Effects of neonatal administration of the reversible goitrogen propylthiouracil on the testis interstitium in adult rats

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The effects of neonatal treatment with the reversible goitrogen, 6-*n*-propyl-2-thiouracil (PTU) on the volumes of testicular interstitial components, the number and average volume of Leydig cells, and the steroid secretory capacity of testis and Leydig cells of rats at 135 days of age were investigated. Rat pups were hypothyroid from birth to 25 days of age following the addition of 0.1% (w/v) PTU to the drinking water of the mother. Treatment was stopped at 25 days and the pups subsequently returned to a euthyroid state. Control rat pups were raised without adding PTU to drinking water of the mother. On day 135, one testis from each rat ($n = 5$ per group) was fixed by whole body perfusion for microscopy and stereology, and the ipsilateral testis was used for steroid secretion analysis using an *in vitro* testis perfusion system. Average testis volume was 84% greater in PTU-treated rats than in controls. This increase resulted from increases in both the seminiferous tubule (86%) and the interstitial (80%) volumes. Moreover, absolute volumes of all testis components in PTU-treated rats were significantly ($P < 0.05$) greater than those of controls; the highest volume increase was observed in the lymphatic space (147%). The number of Leydig cells per testis was nearly doubled, but the average volume of a Leydig cell was decreased by 25% in PTU-treated rats compared with controls. Steroid secretion per testis was not significantly different between control and PTU-treated rats; however, steroid secretion per Leydig cell was significantly lower in PTU-treated rats than in controls. These results demonstrate that the neonatal administration of PTU causes Leydig cell hyperplasia. However, the normal androgenic status of these animals is maintained by hypotrophy associated with reduced steroid secretion of individual Leydig cells at maturity.

Introduction

A deficiency of thyroid hormone during neonatal–prepubertal life increases adult testicular size and sperm production in rats (Cooke and Meisami, 1991; Cook *et al.*, 1991; Kirby *et al.*, 1992). Studies have associated these changes with an increase in the number of Sertoli and germ cells (Van Haaster *et al.*, 1992; Hess *et al.*, 1993). In achieving this unprecedented increase in spermatogenic capacity by treating with the reversible goitrogen, 6-*n*-propyl-2-thiouracil (PTU), changes have also been observed in the hormonal profiles during testicular development and adulthood (Kirby *et al.*, 1992). However, the effect of transient thyroid hormone deficiency during neonatal–prepubertal life on the components of the testis interstitium in general, and Leydig cells in particular, at maturity has not been investigated.

It is logical to hypothesize that the increased testis size and sperm production in these adult PTU-treated rats could be associated with increased volumes of components of the testis

interstitium including the volume of Leydig cells, which could be achieved via hypertrophy or hyperplasia or both mechanisms. If Leydig cell volume is increased in PTU-treated rats, an increase in circulating testosterone concentrations would be expected. However, the circulating concentrations of testosterone are similar in both control and PTU-treated rats (Cooke and Meisami, 1991; Kirby *et al.*, 1992). Testosterone production by Leydig cells depends on LH. However, serum concentrations of LH are significantly lower in PTU-treated rats than in controls at adult ages (Kirby *et al.*, 1992). The circulating concentrations of androgens would be maintained in the presence of low concentrations of circulating LH by alterations in the structure or function of Leydig cells in the PTU-treated rats.

This study was designed to investigate the effects of transient neonatal hypothyroidism, induced by PTU treatment, on the structure and function of the testis interstitium in adult rats. The volume changes in the testis interstitium and its components were determined, together with Leydig cell number, average volume and steroid secretory capacity in adult PTU-treated rats.

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Materials and Methods

Animals and treatments

Sprague–Dawley rats derived from parental stock purchased from Harlan (Indianapolis) were bred and maintained as described by Cooke and Meisami (1991) and Cooke *et al.* (1992). After birth, the sex of pups was determined and they were weighed. Only male pups were used in the study, and litter size was adjusted to 6–8. Pups were weighed every week and maintained in accordance with the NIH Guide for the Care and Use of Laboratory Animals.

Certain litters were randomly designated as controls; control mothers and their pups were untreated and received food and water *ad libitum*. For treated litters, 0.1% (w/v) PTU (Sigma, St Louis, MO) was added to the mother's water beginning immediately after birth. PTU ingested by the mother is transferred to the pups through the milk, resulting in hypothyroidism in the pups (Kirby *et al.*, 1992). The 0.1% PTU used in the present experiments is sufficient to suppress thyroxine maximally during the treatment period (Kirby *et al.*, 1992) and leads to maximal increases in adult testis size (Cooke and Meisami, 1991; Cooke *et al.*, 1993).

For treated litters, food and PTU-containing water were offered *ad libitum*. PTU has a bitter taste, so diet cherry Kool-Aid was added to the PTU-containing water to increase palatability (Cooke and Meisami, 1991). Maternal water consumption by PTU-treated dams was monitored daily from birth of pups to day 25 to ensure adequate maternal water consumption and to prevent dehydration of the dam and pups. At 25 days of age, PTU treatment was stopped and pups were weaned and housed 3–4 per cage. Pups of both control and treated groups were given tap water and food *ad libitum*. Rats were killed by an overdose of inhalation of ether on day 135.

Tissue preparation

At 135 days of age, one testis–epididymis was removed from each control and PTU-treated rat ($n = 5$ group), and placed in ice-cold 0.25 mol sucrose l^{-1} . The epididymis was dissected from the testis, the testis was weighed and the fresh testis volume was calculated as described by Mendis-Handagama and Ewing (1990) and Mendis-Handagama (1992). This testis was then used to determine testosterone secretion under the *in vitro* perfusion conditions described subsequently.

The ipsilateral testis of each rat was fixed and processed for microscopy and morphometry as described by Mendis-Handagama and Ewing (1990) and Mendis-Handagama (1992), using a fixative containing 2.5% glutaraldehyde in 0.1 mol cacodylate buffer l^{-1} (pH 7.4).

Microscopy and morphometry

Sections of 1 μm were made from the blocks of polymerized testis tissue and were stained with modified methylene blue-azure II stain (Dawes, 1980). These sections were viewed under an Olympus BH-2 photomicroscope at $\times 40$ and $\times 100$. Volumes of various testis components, including the seminiferous tubules, interstitium, Leydig cells, blood vessels, lymphatic

space, macrophages and connective tissue cells (i.e. endothelial cells, fibroblasts, peritubular myoid cells and pericytes) were determined as described by Mendis-Handagama *et al.* (1988). In brief the volume density of each testis component (V_v , defined as the volume of each component per unit testis volume) was determined by point counting using an ocular test grid containing 121 test points fitted to the eye piece of the above microscope. The volume of each testis component per testis (V) was calculated by multiplying the volume density by the volume of the testis (V_T).

$$V = (V_v) \times (V_T)$$

The numerical density of Leydig cells (N_v ; defined as the number per unit volume of testis) in control and PTU-treated rats was determined by using the disector method (Sterio, 1984) as described by Mendis-Handagama and Ewing (1990), Mendis-Handagama *et al.* (1990a) and Mendis-Handagama (1992). The number of Leydig cells per testis (N) was obtained by multiplying the numerical density of Leydig cells by the volume of testis.

$$N = (N_v) \times (V_T)$$

The average volume of a Leydig cell (V_{LC}) was calculated by dividing the volume density of Leydig cells (V_{vLC}) by the numerical density of Leydig cells (N_{vLC}).

$$V_{LC} = V_{vLC}/N_{vLC}$$

In vitro perfusion of testis and assay of steroids

Both control and PTU-treated testes were perfused *in vitro* with artificial medium containing ovine LH (100 ng ml^{-1}) as described by VanDemark and Ewing (1963) and Ewing *et al.* (1975). Testicular venous effluent was collected for 4 h to measure the following testicular steroids: testosterone, androstenedione, 17α -hydroxyprogesterone and progesterone. Steroid secretory capacity per Leydig cell was calculated by dividing the amount of steroid secreted per testis by the number of Leydig cells per testis as described by Mendis-Handagama *et al.* (1988).

A Waters Associates Model 6000A pump, U6K injector, 30 cm $\times$ 3.9 mm i.d. Bondapak C_{18} column (Waters Assoc. Milford, MA) and a 7.0 cm $\times$ 2.0 mm i.d. Whatman CO:PELL ODS precolumn (Whatman, Clifton, NJ) were used for the chromatography. Detection of Δ^4 -3-ketosteroids was accomplished by monitoring absorbance at 240 nm with a Spectroflow Model 770 UV analyser (Schoeffel, Westwood, NJ). Peak areas were estimated by a Spectra-Physics (Stirling, NJ) which also carried out the post-run calculations for mass estimation. The HPLC solvents used were tetrahydrofuran and methanol (Photrex grade; T. J. Baker, Phillipsburgh, NJ). Mixtures of the HPLC solvents with double glass distilled water were filtered, vigorously stirred and vacuum degassed for at least 1 h before use.

The separation and measurement of testosterone, androstenedione, 17α -hydroxyprogesterone and progesterone in venous effluents from *in vitro* perfused rat testes were performed by isocratic high-performance liquid chromatography

Fig. 1. Low power photomicrographs of the testis interstitium (INT) of (a) control and (b and c) propylthiouracil (PTU)-treated rats. Leydig cells (arrowheads) are the most abundant cell type in the testis interstitium. Lymphatic space (Ly) was increased after PTU treatment as shown in (b) and (c). ST: seminiferous tubule. Bar represents 50 μ m.

(HPLC) with on-line ultraviolet (UV) absorbance detection (at 240 nm) method as described by Cochran *et al.* (1979). Non-radioactive 11β -hydroxyandrostenedione was used as an internal standard. Prepared samples with internal standard were injected into the chromatograph and eluted with tetrahydrofuran:methanol:water (16:28:56, v:v:v). The sensitivity of the assay was 10 ng for testosterone, androstenedione and 17α -hydroxyprogesterone and 25 ng for progesterone. All of the samples were run in one assay. The intra-assay coefficient of variation was 3.2%.

Statistical analysis

Differences between the means were compared using Student's *t* test. Data were expressed as mean (SEM). Differences were considered significant when $P < 0.05$.

Results

Histology

The perfusion-fixed testicular tissue was well preserved and suitable for morphometric analysis. Tissue relationships were well maintained at the light microscope level (Figs 1, 2), and also at the electron microscope level (results not shown).

In control rats, testicular interstitial tissue contained prominent clusters of Leydig cells often surrounding blood vessels or occupying positions more centrally placed within the interstitial tissue (Figs 1a and 2a). Leydig cells exhibited a variety of shapes including circular, ovoid or irregularly elongated profiles. Leydig cell cytoplasm was densely stained because it

contained many inclusion bodies, which represented mitochondria. This characteristic staining property of Leydig cells was useful in distinguishing Leydig cells from other types of cell in the interstitium of the testis, especially in instances when cytoplasmic fragments of Leydig cell profiles were present without a nucleus. Moreover, cytoplasmic lipid inclusions were not commonly noted in these Leydig cells. The nuclei of these Leydig cells, when present, frequently contained a prominent nucleolus and dense patches of heterochromatin associated with the nuclear membrane. There were fewer macrophages than Leydig cells, and macrophages were readily identified and distinguished from all other cells including Leydig cells, by their pale-staining cytoplasm which contained many cytoplasmic granules and vesicles, indicative of their well-developed lysosomal components associated with the phagocytic function of these cells (Fig. 2). Endothelial cells of blood vessels and lymphatics, fibroblasts, peritubular myoid cells, and pericytes associated with capillaries were collectively identified and quantified as connective tissue cells. A representative testis interstitial area from a control rat is shown (Fig. 1a) and a higher magnification of the same area of the interstitium is presented (Fig. 2a) to demonstrate the various interstitial components quantified.

The basic morphology of the testis interstitium in PTU-treated rats appeared normal, except for the pronounced enlargement of lymphatic space (Figs 1b, c; 2a and 3). The mature Leydig cells present in the testis interstitium were similar in morphology to those described for the control rats. However, in PTU-treated rats, Leydig cell profiles in section were smaller than those in controls (Figs 1 and 2). In addition to mature Leydig cells, small numbers of immature Leydig cells were also observed in the testis interstitium of PTU-treated

Fig. 2. Higher power photomicrograph of the testis interstitium shown in Fig. 1(a) (control) and (b) (propylthiouracil (PTU)-treated rats). Arrows without labels depict Leydig cells. Note that the Leydig cell profiles are smaller in PTU-treated group compared with controls. m: macrophages, ct: connective tissue cells; bv: blood vessels, Ly: lymphatic space; ST: seminiferous tubules. Bar represents 20 μ m.

rats (Fig. 3). By comparison with adult/mature Leydig cells of both control and PTU-treated rats, these immature Leydig cells exhibited a very different morphology and were similar in morphology to Leydig cells in the fetal/neonatal testis (Lording and de Kretser, 1972; Mendis-Handagama *et al.*, 1987). The most distinct feature was the presence of an abundant supply of cytoplasmic lipid droplets (Lording and de Kretser, 1972; Mendis-Handagama *et al.*, 1987; Fig. 3), a feature not commonly observed in the adult rat Leydig cells. The shapes of these immature Leydig cells varied; elongated, ovoid and circular profiles were observed. These immature Leydig cells were frequently observed in association with the peritubular tissue (Fig. 3).

The morphology and location of macrophages were similar in control and PTU-treated rats. Moreover, connective tissue cells in the testis interstitium of PTU-treated rats showed no difference from those in control testes.

Morphometry

Average fresh testis volume in control and PTU-treated rats was 1970 mm³ (SEM = 110) and 3630 mm³ (SEM = 275), respectively, corresponding to an 84% increase in volume compared with controls. The absolute volumes of seminiferous

Fig. 3. Immature Leydig cells (double arrows) in the testis interstitium of propylthiouracil (PTU)-treated rats. Their cytoplasm is abundant with lipid droplets, a feature not observed in adult Leydig cells of rats. Note that in (a) the shape of the cell is somewhat elongated. Moreover, these immature cells are frequently located adjacent to the peritubular tissue. LC: Leydig cells, Ly: lymphatic space, bv: blood vessels, m: macrophages. Bar represents 20 μ m.

tubules, testis interstitium, Leydig cells, blood vessels, lymphatic space and connective tissue cells were significantly greater in PTU-treated rats than in controls (Table 1). By contrast, the absolute volume of macrophages was not significantly different between control and PTU-treated rats (Table 1). The percentage volume increases of the tubular compartment (i.e. seminiferous tubules) and intertubular compartments (i.e. interstitium) were 80 and 86%, respectively. These increases in volume percentages are similar to the percentage increase in testis volume (84%) after PTU treatment. However,

Table 1. Absolute volumes of testis components in 135-day-old control and propylthiouracil (PTU) treated rats

Tissue	Control (mm ³)	PTU-treated ^a (mm ³)	Increase in volume (%) in PTU-treated rats
Seminiferous tubules	1776 (88)	3198 (228)*	80
Interstitial	206 (21)	383 (31)*	86
Leydig cells	44 (4)	61 (6)*	39
Blood vessels (lumen)	30 (4)	49 (8)*	63
Lymph	81 (6)	200 (21)*	147
Connective tissue cells ^b	46 (3)	67 (4)*	46
Macrophages	5 (0.9)	7 (1.7)	40

^aPTU-treated from birth to 25 days.

^bFibroblasts, endothelial cells, peritubular myoid cells, pericytes.

Data are shown as mean (SEM), and *n* = 5 rats per group.

*Significant differences between treatment groups (*P* < 0.05).

Table 2. Number of Leydig cells and average volume in control and propylthiouracil (PTU) treated rats at 135 days

Parameter	Control	PTU-treated ^a
Number of Leydig cells per testis (× 10 ⁶)	26 (2)	50 (5)*
Average volume of a Leydig cell (μm ³)	1642 (28)	1228 (11)*

^aPTU-treated from birth to 25 days.

Data are shown as mean (SEM), and *n* = 5 rats per group.

*Significant differences between treatment groups (*P* < 0.05).

the percentage volume increases of components of the testis interstitium were different among components (Table 1), the greatest increase (147%) occurring in the lymphatic space.

The mean number of Leydig cells per testis showed an 84% increase in PTU-treated rats compared with controls (Table 2). However, the average volume of Leydig cells in PTU-treated rats was 25% lower than that of controls.

No significant differences were observed between control and PTU-treated groups for LH-stimulated secretion of testosterone, androstenedione, 17α-hydroxyprogesterone or progesterone (Fig. 4). Nevertheless, steroid secretion per Leydig cell was significantly lower in PTU-treated rats than in controls (Fig. 5).

Discussion

The study reported here demonstrates that transient neonatal hypothyroidism in rats causes pronounced changes in the number and volume of Leydig cells and volumes of other interstitial components, namely, blood vessels, connective tissue cells and the lymphatic space at maturity. These findings are consistent with observations that transient neonatal hypothyroidism causes an unprecedented increase in adult testis size and sperm production (Cooke *et al.*, 1991 and 1992; Kirby *et al.*, 1992). The magnitude of the increase in volumes of testis interstitium (86%) and seminiferous tubules (80%) is similar to the overall increase in testis volume in PTU-treated

Fig. 4. LH-stimulated steroid secretion per testis per hour of *in vitro* perfused testes of (□) control and (■) propylthiouracil (PTU)-treated rats. Testosterone (T), androstenedione (Androst), 17α-hydroxyprogesterone (17-OH-P) and progesterone (Prog) secretory capacity per testis is not significantly (*P* > 0.05) different between control and propylthiouracil (PTU)-treated rats.

rats (84%), indicating that the unprecedented increase in testis size after PTU treatment results from equal increases in both the tubular and intertubular compartments of the testis. Nevertheless, different components of the testis interstitium showed different volume changes.

The volume of the lymphatic space showed the largest increase (approximately 2.5-fold) of all components in the testis interstitium in the PTU-treated rats. This increase in lymphatic space following PTU treatment may be related to the increase in seminiferous tubule cross-sectional area and to the greater total number of Sertoli cells (Van Haaster *et al.*, 1992; Hess *et al.*, 1993). Because the treated Sertoli cells appear to function normally, their increase in number along the basement membrane would probably cause an increase in

Fig. 5. LH-stimulated steroid secretion per Leydig cell per hour of (□) control and (■) propylthiouracil (PTU)-treated rats. *Testosterone (T), androstenedione (Androst), 17 α -hydroxyprogesterone (17-OH-P) and progesterone (Prog) secretory capacity per Leydig cell is significantly greater ($P < 0.05$) in controls compared with PTU-treated rats.

the secretion of fluid both proleminally and into the interstitium. Hess *et al.* (1993) showed that on a Sertoli cell basis, abundance of mRNA for androgen binding protein (ABP) was similar in control and PTU-treated testes. Thus, if 20% of the ABP is secreted into the interstitium (Gunsalus *et al.*, 1980) and there is an increased number of Sertoli cells, it is probable that there is an overall increase in the amount of fluid secreted by seminiferous tubules. Although the average volume of Leydig cells was lower to maintain normal concentrations of serum testosterone following PTU treatment, the testis appears to have responded to a greater number of Sertoli and germ cells by greater volumes of both the seminiferous tubules and the lymphatic system, possibly to accommodate an increase in fluid secretion.

The study reported here demonstrated for the first time a two-fold increase in the number of Leydig cells per testis in PTU-treated rats compared with that of controls. Increases in the number of Leydig cells were observed after chronic LH treatment (Christensen and Peacock, 1980). However, studies have shown that serum LH concentrations in PTU-treated rats were significantly lower than in controls (Kirby *et al.*, 1992), showing that LH is not the cause of Leydig cell hyperplasia in the PTU-treated rat. Leydig cell hyperplasia was also observed in many instances of seminiferous tubule damage, such as in experimental cryptorchidism (Mendis-Handagama *et al.*, 1990b). This situation in part could be explained by the presence of mitogenic activity in testicular interstitial fluid, which has been shown to increase after seminiferous tubule damage (Drummond *et al.*, 1987). In the study reported here, we observed Leydig cell hyperplasia without any visible seminiferous tubule damage; testicular sperm production is increased by almost 140% in PTU-treated rats compared with controls (Cooke *et al.*, 1991, 1992;

Kirby *et al.*, 1992), which confirms this observation. Moreover, studies *in vitro* have shown that growth factors that are known to be produced in the testis *in vivo* can affect the proliferative activity of Leydig cells (Khan *et al.*, 1992). It is not known whether the transient neonatal hypothyroidism increases the mitotic activity of testicular interstitial fluid or whether any growth factors could induce Leydig cell proliferation. However, it was shown that hypothyroidism causes proliferation of types of connective tissue cell such as fibroblasts, in the testis interstitium (Amin and El-Sheikh, 1977) in neonatal rats. It is therefore likely that in the present study the transient neonatal PTU treatment may have resulted in more Leydig cell precursors in prepubertal testes of PTU-treated rats than in controls. This situation may allow the PTU-treated rats to have significantly greater numbers of Leydig cells at maturity, because Leydig cells have been shown to differentiate from connective tissue cell precursors during development (Lording and de Kretser, 1972; Christensen, 1975), and after ethane dimethane sulfonate (EDS) treatment (Kerr *et al.*, 1985; Jackson *et al.*, 1986).

The presence of immature Leydig cells, which were very similar in morphology to fetal Leydig cells in the testis interstitium of PTU-treated rats, suggests that at least one mechanism for increasing the number of Leydig cells in PTU-treated rats may be similar to the differentiation pattern that is observed in fetal testes and the testes of EDS-treated rats, that is, differentiation of connective tissue cell precursors to Leydig cells via intermediate stages. Among different types of connective tissue cells in the testis interstitium, peritubular cells were also shown to be precursors of Leydig cells in several studies on pubertal maturation (de Kretser, 1967; Fawcett, 1973; Christensen, 1975; van Straaten and Wensing, 1978) as well as after EDS treatment (Kerr *et al.*, 1985; Jackson *et al.*, 1986). This contention is also supported by the observation that interstitial cells suggested to be Leydig cell precursors were noted within the peritubular tissue of human testes in cases of cryptorchidism (Mori *et al.*, 1978). On the basis of these observations, it is believed that newly differentiated Leydig cells may arise at least partly from peritubular cells. They subsequently leave the lamina propria to gain access to the surrounding interstitial tissue. Observations of immature Leydig cells adjacent to the peritubular region in the PTU-treated rat testes in the study reported here support this view. However, the possibility cannot be ruled out that there is differentiation of other types of connective tissue cell in the testis interstitium into Leydig cells.

An increase in the number of Leydig cells could also occur as a result of division of existing Leydig cells, although the rate of proliferation is very low (Teerds *et al.*, 1989). The occurrence of Leydig cell mitosis in mammalian testes ranges between 1 in 17 500 (Mendis-Handagama, 1991) and 1 in 20 000 (Christensen and Peacock, 1980), and it is believed that a mitotic figure in a Leydig cell lasts less than an hour (Christensen and Peacock, 1980). This explains why it is extremely difficult to observe Leydig cells in mitosis. However, it is possible to induce proliferation of Leydig cells in adult testes by treating rats daily with hCG, which results in a significant increase in the number of Leydig cells that have incorporated [³H]thymidine and the number of mitotic figures (Teerds *et al.*, 1988). Although Leydig cells in mitosis were not

observed in PTU-treated rats in this study, it is possible that proliferation of differentiated Leydig cells increases the number of Leydig cells in PTU-treated rats.

The study reported here clearly shows, for the first time, that the LH-stimulated secretion of testosterone as well as of other steroids (androstenedione, 17 α -hydroxyprogesterone and progesterone) by the *in vitro* perfused testes of control and PTU-treated 135-day-old rats was the same, despite the fact that the testes of PTU-treated rats at this particular age contained twice the number of Leydig cells. These findings are consistent with the previous observations that testosterone concentrations in serum were not different in adult control and PTU-treated rats up to 180 days of age (Cooke and Meisami, 1991; Kirby *et al.*, 1992). Although the total number of Leydig cells was almost double in PTU-treated rats compared with controls, these cells could not produce a twofold increase in steroid concentrations compared with controls. This observation suggests that individual Leydig cells in PTU-treated rats were less differentiated in secreting steroids than were the Leydig cells in controls. It is well known that thyroid hormone is essential for cellular differentiation (Guyton, 1991). However, although PTU-treated rats were hypothyroid during the neonatal–prepubertal period, they had normal thyroid hormone concentrations at maturity.

The average volume of Leydig cells has a positive and linear correlation with its testosterone secretion (Mendis-Handagama *et al.*, 1988) and the observation of a substantial reduction in the average volume of Leydig cells is apparently the cause of the reduced steroid secretion. This finding is similar to the situation in Leydig cells in the experimentally cryptorchid mouse (Mendis-Handagama *et al.*, 1990a), in which Leydig cell hyperplasia occurs together with hypotrophy. Moreover, Leydig cell hypotrophy of PTU-treated rats was associated with a reduced capacity of Leydig cells to secrete androgens, similar to observations with cryptorchid mouse Leydig cells (Mendis-Handagama *et al.*, 1990a). The reduced volume of individual Leydig cells after PTU treatment may partly be explained by the lower concentration of LH in serum (Kirby *et al.*, 1992), which could prevent these cells from reaching their normal size and maximal secretion. The exact cause of lower LH concentrations in serum of PTU-treated rats is not known. Since the total steroidogenic capacity of the testes is maintained following PTU treatment, it is clear that this is achieved via increasing the number of Leydig cells per testis and thereby acquiring a similar quantity of cytoplasmic organelles required for steroid biosynthesis. Since close relationships have been shown between testosterone secretion and some Leydig cell organelles such as the smooth endoplasmic reticulum (Zirkin *et al.*, 1980; Mendis-Handagama *et al.*, 1988) and peroxisomes (Mendis-Handagama *et al.*, 1988), it would be appropriate to examine the organelle complement of both the control and PTU-treated rat Leydig cells to test this hypothesis.

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