Testicular responsiveness to hCG during infancy measured by salivary testosterone

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Abstract. To investigate the role of gonadotropins in postnatal testicular activation, testosterone responsiveness to human chorionic gonadotropin was studied in 11 male infants (aged 5-180 days). The boys were given a single im injection of 5000 IU/1.7m² hCG, and serum and salivary testosterone responses were then measured for 7 days. The results were compared with the serum testosterone responses of 8 older prepubertal boys (aged 1.7-10.4 years) studied with the same protocol. The mean (±SEM) basal serum testosterone levels were 2.67±1.27 nmol/l in the infants and 0.90±0.02 nmol/l in the prepubertal boys (p<0.05). Both groups gave a significant response to hCG stimulation (p<0.001, ANOVA, one-way). The stimulated concentrations of serum testosterone were higher in the infants than in the prepubertal boys (p<0.001). The mean basal level of salivary testosterone was 30.5±7.0 and the mean maximal level was 97±10.3 pmol/l in the infants (p<0.001). No age-related changes were observed in either basal or hCG-stimulated levels. In infants the mean (±SEM) maximal hCG-stimulated increase was 25±10-fold in serum and 8±4-fold in saliva (p=0.13). A clear stimulatory effect of hCG on testicular testosterone production was found, suggesting that the postnatal increase in serum testosterone concentration in male infants is gonadotropin-mediated. Salivary testosterone concentrations can be increased by hCG, indicating that measurements of salivary testosterone may provide an optional, non-invasive method for assessing gonadal function in children.

Pituitary gonadotropin secretion increases during the early months of postnatal life after a transient decrease in levels in the first few days of life (1-4). This is accompanied in the male by a clear peak in serum testosterone (T) concentrations (5,6), suggestive of gonadotropin-mediated increase in testicular steroidogenic activity. However, the exact mechanisms responsible for the testicular activation have so far remained unclear. The increase in pituitary-testicular activity in the early months of life has been assumed to be due to elimination of the inhibitory action of placental steroids from the circulation of the neonate. However, it is uncertain whether T production in infancy is really a response to stimulation by gonadotropins, since Bidlingmaier et al. (7) were unable to demonstrate a 2- to 3-month peak in testicular T concentration. Furthermore, a postnatal increase in testicular activity was not revealed by longitudinal measurements of salivary T (8), which are thought to reflect the circulating levels of the biologically active free (non-protein bound) fraction of T (9,10). This is in agreement with findings of simultaneous increase in serum T and in binding capacity or sex-hormone-binding globulin (SHBG) concentrations postnatally in male infants, leading to an increasing degree of binding of T to plasma proteins (1,11). Therefore, the postnatal increase in plasma gonadotropin and T concentrations could simply represent adaptation of the pituitary-testicular axis to increased SHBG levels. Nevertheless, a sex difference in free T levels is clear at birth, and a true decline in these levels occurs gradually during the first 6 months of life (8,11).

The objective of the present study was to evaluate whether gonadotropins are able to stimulate T production in infancy, especially the free (non-protein bound) fraction of T. In the present study we examined serum and salivary T responses to
human chorionic gonadotropin in infant boys. The results were also compared with serum T responses to hCG in prepubertal boys.

Patients and Methods

Patients and protocols
Eleven male infants (aged 5-180 days, mean 88) and 8 older prepubertal boys (aged 1.7-10.4 years, mean 6.0) were studied. Eight of the infants and 7 of the older prepubertal boys had unilateral incomplete descent of the testis, and 3 infants and one prepubertal boy had bilateral incomplete descent of the testis. The testes were of normal size and consistency at palpation; only one testis was not palpable. On physical examination, the boys were otherwise healthy. Each boy was given a single intramuscular injection of 5000 IU hCG/1.7 m² (Pregnyl®, Organon, Oss, The Netherlands) between 08.00 and 10.00 h. In 6 infants and in all 8 older prepubertal boys, peripheral blood samples were taken immediately before the hCG injection and then on days 1, 2, 3, 4, 5, and 7. In all infants, saliva was collected before the injection and then daily for 7 days. Saliva (about 0.5 ml) was collected before feeding with a plastic Pasteur pipette (Falcon) from under the tongue. If necessary, a small crystal of citric acid was placed on the tongue. Samples were stored at −20°C and all samples from an individual subject were run in the same assay. Informed consent was obtained from at least one of the two parents. The protocol was approved by the Ethical Committee of the hospital.

Methods
Serum and salivary T were quantified by RIA as described by us before (8,12).

Statistics
Student’s t-test (unpaired) was used for single variables between the groups. Analysis of variance (ANOVA) was used for repeated measurements (i.e. responses) within each group (one-way) and between groups (two-way).

Results

Serum T responses
The mean (±SEM) basal levels of serum T were 2.67±1.27 nmol/l in the infants and 0.09±0.02 nmol/l in the prepubertal boys (p<0.05) (Fig. 1). In both groups the response to hCG stimulation was significant (p<0.001, ANOVA, one-way). The stimulated T concentrations were higher in the infants than in the prepubertal boys (p<0.001, ANOVA, two-way). The shapes of the response curves were similar in the two groups and even on day 1 the T concentrations were significantly elevated above the basal levels in both groups.

Salivary T responses
The mean basal levels of salivary T were 30.5±7.0 pmol/l and in all infants they were below 50 pmol/l (Fig. 2). In infants there was no clear increase in the basal salivary T levels with age. A significant salivary T response to hCG was seen in the infants (p<0.001, ANOVA, one-way). In infants the mean maximal levels were 97±10.3 pmol/l and there was no clear increase in the T responsiveness to hCG with age. The shape of the response curve in salivary T was similar to that in serum; however, the mean maximal level was seen on day 3 in saliva, and on day 4 in serum.
Discussion

The testis tissue clearly receives strong gonadotropin support between the ages 1 and 3 months, as demonstrated by measurements of circulating luteinizing hormone and follicle-stimulating hormone (2-4). The increased gonadotropin secretion is obviously responsible for the postnatal increase in testicular volume (13,14), but the effect of gonadotropins on testicular T production in infancy has been poorly documented. The present study clearly demonstrates the stimulatory effect of hCG on testicular T production, suggesting that the postnatal increase in serum T concentrations in male infants is gonadotropin-mediated.

For ethical reasons we were able to study testicular responsiveness to hCG only in infants with incomplete testicular descent. There is evidence that some infants with incomplete testicular descent have lower T levels than normal infants (15). However, if hCG is able to stimulate T production in these subjects, it should also be able to stimulate it in normal subjects.

In absolute terms, basal serum T levels and serum T responses to hCG were higher in infancy than at prepuberty. The reason may be that during the postnatal period the Leydig cell population has not yet dedifferentiated to the "quiescent stage" (16). However, in relative terms serum T responses above the basal level were 146-fold and 25-fold in prepubertal boys and in infants, respectively. This could be explained by the higher circulating gonadotropin concentrations in infancy, since in the adult testis, at least, excessive amounts of gonadotropins are known to desensitize testicular steroidogenesis (17-19). The differences in T responsiveness to hCG between the neonatal and prepubertal periods could also be explained by functional differences between fetal- and adult-type populations of Leydig cells, which presumably occurred in different proportions in the two groups studied.

The finding that salivary T concentrations can be increased by hCG indicates that salivary T measurements provide an optional, non-invasive method for assessing gonadal function in children.

Acknowledgments

This study was supported by research contracts from the Finnish Life and Pension Insurance Companies and the Academy of Finland.

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Received July 12th, 1990.
Accepted October 1st, 1990.

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