Effect of HCG on testicular function in juvenile rhesus monkeys (*Macaca mulatta*)

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Although information exists on the morphological aspects of the immature and adult monkey testis (van Wagenen & Simpson, 1965; Baker, 1972) and peripheral plasma testosterone levels in the adult monkey (Goodman, Hotchkiss, Karsch & Knobil, 1974; Michael, Setchell & Plant, 1974), little consideration has been given to the functional postnatal differentiation of the testis in nonhuman primates. In the present study, therefore, we describe the histological changes and corresponding changes in testicular and plasma testosterone levels in the testis of young rhesus monkeys treated with HCG.

The 7 juvenile male monkeys used, 10–14 months old and weighing 1.4–1.7 kg, were maintained under standard colony conditions. One testis of each animal was removed under ether anaesthesia on Day 1 of the experiment to serve as the initial control. Four of the unilaterally castrated monkeys received a s.c. injection of 300 i.u. HCG (Physex Leo) per day for 5 days (Days 2 to 6). The other three monkeys received no treatment. On Day 7, the remaining testis was removed and a weighed portion of the decapsulated gonad was processed for testosterone estimation. A piece from the same testis was fixed in Bouin's solution for histological examination of paraffin-wax sections cut at 8 μ m and stained with Harris's haematoxylin and eosin. Blood samples were drawn from the saphenous vein of the unanaesthetized animal on Days 1 and 7, before removal of the testis. Bleeding and surgery were always performed between 10.00 and 11.00 hours.

For the analysis of testosterone, testicular homogenates were extracted with redistilled analytical grade methanol (1 : 25 w/v), centrifuged and the precipitate re-extracted with methanol. The two extracts were pooled. Similarly, plasma aliquots were extracted with methanol (1 : 8 v/v). The tissues were then taken to dryness under nitrogen at 40°C. The dried extracts were reconstituted with appropriate volumes of the assay buffer. Testosterone was measured by the radioimmunoassay described by van Damme, Robertson, Romani & Diczfalussy (1973). All plasma and tissue determinations were made in triplicate. Recovery of [3 H]testosterone and unlabelled testosterone standards added to plasma or tissue homogenates was about 93%. To check the reliability of our extraction procedure, celite chromatography of the samples was carried out as outlined by Abraham (1973), and after extraction of the plasma and tissue homogenates with diethyl ether. The testosterone values obtained by the two methods did not differ significantly.

The histological appearance of the control testes (recovered on Day 1) was fairly uniform in all the animals. As described for monkey (van Wagenen & Simpson, 1965; Baker, 1972) and man (Vilar, 1970), the seminiferous epithelium was composed of pre-Sertoli cells, gonocytes and a few spermatogonia, while the undifferentiated mesenchymal cells (fibroblasts) and fine stromal tissue constituted the bulk of the intertubular compartment. Unilateral castration did not produce any marked effect either on the weight of the remaining testis (Table 1) or its histological characteristics at the end of the 6-day period, in any of the 3 untreated animals. Administration of HCG resulted in an increase in the testicular weight accompanied by a hyperplasia of the intertubular tissue. In many areas of the testis the interstitial cells were characterized by their large size and eosinophilic cytoplasm. The nucleus of such cells was spherical and contained one or more distinct nucleoli, i.e. a transformation of mesenchymal cells into Leydig cells. Such changes in the intertubular tissue of the testis have been shown to occur at the time of puberty or under gonadotrophic stimulation in other mammalian species, including man (Albert, 1961; Abramovich, Baker & Neal, 1974). In the HCG-stimulated testis there was also a proliferation of the germinal cells and a number of mitotic figures could be seen in the seminiferous tissue. In 20 transverse sections of the seminiferous tubules examined, the cell counts

Table 1. Changes in testicular weight and plasma and testicular concentrations of testosterone in juvenile rhesus monkeys after treatment with 300 i.u. HCG

Treatment	Animal	Testosterone concentrations					
		Testicular wt (mg)		Testicular (ng/g)		Plasma (ng/100 ml)	
		Day 1*	Day 7†	Day 1*	Day 7†	Day 1*	Day 7†
None	748	126	124	6.3 ± 0.9	8.8 ± 1.3	67.8 ± 3.8	61.3 ± 3.2
	750	170	178	3.8 ± 0.3	4.4 ± 0.4	44.2 ± 6.1	46.8 ± 2.7
	764	224	220	12.6 ± 1.7	11.0 ± 1.1	34.5 ± 2.2	34.5 ± 3.2
Group Mean ± S.E.M.		173.3 ± 28.3	173.0 ± 29.7	7.5 ± 2.6	8.0 ± 1.9	48.8 ± 9.8	47.5 ± 13.4
HCG (300 i.u./day on Days 2 to 6)	741	196	275	5.7 ± 0.2	84.6 ± 6.0†	45.3 ± 1.5	51.5 ± 2.6
	742	181	249	2.5 ± 0.3	102.7 ± 4.4†	26.0 ± 2.9	52.4 ± 1.1†
	747	275	330	2.0 ± 0.4	137.2 ± 15.6†	28.2 ± 0.3	60.1 ± 2.9†
	763	116	180	6.6 ± 0.8	315.4 ± 9.4†	52.3 ± 2.6	118.5 ± 5.4†
Group Mean ± S.E.M.		192.0 ± 32.6	258.5 ± 31.1	4.2 ± 1.1	159.9 ± 52.9	37.9 ± 6.4	70.6 ± 16.0

Values for testosterone levels in plasma and testis of individual monkeys are based on the Mean ± S.E.M. of 3 determinations.

* Values obtained from one testis removed on Day 1.

† Values obtained from the remaining testis on Day 7.

‡ P < 0.01 compared with the values on Day 1.

indicated a 3-fold increase in the spermatogonia with a gonocyte to spermatogonium ratio of 1.8 : 1.3 per section of seminiferous tubule. The equivalent ratio in the untreated animals was 2.1 : 1.3. The effect of HCG treatment on the seminiferous tubules of the immature monkey may be related to some FSH-like activity in HCG, since even highly purified HCG preparations are known to have some FSH-like activity (Qazi, Mukherjee, Javidi, Pala & Diczfalusy, 1974), or it could be a consequence of endogenous androgen production.

The testicular and plasma testosterone concentrations were low in the intact juvenile monkeys (Table 1), and changed little after unilateral castration. Similar plasma testosterone values have been reported before the onset of puberty in man (Kelch, Grumbach & Kaplan, 1972) and in adult castrated monkeys (Goodman *et al.*, 1974; Michael *et al.*, 1974). It is possible that the plasma levels of testosterone in the juvenile monkeys reflect androgen secretion by an extra-gonadal source such as the adrenal cortex. The mean $\pm$ S.E.M. plasma testosterone level was 51.3 ± 10.90 ng/100 ml in 3 18- to 24-month-old monkeys which had been castrated for more than 6 months. In HCG-treated animals, the testosterone concentrations in the testicular tissue were 17 to 70 times higher than the values recorded before treatment. These results accord with earlier findings of an HCG-induced stimulation of testosterone production by the testicular tissue (de Jong, Hey & van der Molen, 1974; Abramovich *et al.*, 1974). In our experiments, the increase in plasma testosterone levels after HCG treatment was about twofold except in one monkey (No. 741) in which there was little difference. The increase in plasma testosterone levels after HCG treatment is reported to be much greater in adult monkeys (Plant & Michael, 1973) and men (Rosemberg, Crigler, Lee, Jan & Butler, 1973). It is, therefore, suggested that the HCG-dependent increase in testosterone production may not be reflected as markedly in the peripheral circulation of the juvenile as in the adolescent or the adult.

This study was supported by a grant from the Population Council, New York (M73 056C). We are grateful to Dr E. Diczfalusy, Swedish Medical Research Council, Karolinska sjukhuset, Stockholm 60, Sweden, for antitestosterone serum.

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Received 13 February 1976