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Regulation of testosterone production in fetal testicular cells: effect of androgens

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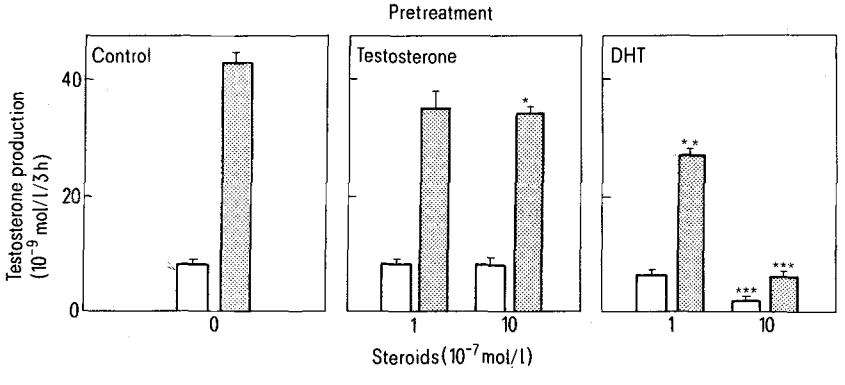
Summary. Pre-incubation of dispersed testicular cells from 18-day-old mouse fetuses in presence of testosterone or dihydrotestosterone resulted in a significant decrease of the hCG-stimulated testosterone production. These results suggest that during late fetal life testosterone production may be inhibited by an ultra-short loop feedback of androgens.

In vivo and in vitro studies have suggested that in immature and adult rats testicular testosterone production may be locally[1-3] controlled by androgens via a short-loop negative feedback mechanism. In the fetus, high concentrations of testosterone have been reported to exert a direct inhibitory influence on testicular testosterone synthesis in organ culture[4]. However, physiological concentrations of this androgen did not produce a negative effect[5]. The present study was undertaken to explore the influence of androgens such as testosterone and dihydrotestosterone (DHT) on basal and hCG-stimulated testosterone production by dispersed fetal testicular cells.

Materials and methods. Testes were excised from 18-day-old mouse fetuses. Fetal testicular cell suspensions were obtained from 150–200 fetal testes by mechanical dissection and collagenase dispersion using the method described earlier[6]. The cell suspension contained about 10% Leydig cells as identified by their bright yellow ring[7]. Aliquots of this cell suspension (10^5 cells/500 µl) were incubated with or without increasing concentrations of testosterone or dihydrotestosterone. After 30 min of incubation at 37 °C, the cellular suspension was centrifuged for 10 min at 100 × g and the supernatant discarded. The cells were then washed twice and incubated for 3 h at 37 °C in the absence or presence of 25 pM hCG (2nd IS for chorionic gonadotrophin bioassay). Since testosterone values obtained using unextracted medium did not differ from those obtained after ether extraction and the chromatographic procedure, testosterone was measured directly in the incubation media by radioimmunoassay as previously reported[6]. Testosterone levels were compared between groups by 1-way analysis of variance.

Results. The effect of pretreatment of fetal testicular cells with testosterone or DHT on basal and hCG-stimulated testosterone production is shown in the figure. The exposure of fetal cells to testosterone had no discernible effect on the quantity of testosterone produced under basal conditions during a 3-h incubation period. Pre-incubation of the cells with 10^{-7} M testosterone did not significantly affect the stimulatory effect of hCG on testosterone production, but the subsequent hCG-response of cells pre-exposed to 10^{-6} M testosterone was reduced ($p < 0.01$). Basal testosterone levels were not modified when the cells were pre-exposed to 10^{-7} M DHT, but were decreased with a higher concentration of DHT (10^{-6} M; $p < 0.001$). DHT had a significant inhibitory effect on hCG-stimulation of testosterone secretion in cells pre-incubated either with 10^{-7} M DHT ($p < 0.005$) or 10^{-6} M DHT ($p < 0.001$).

Discussion. The results of the present study show that both testosterone and DHT can influence the steroidogenic activity of the fetal testis. This effect is mainly characterized by a decrease of the stimulatory influence of hCG. Other authors[5] reported that physiological concentrations of testosterone were ineffective in inducing inhibition of testosterone production by whole testis in organ culture. Our data show that doses in the same range are able to reduce testosterone production by dispersed fetal testicular cells significantly, suggesting that this model is more appropriate for studying the regulation of testosterone synthesis in the fetal testis. Furthermore, it is interesting to note that the lowest concentration of androgen which is effective in our model is only 20 times greater than



Effect of a 30-min pre-exposure without or with T or DHT on T production by fetal testicular cells incubated 3 h in the absence (open bars) or presence of 25 pM hCG (solid bars). The results are the means of triplicate incubations. *$p < 0.05$, **$p < 0.001$, ***$p < 0.001$ as compared with appropriate control group.

that found in the male fetal circulation[8]. Thus one can speculate that the androgen concentration which exists in the microenvironment of the fetal Leydig cells may exert a local negative influence by the way of an ultra-short loop feedback. Since the fetal testis is able to metabolize testosterone into both estradiol[9] and DHT[10], it is difficult to predict whether this inhibition is mediated by testosterone itself or via aromatization and/or formation of 5α-reduced products. The fact that DHT is more effective in reducing testosterone production than testosterone itself suggests that aromatization is not a prerequisite for androgen action. Whether such an effect results from a direct influence on the fetal Leydig cell is unclear, since more than one type of cell is present in the testicular cell suspension used. This mechanism might offer an explanation for the decrease in circulating testosterone in the face of high concentrations of gonadotropins during late fetal life[11,12] and could take a part in desensitization of the fetal testis as previously suggested[6].

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Prolactin concentrations in mouse milk during lactation

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Summary. Prolactin levels in mouse milk increased from the day of parturition to give a concentration of 230 ng/ml on days 2 and 3 of lactation. Thereafter, levels dropped to 140 ng/ml by day 6 and were maintained at this concentration until weaning.

Milk contains a variety of steroid and polypeptide hormones[2] as well as growth factors[3]. Although we don't know the role (if any) played by these milk hormones in the neonate, many of them have been shown indirectly to cross the mucosa of the gastrointestinal tract and exert a biological effect in the neonate[4,5]. As our knowledge and understanding of milk hormones is still in its infancy, more information is required on the levels of these hormones in milk throughout lactation. As part of our study in mice we have monitored the level of prolactin (PRL) in mouse milk.

Materials and methods. Lactating CBA mice were used in this study to provide milk samples. Litter size was standardized to 5–7 pups and mothers were separated from their pups for at least 1 h before being milked at midday. Lactating mice were given i.p. injections of pentobarbitone sodium (60 μg/g) and oxytocin (0.62×10^{-3} units/g) and milk was collected using a suction apparatus[6,7]. Milk samples were immediately defatted and stored at −20 °C for a maximum period of 2 weeks.

The highly sensitive homologous radioimmunoassay for mouse PRL used in this study has already been described[1,8]. Iodination of PRL was by a modification of the chloramine-T method[8] and separation of free iodine from labelled PRL was accomplished on a Biogel P60 column (BioRad Laboratories, Bromley, Kent). The iodinated PRL had a mean sp. act. of 105 μCi/μg and was stored at −20 °C for up to 1 month. Prior to use in the radioimmunoassay the ^{125}I PRL was further chro-

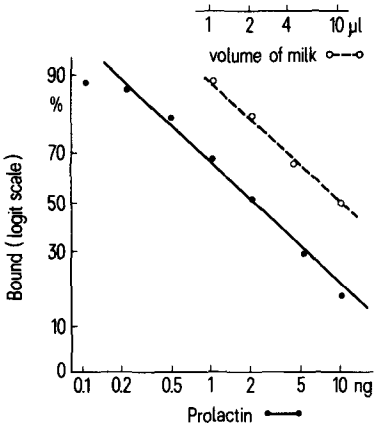

Figure 1. Dilution dose response curves for mouse prolactin standards and samples of mouse milk. Ordinate: logit scale of % ^{125}I-prolactin bound. Abscissa: $\log_e$ standard concentration or volume of milk sample.

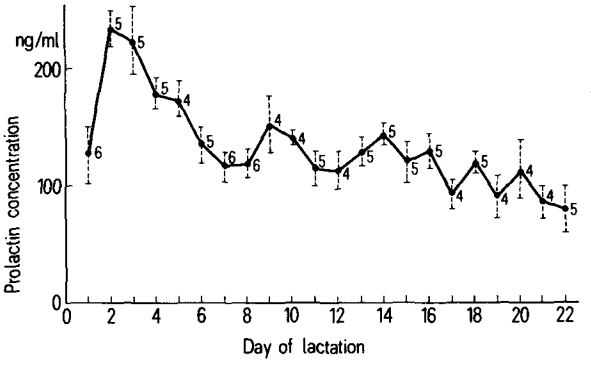

Figure 2. Prolactin concentrations in mouse milk throughout lactation. Variation bars represent SEM and n-values are included for each determination.