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ORIGINAL ARTICLE

Neonatal hypothyroidism-induced changes in rat testis size; dependence on temperature

S. K. Lagu, N. G. Bhavsar, R. K. Sharma & A. V. Ramachandran

Division of Reproductive Biology and Endocrinology, Department of Zoology, Faculty of Science, M. S. University of Baroda, Vadodara.390 002, Gujarat, India.

Correspondence to: Prof. A. V. Ramachandran
Division of Reproductive Biology and Endocrinology,
Department of Zoology, Faculty of Science,
M. S. University of Baroda,
Vadodara.390 002
Gujarat, INDIA
PHONE: No. +91-265-2780455, +91-265-2791898
EMAIL: av_rama@rediffmail.com
avrcn@rediffmail.com
lagusonali@hotmail.com

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Abstract

OBJECTIVE: Effects of transient neonatal hypothyroidism (HPOT) on adult testis size and serum hormone profiles were evaluated in the Charles foster strain of rats, maintained under the temperature of 21°C (HPOTL) or of 34°C (HPOTH).
METHODS: Hypothyroidism was induced in suckling pups in preweanling period (1–21 days), by administering 0.1% 6-propyl-2-thiouracil in drinking water to mothers. Body mass and testis characteristics, the steroidogenic potential [measured by histochemical localization of 3α-, 3β- and 17β-hydroxysteroid dehydrogenases (HSDHs)] and serum concentrations of thyroid-stimulating hormone (TSH), triiodothyronine (T3), thyroxine (T4), luteinizing hormone (LH), testosterone (T) and corticosterone (Cort) were evaluated on the 35th, 45th, 60th and 90th day of age.
RESULTS: The HPOTH rats showed lower testis masses, while the HPOTL rats showed higher testis masses and lower body masses after 90 days. Histologically, the testes of the HPOTH rats demonstrated increased germ cell degeneration after 35 and 45 days and reduced tubular size, germ cell numbers and sperm density after 90 days. In contrast, the testes of the HPOTL rats showed reduced tubular diameters after 35 and 45 days, and increased tubular diameters, germ cell numbers and sperm density after 90 days. Serum TSH, T3, T4, LH and T concentrations and 3β- and 17β-HSDH activities were reduced in both groups of the HPOT rats after 35 and 45 days. Intratubular steroidogenesis and a prominent Δ5 pathway were also found in the HPOT animals.
CONCLUSION: The temperature has a definite influence on the thyroid hormone action, postnatal growth and function of rat testes.

Abbreviations:
3α HSDH – 3α hydroxysteroid dehydrogenases
3β HSDH – 3β hydroxysteroid dehydrogenases
17β HSDH– 17β hydroxysteroid dehydrogenases
TSH – Thyroid-stimulating hormone
T3 – Triiodothyronine
T4 – Thyroxine
LH – Luteinizing Hormone
T – Testosterone
PTU – 6-Propyl-2-thiouracil
C – Control
HPOT – Hypothyroid; hypothyroidism
CL – Control (Low temperature)
CH – Control (High temperature)
HPOTL – Hypothyroid rats (Low temperature)
HPOTH – Hypothyroid rats (High temperature)
HHA – Hypothalamo-hypophyseal-adrenal

Introduction

There have been many attempts to relate thyroid hormones to the development and functions of the testes. Some early studies showed the growth of testis and epididymis to be only slightly retarded in hypothyroid (HPOT) rats [l, 2], but some later reports demonstrated that thyroidectomy, performed in immature male rats, can cause a severe inhibition of spermatogenesis and of Leydig cell development [3, 4]. Thyroidectomy or goitrogen treatment was reportedly ineffective on the function of testes in adult rats [5, 6]. However, Baksi [7] showed degenerative changes in the testis, due to hypothyroidism (HPOT). Recently, differential effects on testis development have been shown by different schedules of goitrogen treatment. Accordingly, chronic HPOT, induced by methimazole administration from birth to puberty, caused a reduction in diameters of the seminiferous tubules and their delayed maturation with a reduced number of germ cells, leading to reduced final testis size [3, 8–12]. In contrast, thyroidectomy, performed in one month old pre-pubertal rats, or goitrogen, administered in post-pubertal periods, did not affect either testis growth or fertility [4, 5, 13, 14]. However, an induction of transient HPOT during the first three weeks after birth by an administration of propylthiouracil (PTU) – a goitrogen – was shown to increase the size of the testes and of the reproductive organs [11, 15–23], enhance sperm production [20, 23], and increase Sertoli and germ cell numbers [24, 25] in adult animals. These reports are based on studies, conducted on Long-Evans and Sprague-Dawley strains of rats, maintained under a light:dark photoperiod (LD) schedule of 12:12 (LD 12:12) and temperature of 21°C. Since no study has yet been reported to gauge the effect of either the photoperiod or temperature on HPOT, the present study was planned, involving two groups of animals; one group maintained in temperature of 34 ± 2°C and the other one – in temperature of 21 ± 2°C, under similar photoperiods of LD 8:16 and subjected to transient neonatal HPOT.

Materials and methods

2.1. Procurement of rats

Mated female rats of Charles foster strain were purchased from the Sarabhai Research Centre, Baroda, and were maintained in a well-ventilated animal house. They were adapted to a photoperiod of 8:16 LD and maintained at temperature of either 34 ± 2°C or 21 ± 2°C. The animals were given standard food and water ad libitum. When the mated females delivered pups, the males and females were separated and experimental schedules were started in the male pups. Efforts were made to maintain the litter size at constant level in order to minimize the variations related to the number of pups per rat mother. Throughout the experiment, the animals were maintained under the above mentioned environmental regimen. Replicates of all the experiments were parallely conducted.

2.2. Experimental Set-up:

The neonatal pups were divided into the following groups:

Groups 1 and 2 (C):

Newborn rat neonates, maintained as control (C), under 8:16 LD and temperature of 21 ± 2°C (CL) or 34 ± 2°C (CH), given normal drinking water.

Groups 3 and 4 (HPOTL and HPOTH):

Newborn rat neonates, subjected to transient HPOT by feeding mothers with 0.1% 6-propyl-2-thiouracil (PTU) in drinking water for 21 days of the pre-weanling period and maintained under 8:16 LD and temperature of 21 ± 2°C (HPOTL) or 34 ± 2°C (HPOTH).

2.3. Parameters and Methods of Evaluation

Particular treatments were discontinued from the 22nd day, and after 35, 45, 60 and 90 days of age; the rats from all the groups were weighed. Six rats from each group were weighed and sacrificed under mild anaesthesia, taking maximum care to minimize any stress while handling. The viscera were cut open and the testes were excised, blotted free of tissue fluids and weighed up on a Mettler balance with 0.0l mg accuracy. The absolute weights, so obtained, were converted to relative weights and expressed as the percentages of body weights.

2.3.1. Histochemical localization

Steroid dehydrogenases: The testis was excised immediately after decapitation under mild anaesthesia and transferred to a cryostat microtome, maintained at –20°C. Fresh frozen sections, 15–20 μm thick, were cut, put on a clean slide and thawed. 17β-hydroxysteroid dehydrogenase was localized, by the method of Kellog and Glenner [26], employing testosterone as substrate. 3β-hydroxysteroid dehydrogenase was localized, according to the method of Wattenberg [27], using dehydroepiandrosterone (DHEA) and pregnenolone (P) as substrates, while 3α-hydroxysteroid dehydrogenase was histochemically localized, following the method of Balough [28], using androsterone (A) as

substrate. Incubation of the sections was carried out at 42°C. Nicotinamide-adenine dinucleotide (NAD) was used as coenzyme and nitro-blue tetrazolium (NBT) salt as hydrogen acceptor. The stained sections were thoroughly washed in distilled water and fixed in 10% formalin for 15 min., washed again in distilled water and mounted in glycerin jelly. In all the histochemical observations, the tissue sections, incubated in substrate blank medium, served as controls.

2.3.2. Histology

The testes were fixed immediately in Bouin's fluid and processed for histological studies. Paraffin 5 µm-thick sections were cut on a microtome and stained with Haematoxylin-Eosin (HE). The diameters of seminiferous tubules were measured, using an ocular micrometer and expressed in µm. Numerical densities of Leydig and Sertoli cells (no. of cells/mg testis tissue) were obtained by Floderus equation [29]. The total count was obtained, using the following equation:

$$\mathbf{Nr = Na/(D+T -2h).}$$

Nr – Numerical density
Na – The No. of cell nuclei in a unit area of tissue section
D – The average diameter of a cell nucleus
h – The height of the smallest recognizable section of cell nuclei
T – Section thickness

Since **h** was assumed to be 0.1 x nuclear diameter [30], **D** was obtained by measuring its long and short axes.

2.3.4. Hormone Assays

The blood for hormone assays was collected from the brachial vein before sacrificing the animals. Triiodothyronie (T3) and thyroxine (T4) concentrations were assayed by ELISA, using a kit, purchased from Glaxo (product code H-T3H-0010 and H-T4H-0010) and expressed in ng/ml of serum.

Rat thyroid-stimulating hormone (TSH) (NIDDK-rTSH-I-9) and LH (NIDDK-rLH-I-7) were iodinated by the chloramine T method with carrier free 125I, obtained from the Amersham International plc, as described before [31].

Pure rat hormones (2.5 µg) were incubated with chloramine T in concentrations of 5 µg for TSH and 4 µg for LH, for 45 s. The reaction was stopped with 20 µl sodium metabisulphite (80 µg), and purified in a PD-10 column, presaturated with barbitone buffer (0.07 M) and precoated with BSA (1%) (Pharmacia LKB Biotechnology, Bromma, Sweden), eluted in barbitone buffer, and tubes, with the peak specific activity of 60–80 µCi/µg, were used for RIA.

RIA of TSH and LH: Peptide hormones were measured in liquid-phase RIA, using specific antibodies and reference preparations from NIDDK. There were two types of used antisera: anti-rTSH-S-5, and anti-rLH-S-I0. The hormones and antisera were dissolved in peptide assay buffer (pH 7.4), containing sodium dihydrogen phosphate (3.05 g/l), sodium chloride (8.8 g/l), sodium azide (100 mg/l) and BSA (5 g/l). EDTA (0.025 M) was also added. The tracer was diluted in the peptide assay buffer, containing 0.5% normal rabbit serum, to approximately 20,000–30,000 c.p.m/l00 µl tracer. Anti-rabbit γ-globulin (ARGG) in peptide assay buffer (l:26) and 8% polyethylene glycol (PEG) in saline were used for the separation of bound and free hormones.

200 µl of each antiserum, a sample and a tracer in 600 µl assay buffer were incubated for 24 h at 4°C, followed by an addition of 100 µl ARGG, to continue the incubation for another 24 h. At the end of the second incubation, l ml of PEG was added and centrifuged at 1500 g for 45 min. Radioactivity in the pellet was counted for 1 min in a microprocessor-based LKB gamma counter. The sensitivity of the assays was as follows: for TSH – 0.01 ng/ml and for LH – 0.14 ng/ml. Interassay variations were: for TSH – 8.2% and for LH – 9.9%. Intra-assay variations were: for TSH – 4.7–6.9% and for LH – 4.9–8.4%. The antibodies were highly specific to respective rat antigens with very minimal cross-reactivity with other peptides, as per NIDDK specifications. The maximum binding of the antibodies, recorded by us, was 39% for TSH and 32% for LH.

RIA of Testosterone: Testosterone was assayed by liquid-phase RIA, using the protocol and reagents from the World Health Organisation. The maximum binding of the testosterone antiserum was 40% and its cross-reactivity to cortisol, dehydrotestosterone, androstenedione and Δ5-androstenediol was 0.001%, 14%, 0.8% and 6%, respectively. The interassay and intra-assay variations were 6% and 4%, respectively. The sensitivity of the assay was 0.3 pg/ml.

2.4. Statistical Analysis

All the data have been expressed as means ± SEM. The data were analysed by Student's t-test and the two-way analysis of variance (ANOVA) with 95% confidence limit.

Results

Body and Testis masses

The changes in body mass and in the absolute and relative masses of testes in all the four groups of animals are given in Table I. Whereas body mass of the HPOTL rats remained significantly lower throughout the experiment, when compared to that of CL rats, HPOTH rats showed growth catch up between 60 and 90 days to reach the mass of the CH rats. The absolute and relative masses of testes of the HPOTL rats showed a significant increase between 60 and 90 days and, after 90 days, they were higher than those in CL rats, while in HPOTH rats, the values of those parameters remained significantly lower than those in CH rats at each time point.

Table I: Chronological alterations in body mass (g) and in the absolute (g) and relative masses (g/100 g) of testes in Control and Hypothyroid rats.

Treatment	**Body Mass**				Testis Mass				Relative Testis Mass			
	------- Age in days -------				------- Age in days -------				------- Age in days -------			
	35	45	60	90	35	45	60	90	35	45	60	90
C_L	85.513 ± 1.902	117.16 ± 0.307	193.66 ± 5.493	322.49 ± 4.078	0.803 ± 0.064	1.280 ± 0.043	2.28 ± 0.050	3.067 ± 0.063	0.944 ± 0.025	1.09 ± 0.040	1.178 ± 0.073	0.933 ± 0.039
$HPOT_L$	37.83^c ± 2.242	74.67 ± 0.760	164.2^b ± 0.544	287.3^b ± 10.02	0.232^c ± 0.031	0.750^c ± 0.019	2.215 ± 0.169	4.765^c ± 0.117	0.613^c ± 0.046	1.011^a ± 0.028	1.340^a ± 0.050	1.522^c ± 0.061
C_H	58.20 ± 0.254	53.20 ± 0.123	89.70 ± 3.588	120.34 ± 4.184	0.477 ± 0.022	0.727 ± 0.032	1.397 ± 0.083	2.438 ± 0.123	0.83 ± 0.006	1.37 ± 0.091	1.56 ± 0.102	2.03 ± 0.161
$HPOT_H$	19.76^c ± 0.057	24.50^c ± 0.115	32.50^c ± 0.097	120.13 ± 0.182	0.080^c ± 0.006	0.149^c ± 0.054	0.344^c ± 0.054	1.714^c ± 0.096	0.408^c ± 0.031	0.608^c ± 0.064	1.058^c ± 0.092	1.423^b ± 0.102

C_L – Control (Low temperature), **C_H** – Control (High temperature), **$HPOT_L$** – Hypothyroid rats (Low temperature), **$HPOT_H$** – hypothyroid rats (High temperature).
Values expressed as mean ± SEM of six animals. $^a p < 0.05$, $^b p < 0.005$, $^c P < 0.0005$

Table II: Histometric enumerations of seminiferous tubules in Control and hypothyroid rats on the 90th day of experiment.

Treatment	T_v in cc	S_D in cm	S_L in cm	bm in cm^2	SC_N x 10^6	LC_N x 10^6	TGC_T x 10^6	TGC_M x 10^6
C_L	1.503 ± 0.030	0.0279 ± 0.0006	2321.03 ± 94.200	204.045 ± 5.230	32.49 ± 1.800	29.61 ± 1.4	311 ± 6.300	13.39 ± 0.260
$HPOT_L$	2.176^c ± 0.100	0.035^c ± 0.0014	2197 ± 65.200	238.89^c ± 2.600	65.42^c ± 1.500	35.38 ± 1.35	494.0^c ± 8.600	22.48^c ± 0.390
C_H	1.023 ± 0.009	0.971 ± 0.008	1579.34 ± 63.489	138.84 ± 2.651	21.61 ± 1.125	12.82 ± 0.087	206.85 ± 4.689	9.11 ± 0.186
$HPOT_H$	0.782 ± 0.003	0.742 ± 0.009	789.22 ± 32.952	85.79 ± 0.976	19.2 ± 0.0863	13.28 ± 0.0938	144.98 ± 3.571	8.07 ± 0.097

C_L – Control (Low temperature), **C_H** – Control (High temperature), **$HPOT_L$** – Hypothyroid rats (Low temperature), **$HPOT_H$** – hypothyroid rats (High temperature).
Values expressed as mean ± SEM of minimum fifteen observations. $^a p < 0.05$, $^b p < 0.005$, $^c P < 0.0005$
T_V – Volume of Testis, **S_D** – Seminiferous tubule diameter, **S_L** – Length of seminiferous tubule, **bm** – basement membrane area of the seminiferous tubule, **SC_N** – Total Sertoli cell number in testis, **LC_N** – Total Leydig cell number in Testis, **TGC_T** – Theoretical germ cell number per testis, **TGC_M** – Theoretical germ cell number per meter of seminiferous tubule.

Histology and Histometry

Low Temperature Regimen: In the HPOT animals, after an initial retardation in the spermatogenic process, marked by a smaller number of meiotic stage germ cells, it was fully established after 45 days, marked by the appearance of spermatids. Interstitial Leydig cells were also relatively well formed. After 60 and 90 days, the HPOTL animals showed prominently enlarged tubules with fully established spermatogenesis with a higher number of germ cells and higher sperm density. Leydig cells were very prominent.

Histometric calculations revealed the testicular volumes, the tubular diameters, the thickness of the germinal epithelium, seminiferous tubular volumes, the total basement membrane area, the total Sertoli germ and Leydig cell numbers to be significantly increased in the HPOTL rat testes (Table II).

High Temperature Regimen: The testes of the HPOTH animals showed pronounced germ cell degeneration with concentration of Leydig cells after 35 days, compared to respective values in corresponding control testes. After 45 days, there was a noticeable recovery with an appearance of zygotene spermatocytes in the HPOTH animals, though the CH testes showed a progression of spermatogenesis up to the spermatid stage. The progression of spermatogenesis was poor after 60 days and the sperms appeared after 90 days only, when compared to the fully established spermatogenesis in CH after 60 days. There was also a significantly smaller density of germ cells and Leydig cells in the HPOTH testes.

All the histometric features, like the testis and tubular volumes, the seminiferous tubular lengths, the basement membrane area and the total number of Sertoli, germ and Leydig cells were all significantly lower in the CH animals, compared to respective values in the controls. The testes of the HPOTH animals still showed a significant decrease in size (Table II).

Histochemical Observations

Except for a slightly higher activity in low temperature, there was no other difference with reference to the pattern of enzyme activity between CL and CH animals. In general, 3β-HSDH activity expressed itself in Leydig cells after 35 days, with DHEA as a substrate. The enzyme activity increased gradually thereafter to an optimum level after 60 to 90 days. 17β-HSDH was strongly expressed in the tubules after 35 and 45 days

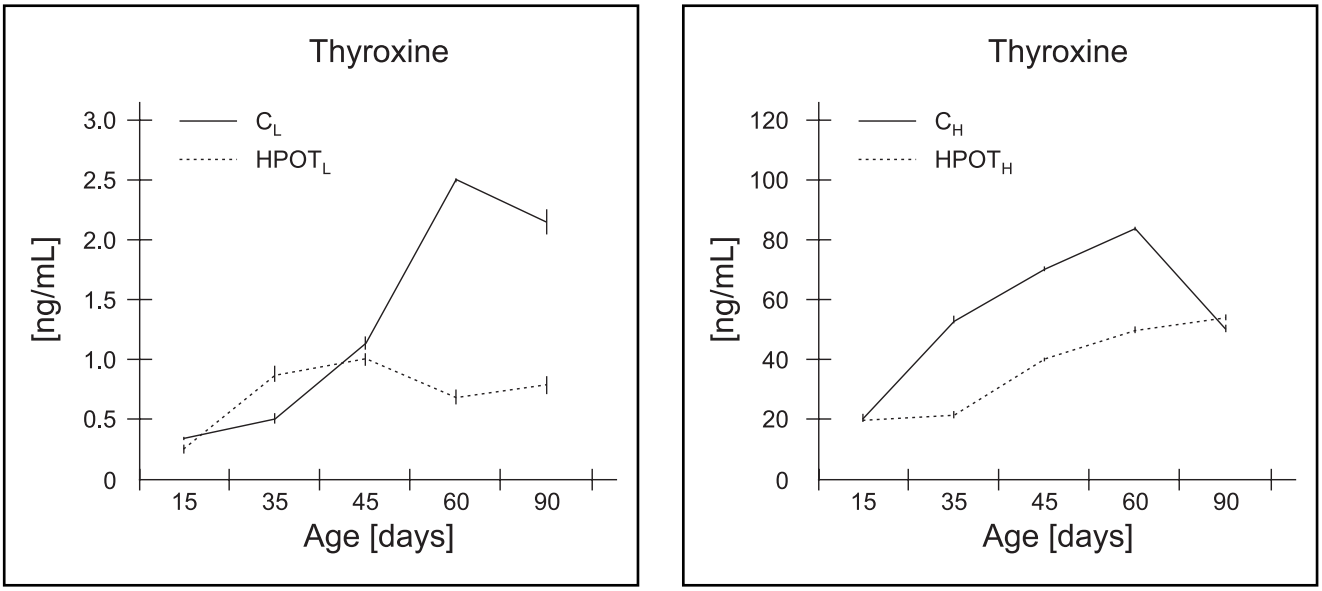

Figure 1. Chronological alterations in serum T4 levels (ng/ml) in control and hypothyroid rats. ✻– vs. L-T4-treated group, p<0,05;

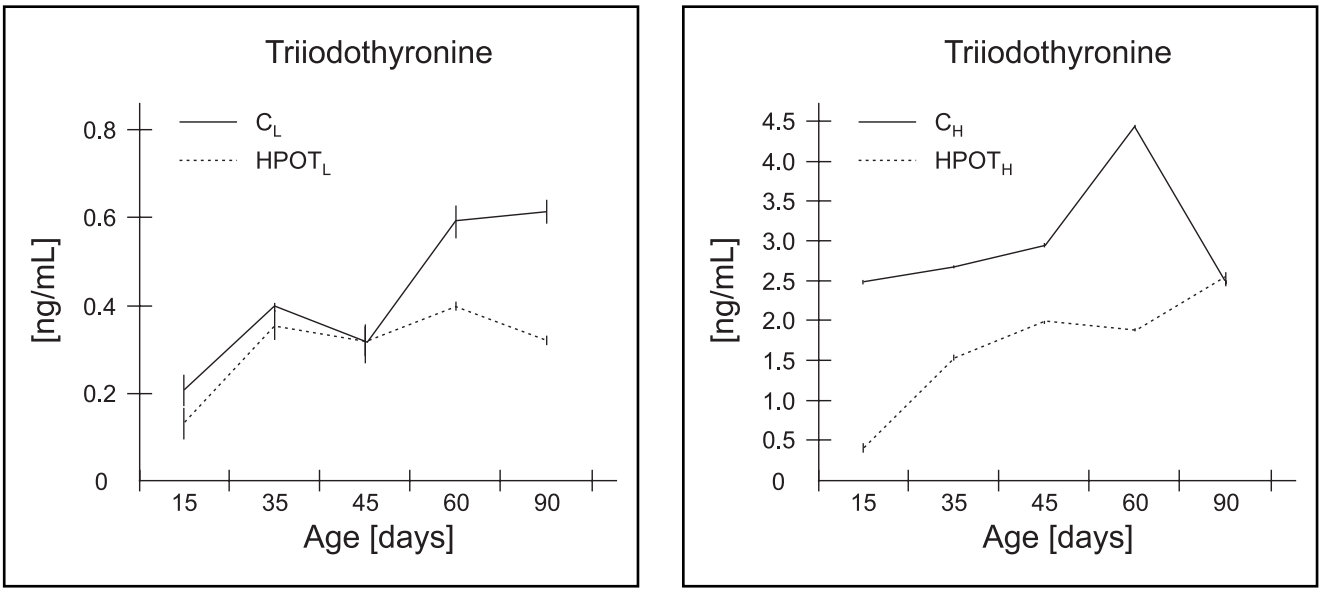

Figure 2. Chronological alterations in serum T3 levels (ng/ml) in control and hypothyroid rats.

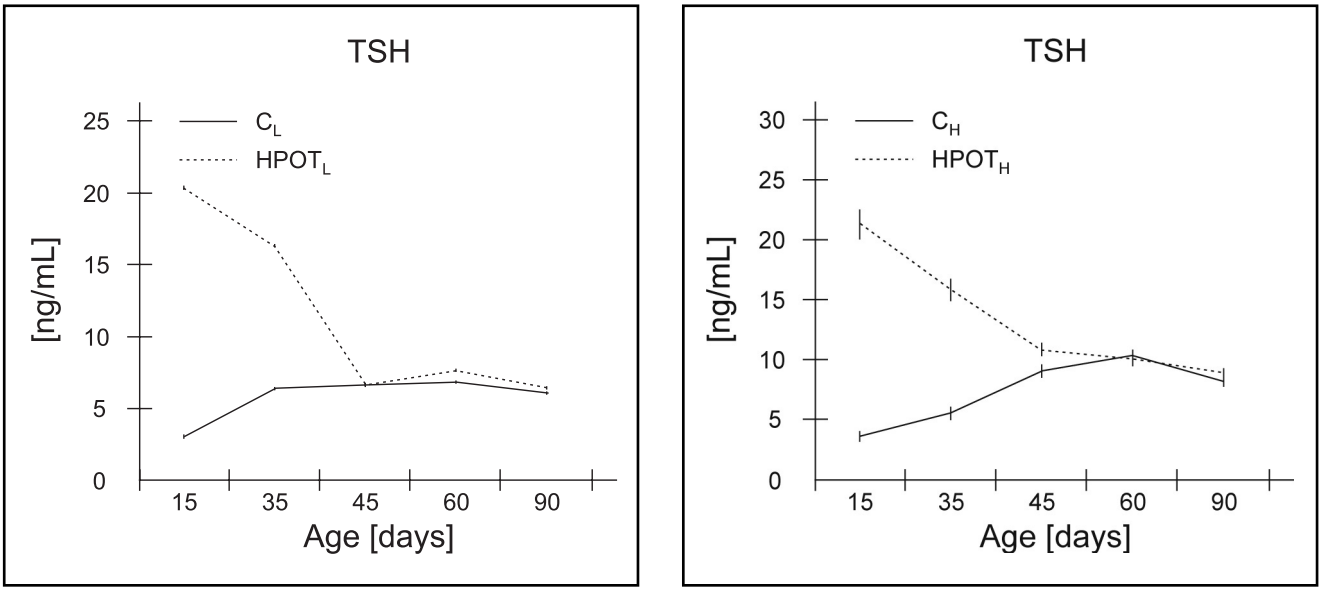

Figure 3. Chronological alterations in serum TSH levels (ng/ml) in control and hypothyroid rats.

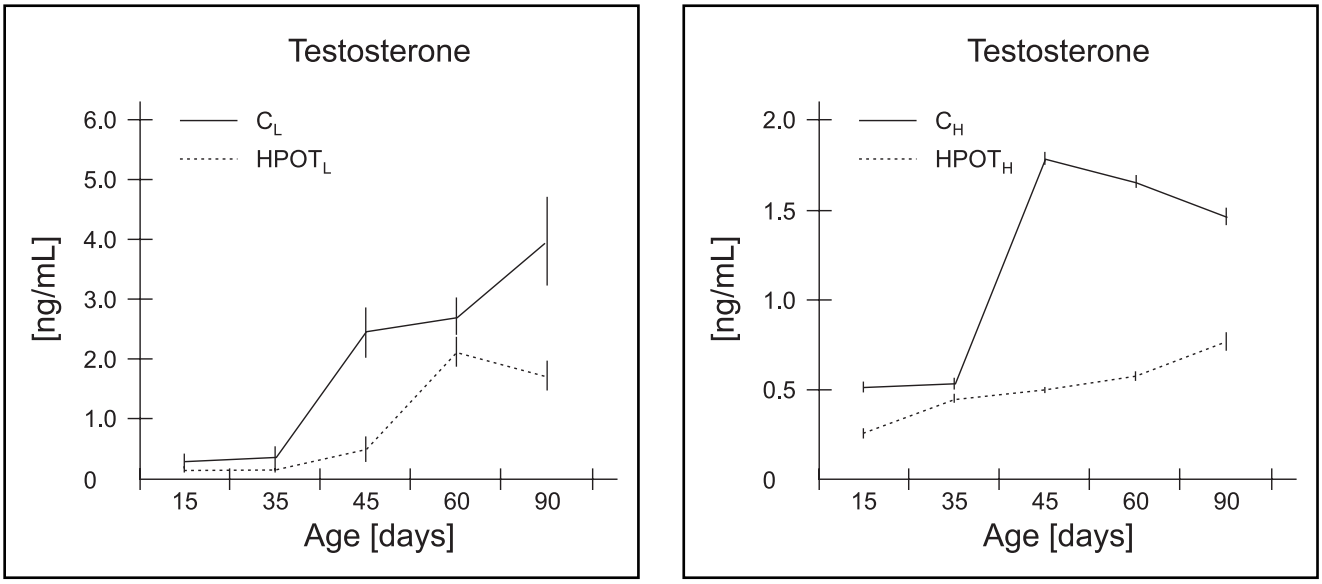

Figure 4. Chronological alterations in serum Testosterone levels (ng/ml) in control and hypothyroid rats.

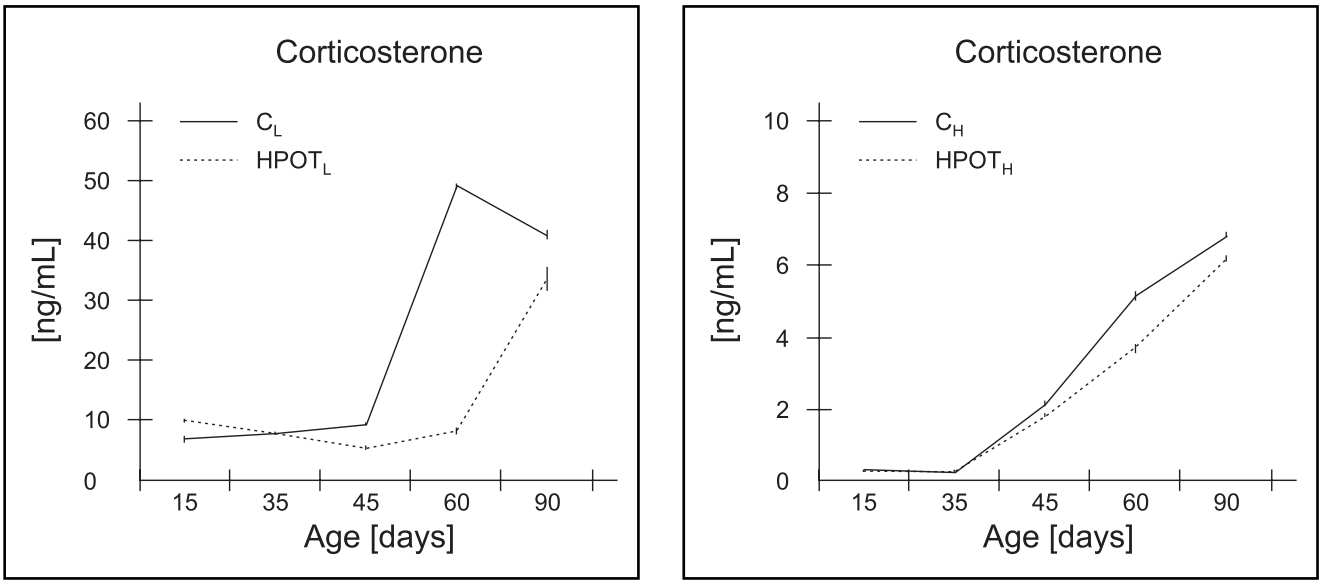

Figure 5. Chronological alterations in serum Corticosterone levels (ng/ml) in control and hypothyroid rats.

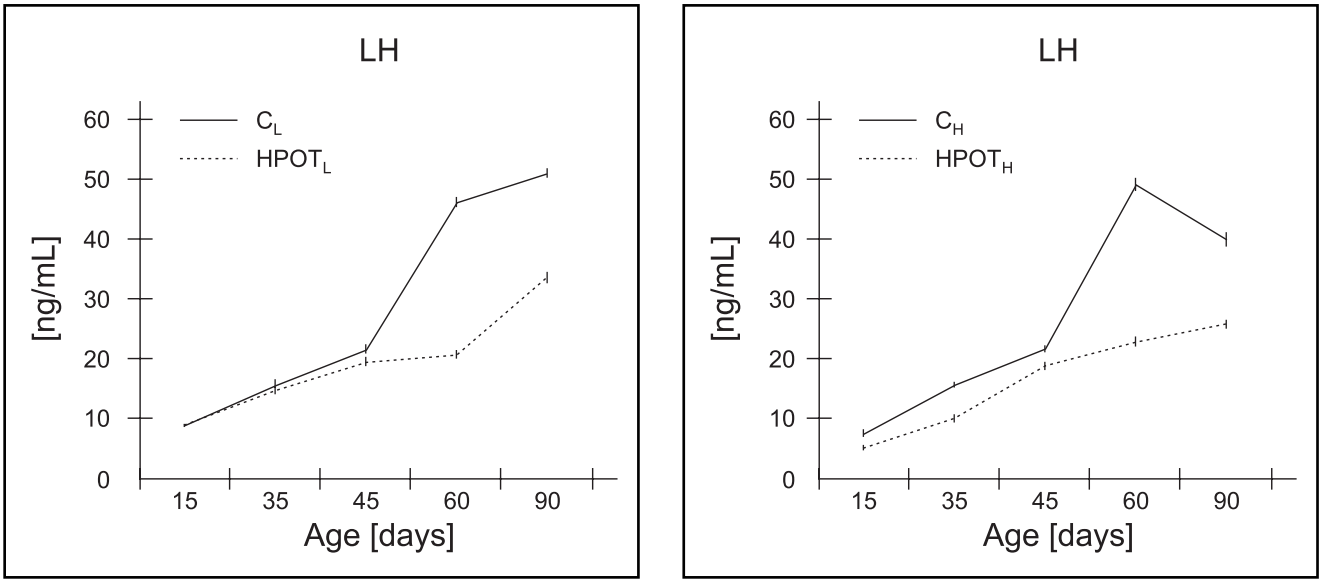

Figure 6. Chronological alterations in serum LH levels (ng/ml) in control and hypothyroid rats.
CL – Control (Low temperature), CH – Control (High temperature), HPOTL – Hypothyroid rats (Low temperature), HPOTH – hypothyroid rats (High temperature). Values expressed as mean ± SEM of our samples. ap < 0.05, bp < 0.005, cP < 0.0005

and, after 45 days, the enzyme activity showed peripheral localisation in the tubules with early stages of germ cells and a luminal localization in the tubules with advanced stages of germ cells. After 60 and 90 days, the enzyme activity disappeared from the tubules and was restricted only to Leydig cells. 3α-HSDH activity was very weak after 35 days and then, it increased gradually in both the tubules and Leydig cells, to fall down to the optimal levels after 60 and 90 days.

In general, the testes of the hypothyroid animals showed slightly lower levels of activity, compared to those in the controls. The general pattern of enzyme activity remained the same as that in the controls, except for a delayed optimal expression of both 3β- and 17β-HSDH after only 90 days and a prominent 3β HSDH activity with pregnenolone as substrate after 35 and 45 days, and the switch over to DHEA-dependant activity appearing only after 60–90 days.

Serum Hormones

In general, the levels of T4, T3, and TSH were higher in CH rats, compared to those in CL. Hypothyroidism induced a similar change of significantly reduced T3 and T4 levels, coupled with increased TSH levels during the treatment period. The levels gradually reverted to control values in the HPOTL rats, while the levels remained permanently subnormal in the HPOTH rats. (Fig. la & l b, 2a & 2b and 3a & 3b).

Testosterone and LH levels were relatively lower in CH animals, compared to those in CL. During the treatment period, the levels of the hormones were significantly low in both the HPOTH and HPOTL rats. Though there was a tendency of recovery towards normal levels during the post-treatment periods, the ultimate adult levels were still significantly low. Serum corticosterone level was relatively high in CL rats, compared to that in CH rats. During the treatment, as well as during post-treatment periods, the levels remained significantly high in both the HPOTL and the HPOTH animals, with pronouncedly high levels in the former (Fig. 4a & 4b, 6a & 6b and 5a & 5b).

Discussion

Many temperature dependent differences could be found in the present observations. Though the initial body mass was low in the HPOT rats on the 35th day, there was growth catch up later on, with the final mass of HPOTH rats on the 90th day, being equal to that of CH rats, while HPOTL rats still showed mass deficit of 11% vs. the corresponding CL rats. In contrast, the testis mass, which was 83% less on the 35th day in HPOTH rats, as compared to respective values in corresponding controls, still showed a deficit of 30% on the 90th day, while the testis mass in the HPOTL rats, which was 71% less on the 35th day, nullified the difference and grew up to a 58% higher mass on the 90th day, compared to respective values in the corresponding controls. Clearly, the growth catch up of testes was rather poor in the HPOTH rats, compared to that in the HPOTL rats.

A definite influence of temperature is clearly indicated by the observed differences in testis mass of CL and CH animals, which was 41% less on the 35th day and 19% less on the 90th day in the latter. Those results clearly suggest a definite influence of ambient temperature on the postnatal growth and development of testes.

Histologically, HPOT is observed to induce some degenerative changes in germ cells, relatively more in HPOTH, in the immature stage (after 35 days). Though such degenerative changes became almost null after 45 days in HPOTL rats, the same tendency persisted in HPOTH rats till 60 days (in a relatively decreased rate). Spermatogenesis was established in CL and CH testes after 45 days and sperm was seen in the tubules after 60 days. The spermatogenic process was delayed in both HPOTL and HPOTH animals and the sperm fully appeared in the tubules only after 90 days. The major point of difference was the thin population of germ cells with reduced sperm density in HPOTH rats and the dense population of germ cells with high sperm density in HPOTL rats, compared to respective values in the corresponding controls. Those differences in the histoarchitecture of the testes, as part of the temperature dependent response to HPOT, are further corroborated by the persistently decreased tubular diameter in the HPOTH animals (16% difference after 35 days, accentuated to 27% after 90 days) and the diametrically opposite increased tubular diameter in the HPOTL animals after 90 days. Histometric evaluations revealed a significant increase in testis and tubular volumes, in Sertoli and Leydig cell numbers and in the total germ cell count per meter length of tubule and per testis in the HPOTL rats and a significant decrease in HPOTH animals. It is apparent from the present set of observations that neonatal HPOT induced an increase in testis size, tubular diameter, and germ cell population, due to the prolonged phase of Sertoli cell proliferation and the consequent increase in its number, together with the fact that increase in Leydig cells are, essentially, temperature dependent. The influence of temperature was also denoted by the significant decrement in all the measurements of CH animals, compared to CL ones. This is the first report, which demonstrates the influence of temperature on the growth and maturation of postnatal testes in relation to altered hormonal status.

A temperature-induced difference was also seen in the hormonal milieu. A cursory consideration of the observations on the hormonal status in the two groups of experiment animals, reveals clear-cut differences.

1. The hypothalamo-hypophyseal-thyroid (HHT) axis is altered to a low set point, as revealed by the low levels of TSH, T4 and T3 in the adult status in HPOTH compared to CH, while the axis is relatively unaltered in HPOTL as, after the initial disturbance, the levels returned to normal in the adult stages.
2. The hypothalamo-hypophyseal-gonadal (HHG) axis is affected similarly under both temperature regimens, as the HPOT animals, in general, showed

a lowered set point, as revealed by the lower titres of LH and testosterone.
3. The hypothalamo-hypophyseal-adrenal (HHA) axis is differentially affected with a permanently elevated set point in HPOTL and a decreased set point in HPOTH.
4. It is inferable from the previous studies that HPOT-induced prolongation of Sertoli cell proliferation and delayed differentiation [24, 25], are more probable in lower temperatures. It is speculated that, despite lower thyroid hormone levels, there is an increased sensitivity (either by an increased receptor sensitivity or an actual increase in the receptor number), which prevents prolongation of Sertoli cell proliferation and, probably, brings about slow differentiation.

The higher degree of germ cell degeneration in the HPOTH rats could be due to a relatively higher level of estradiol produced by the not fully differentiated Sertoli cells, [32] and the relatively lower serum corticosterone levels in the HPOTH rats. The possible role of reduced corticosterone in the increased loss of germ cells is confirmed by our observations that corticosterone excess in the neonatal period decreases germ cell apoptosis and increases germ cell population by the permanently elevated HHA axis [37]. The ultimately increased testis size, seen in the HPOTL animals, could be a cumulative effect of both the increased Sertoli cell number and the reduced germ cell loss, and of the support of germ cells by the Sertoli cells to their full potential.

The lower serum level of T observed in the HPOT groups of animals, is validated by long-term decreased 3β- and 17β-HSDH activities in the interstitium. The reduced 3α HSDH activity and T levels could be related to the reduced gonadotropin levels, seen here, as well as reported by others [21, 25, 33]. Further justification is provided by the reported reduced levels of LH and FSH and the diminished activity of 3β- and 17β-HSDH in Leydig cells of rats rendered hypothyroid in the pre-pubertal period [31]. The same authors also demonstrated a reduced sensitivity of Leydig cells to LH. A remarkable observation, emanating from the present histochemical observations, is the intra-tubular androgenesis. Whereas the control testis showed a distinct compartmentalization, with 3β-HSDH activity in the interstitium and 17β-HSDH in the tubules, the testes of the HPOT animals showed prominent localisation of both enzymes in the tubules. Though both the control and the HPOT animals tended to show an active Δ5 pathway, there was a cooperative interaction between the two components in the controls with androstendione, required for conversion to testosterone in the tubules, being provided by the Leydig cells but, in the HPOT animals, that appeared to be essentially an intra-tubular phenomenon. The control rats depicted a full activity of intra-tubular steroidogenesis after 45 days and the preferred pathway appeared to be Δ4. Both pathways became operative in both compartments of the control animals after 60 days. The HPOT animals of comparable age showed persistent Δ5 pathway in the tubules, as well as its expression in the Leydig cells. A remarkable difference is that, while the Leydig cells of the control rats showed a steroidogenic potential by both pathways, those of the HPOT rats persisted with the Δ5 pathway till the 60th day. Apparently, intra-tubular steroidogenesis and steroid metabolism, though not well recorded in literature [34, 35], as well as the delayed expression of the Δ4 pathway in the HPOT animals, are noteworthy observations. The latter finds a confirmatory evidence by the recent molecular studies, showing T3-induced expression of the thyrotropin inhibitory protein (TIP) and modulation of 3β-HSDH to a more active state by T3 [36]. It can finally be concluded that the HPOT-induced testis growth and functional maturation is temperature modulated and regulated by altered hormonal set points and their molecular mechanisms.

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