Testosterone secretion during gubernacular development and testicular descent in the dog

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Summary. Serum testosterone concentrations ranged from 0.24 to 1.45 nmol/l between Day 53 *post coitum* (*p.c.*) until Day 40 *post partum* (*p.p.*) and did not show variations that could be correlated with the process of testicular descent. The intratesticular androgen appeared to be mainly testosterone, its concentration being about 5000-fold higher than that in serum whereas 5 α -dihydrotestosterone could not be demonstrated. The intratesticular testosterone concentration at the initiation of gubernacular regression (Day 0) was apparently, but not significantly, higher than at Day 49 *p.c.* and at Day 40 *p.p.* The ability of the neonatal canine testis to synthesize testosterone was indicated by increased serum testosterone concentrations after hCG stimulation.

Introduction

During the migration of the testis from an intra-abdominal position towards the scrotum, the extra-abdominal part of the gubernaculum increases in volume, causing the first phase of testicular descent. This is followed by a regression of the gubernaculum that starts at birth and creates space for the second phase of testicular descent. In the dog, the testis passes through the inguinal canal at Day 3–4 *post partum* (*p.p.*) (Wensing, 1968; Baumans, Dijkstra & Wensing, 1981). The testis induces the growth as well as the regression of the gubernaculum and consequently regulates the first and second phases of testicular descent (Baumans *et al.*, 1982). The first phase of testicular descent seems to be regulated by an unidentified testicular factor, but there are indications that testosterone plays an additional role in this phase. Testosterone also induces gubernacular regression, and so regulates the second phase of testicular descent (Baumans *et al.*, 1983).

One of the most important enzymes of testosterone synthesis, Δ^5 -3 β -hydroxysteroid dehydrogenase (3 β -HSD), is detectable in the Leydig cells in the late fetal and neonatal period and suggests that testosterone may be synthesized in the Leydig cells of the dog (Baumans *et al.*, 1981). The immature testes of several mammals are steroidogenically active and capable of responding to human chorionic gonadotrophin (hCG) *in vitro* and *in vivo* (Nussdorfer, Robba, Mazzochi & Rebuffat, 1980; Arslan *et al.*, 1981). Many authors have demonstrated that the immature testes from several mammals are able to convert pregnenolone and/or progesterone into testosterone (see Terada, Wakimoto, Mizutani & Matsumoto, 1979).

The purpose of this study was to investigate serum testosterone concentration during the period of testicular descent, and since peripheral testosterone concentration does not reflect rapid and/or brief changes in testicular secretion in the dog (Comhaire, 1983), testosterone concentration was also estimated in testes of fetal and neonatal dogs. The ability of the neonatal canine testis to respond to hCG stimulation *in vivo* with an increase in serum testosterone concentration was also determined.

Materials and Methods

Collection of samples. Blood samples were collected from beagles (strain CPBDoBE67 from the Central Institute for the breeding of laboratory animals (TNO), Austerlitz, The Netherlands) by cardiac puncture from Day 53 *post coitum* (*p.c.*) to Day 40 *post partum* (*p.p.*). These punctures were carried out under neuroleptanalgesia (fluanisone/fentanylcitrate; Hypnorm: Philips-Duphar B.V., Amsterdam, The Netherlands). After centrifugation at 4°C, the serum was stored at -25°C until radioimmunoassay (RIA) of testosterone.

The responsiveness of the Leydig cells to stimulation with hCG was estimated in 8 beagles from 2 litters on the day of birth. The dogs received 30 i.u. hCG (Chorulon: Intervet Nederland B.V., Boxmeer, The Netherlands) i.v. Before hCG injection (0 h) and after 2, 24 and 48 h, 2 ml blood were withdrawn from the jugular vein of each dog and processed for RIA of testosterone.

The testes, removed at Day 49 *p.c.*, Day 0 (day of birth), or Day 40 *p.p.*, exactly as described by Baumans *et al.* (1982), were put on crushed ice within 5 min after excision. After weighing, homogenates of the testes were prepared separately with a Schacht X 10/20 homogenizer (International Laborator Apparaten GmbH, Dottingen, West Germany; 0.5 min) followed by homogenization in a Potter-Elvehjem homogenizer (1 min) in 1 ml buffer (pH 7.4; 0.01 M-Tris-HCl, 0.25 M-mannitol) and centrifuged at 900 *g* for 20 min at 4°C. The supernatant was stored at -25°C until RIA and gas-liquid chromatography.

Radioimmunoassay. The concentration of testosterone in testicular homogenates was estimated by a previously validated RIA method after extraction (Dieleman, Kruip, Fontijne, de Jong & van der Weyden, 1983). For the estimation of testosterone concentration in serum samples the antiserum was diluted 1:100 000 and the amount of tracer was reduced to one-half; the range of the standard curve was 0-175 fmol. The mean recoveries ($n = 25$) after extraction were: serum 81.6 ± 3.0 (s.d.)% and testis homogenate 82.3 ± 3.2 %.

The antiserum, raised in a rabbit against testosterone-3-keto-oxime-BSA conjugates (Verjans, Cooke, de Jong, de Jong & van der Molen, 1973), was generously supplied by F. H. de Jong; the main cross-reactions were 49.7% for 5 α -dihydrotestosterone, 7.64% for 4-androstene-3 β -17 β -diol and 3.35% for androstenedione.

The intra- and interassay coefficients of variation for the RIA of testis homogenate samples were 16 and 14% ($n = 8$) and for the RIA of serum samples 12 and 10% ($n = 8$) respectively; the sensitivity was 35 and 15 fmol per assay tube respectively.

Gas-liquid chromatography. The testis homogenate (1 ml) was extracted 3 times with 1 mol diethyl ether (recovery: 95 ± 2 (s.d.)%), and the combined extracts evaporated under a nitrogen stream. To the residues, 50 μ l TRISIL (Pierce, Illinois, U.S.A.) and 10 ng 5 α -cholestane (Ikapharm, Ramat-Gan, Israel) were added in pyridine; after shaking and subsequent derivative formation at 60°C for 30 min, the samples were evaporated. The samples were dissolved in *n*-hexane for analysis by gas-liquid chromatography.

A GCV (PYE-Unicam, U.K.) gas chromatograph was used with a flame ionization detector. The chromatographic conditions were: injection port 250°C; detector: 260°C, 30 ml hydrogen/min, 300 ml air/min; fused silica capillary column (Chrompack, the Netherlands), 25 m long with an internal diameter of 0.23 mm, coated with CPSIL5, temperature programmed for 15 min at 180°C and after that at a rate of 6°C/min to 240°C; carrier gas N₂ (for analysis) (1 ml/min flow), makeup gas N₂ (30 ml/min flow). Samples were applied by solid injection. Quantitative measurement of the steroids was carried out with 5 α -cholestane as an internal standard; the coefficient of variation for the correction coefficient k for testosterone was 5.4% ($n = 7$) (Schoenmakers, 1979). The retention time proportional to 5 α -cholestane was determined for standard solutions of 5 α -dihydrotestosterone, testosterone and their trimethylsilyl (TMS) derivatives; the retention times were 0.788 ± 0.007 (s.d.) ($n = 8$), 0.845 ± 0.007 ($n = 7$), 0.831 ± 0.004 ($n = 7$), and 0.877 ± 0.004 ($n = 8$) respectively.

Statistical analysis. One-way analysis of variance followed by multiple comparisons according to the method of Scheffé (1959) was applied to screen data for significant differences. $P \leq 0.05$ was considered statistically significant.

Results

Serum testosterone concentrations from Day 53 *p.c.* to Day 40 *p.p.* ranged from 0.24 to 1.45 nmol/l (Text-fig. 1). There was a considerable variation between and within the subjects.

Text-fig. 1. Serum testosterone concentrations in dogs during the period from Day 53 *p.c.* until Day 40 *p.p.* Drawn line indicates the mean.

The apparently higher concentration of intratesticular testosterone at Day 0 (3.35 ± 0.23 (s.e.m.) nmol/g, $n = 12$) was not significantly different from those at Day 49 *p.c.* (2.32 ± 0.54 , $n = 8$) and at Day 40 *p.p.* (1.57 ± 0.61 , $n = 4$).

After hCG treatment at Day 0, serum testosterone concentrations increased significantly ($P < 0.05$) from 0.49 ± 0.09 nmol/l, $n = 7$ (before treatment) to 2.25 ± 0.10 , $n = 8$, and 4.10 ± 0.31 , $n = 8$, at 2 and 24 h respectively after treatment. After 48 h the concentrations were still significantly elevated (3.72 ± 0.29 , $n = 7$).

Gas-liquid chromatography (Text-fig. 2) of testicular homogenates demonstrated the presence only of testosterone. Intermediate androgens such as androsterone, androstenedione and DHEA, have a shorter retention time than testosterone in the g.l.c. system used. The small peaks did not coincide with those of intermediate androgens. A comparison of intratesticular testosterone concentrations ($n = 13$) as determined by gas-liquid chromatography with those determined by RIA showed no significant difference (paired *t* test), and the correlation coefficient was $r = 0.75$ ($P < 0.01$).

Text-fig. 2. Gas chromatogram for the homogenate of half a testis, equivalent to 0.01 g tissue taken from a dog at Day 0 *p.p.* Arrow 0 indicates the addition of 10 ng 5α -cholestane, arrow 1 testosterone-TMS, arrow 2 the location of 5α -dihydrotestosterone-TMS, arrow 3 a blank and arrow 4 the location of 5α -dihydrotestosterone. Chromatographic conditions: fused silica capillary column (CP-Sil-5), temperature programmed for 15 min at 180°C after that at a rate of $6^{\circ}\text{C}/\text{min}$ to 240°C ; flame-ionization detection. The 15-min interval of the chromatogram is not shown.

Discussion

The serum and testicular testosterone concentrations obtained in the present study are in agreement with data published by Hart & Ladewig (1979) and DePalatis, Moore & Falvo (1978). An increase in serum testosterone levels reported to take place during the second phase of testicular descent in the rat and pig (Rajfer & Walsh, 1977; Colenbrander, de Jong & Wensing, 1978) was not detected in the dog. It is possible that an increase of the testosterone output is not reflected peripherally (Comhaire, 1983).

Gas-liquid chromatography analysis of fetal and neonatal dog testes showed that the intratesticular androgen was testosterone; in contrast to the situation in the rat, in which 5α -dihydrotestosterone is the major androgen (Rajfer & Walsh, 1977), 5α -dihydrotestosterone could not be demonstrated in dog testis at these ages. The intratesticular testosterone concentration was about 5000-fold higher than that in the serum. The slight rise of testosterone concentrations at Day 0 coincides with the onset of gubernacular regression in the second phase of testicular descent. If these high intratesticular concentrations reflect high production and release, this is in agreement with the earlier suggested role of testosterone in this process (Baumans *et al.*, 1983).

A possible explanation for the effectiveness of the relatively low testosterone concentration in the peripheral circulation might be a high sensitivity of the gubernaculum to testosterone in the period of regression. Another possibility is that the testosterone concentration might be considerably higher in this structure due to a specific transfer of testosterone from the venous outflow of the testis to the testicular artery (Free & Jaffe, 1975), since its epididymal branches are also an important vascular supply for the gubernaculum.

The ability of the fetal and neonatal canine testes to synthesize steroids is indicated by the histochemical demonstration of Δ^5 - 3β -HSD (Baumans *et al.*, 1981), and also by the increased serum testosterone levels after hCG stimulation (present study).

We conclude that the testis of the neonatal dog is able to synthesize testosterone *in vivo*, while the second phase of testicular descent seems to coincide with a trend towards a higher testosterone production.

We thank Professor Dr A. Rijnberk and Dr Pat Noden for their criticisms; Professor Dr W. A. de Voogd van der Straaten for statistical help; Dr J. Meijer for technical assistance; and Mrs L. J. M. Michielsens for the typing.

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Received 21 February 1984