitochondrial fractions from seminiferous tubules was low and was not significantly increased after HCG treatment. These data strongly suggest that increased androgen production after prolonged administration of gonadotrophins is caused by an enhanced steroid synthesis in interstitial tissue mitochondria. The increased amount of protein in the interstitial mitochondrial fraction and the increased concentrations of cytochrome P_{450} and testodoxine, components of the mitochondrial cholesterol side chain cleaving enzyme system [10, 11], may favour the conclusion that as a result of HCG treatment an increased number of mitochondria in the steroidogenic cells in the interstitial compartment is responsible for the increased steroid production. However, it cannot be excluded that an increased concentration of components of the cholesterol side chain cleaving enzyme complex per mitochondrion and an accumulation of precursors and other co-factors for pregnenolone biosynthesis (*e.g.* cholesterol) will render an important contribution to the HCG stimulation of androgen production. Only little information is available on the effect of long-term HCG treatment on steroid precursor pools in testis of intact adult rats. The biosynthesis of cholesterol appears to be activated [12], but no significant effect on free cholesterol and esterified cholesterol concentrations in isolated interstitial tissue was observed after HCG treatment [13]. A possible effect of trophic hormones on cholesterol uptake by interstitial cells and on an intracellular or intramitochondrial translocation of cholesterol remains to be investigated.

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