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Effects of Luteinizing and Thyroid Hormones on the Aged Leydig Cells in Brown Norway Rats

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Abstract : The present study was designed to investigate the possibility of restoring the testicular steroidogenic ability of the aged Brown Norway rats by administering luteinizing hormone (LH) and thyroxine (Thy). Rats of 3, 6, 12 months (M) of age (n = 8 per group) and four groups of 18 month old rats (n = 8 per group) were used. Eighteen month old rats were implanted subdermally with Alzet mini osmotic pumps containing saline (control), luteinizing hormone (LH, 24 μg/day), thyroxine (Thy, 5 μg/day) and LH and Thy (LH+Thy, 24 μg/day and 5 μg/day), respectively for four weeks (i.e testing was done at 19 months). The results showed that the testis volume was unchanged among all treatment groups. The number of Leydig cell per testis was not significantly different among all treatment groups. The average volume of a Leydig cell was significantly decreased at 12 months, and a further reduction was observed at 19 months (saline-treated); values for 19 month LH- and -LH+Thy-treated rats were not significantly lower than those at 3 and 6 months of age. Testosterone secretory capacity per testis and per Leydig cell in vitro were significantly reduced concomitantly with age advancement from 6 to 19 months (saline-treated) of age. These values of LH- and Thy-treated 19 month old rats were similar to those at 12 months. LH+Thy-treated rats were equally capable to 3 and 6 month old rats in producing testicular testosterone in vitro in response to LH. Serum testosterone was unchanged from 3 M to 12 M rats but was reduced in 19M control rats. Both LH and Thy significantly raised these values above the 19M control levels, but they were still lower than the 3 M through 12 M levels. Additionally, LH+Thy significantly raised the serum testosterone levels to those of 12M rats, but these values were significantly lower than those of 3 M and 6 M rats. In summary, the present study demonstrated that the exogenous supplementation of LH and Thy was effective in restoring the steroidogenic potential of the aged Leydig cells; the most effective treatment was LH+Thy, which upgraded the capacity of aged testes to those of 3 and 6 months.

Key words : Luteinizing hormone, Thyroid hormone, Aged Leydig cell

INTRODUCTION

The most obvious effect of aging on male reproduction is the progressive decrease in sexual activity from adolescence into old age (Kinsey *et al*. 1948) due to the reduced circulating testosterone levels which occur in all mammalian species studied to date, including human (Hollander & Hollander 1958, Kirschner & Coffman 1968, Vermeulen 1976) and rats (Harman *et al*. 1978, Bethea & Walker 1979, Chen *et al*. 1994, Mendis-Handagama & Gelber 1995). Testosterone is necessary in the male reproductive system for many

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functions that include the regulation of spermatogenesis in the testis, maintenance of the accessory sex organs and the erectile function. Testosterone is also required in other organ systems of the mammalian male for their proper functioning. These include but not limited to the brain (for libido and mood), skin (for hair growth and sebaceous gland activity), muscle (to increase muscle strength and volume), liver (to synthesize serum proteins), synovial tissue (to modulate immune responses), bone (to maintain strength and volume), bone marrow (to stimulate stem cells) and kidney (to stimulate erythropoetin) (Morales *et al*. 1996, Anderson *et al*. 1996, Norman & Litwack 1997). Therefore, it is clear that sustaining the normal levels of circulating testosterone is important for the well being of the male. Testosterone is primarily produced by the Leydig cells of the testis. Many studies on the effects of aging on Leydig cell structure and function have revealed that Leydig cells undergo atrophic changes in size (Chen *et al*. 1994, Mendis-Handagama & Gelber 1995) and organelle content (Mori *et al*. 1982, Ichihara *et al*. 1993) with aging thus enab-ling them to go into a malfunctioning status.

It is established that the luteinizing hormone (LH) regulates the Leydig cell structure and function. Leydig cell size (i.e. average volume) is generally dependent on LH (Mendis-Handagama *et al*. 1992, 1998a), and the testosterone secretory capacity of Leydig cells have a positive correlation with its size (Mendis-Handagama *et al*. 1988). Thyroid hormones are known to be important in cellular differentiation (Guyton 1991), and more importantly recent observations have demonstrated that it is critical in the process of differentiation of precursor cells to Leydig cells in the postnatal rat testis (Ariyaratne *et al*. 2000a, Mendis-Handagama *et al*. 1998, Teerds *et al*. 1998). The specific effects of thyroid hormone on mature Leydig cells are not clear at present, it is reported that hypothyroidism in neonatal rats results in reduced size and numbers of identifiable Leydig cells and increase in connective tissue cells/mesenchymal cells in the testis interstitium (Amin & El-Sheikh 1977). As mesenchymal cells in the testis interstitium are the precursors to Leydig cells and if the differentiation of connective tissue cells into Leydig cells requires thyroid hormone (Ariyaratne *et al*. 2000b, Mendis-Handagama *et al*. 1998, Teerds *et al*. 1998), it is possible that the abundance of mesenchymal cells and decreased numbers of Leydig cells in testes of hypothyroid rats seen previously (Amin & El-Sheikh 1977) may be due to the de-differentiation of many Leydig cells towards their connective tissue precursor cells under reduced thyroid hormone levels with aging.

Reduced circulating levels of LH (Riegle & Meites 1976, Riegle & Miller 1978) and thyroid hormone (Cizza *et al*. 1992, 1995) with aging can be explained at least in part by the direct (LH secretion) and indirect (thyrotrophin/TSH action on the thyroid gland) effects that occur as a result of the pituitary aging. Based on these information, we hypothesize that these atrophic and de-differentiated status of Leydig cells in aged testes are caused at least in part by the reduced LH and thyroid hormone levels in the aged males. Therefore, the objective of the present study was to test the possibility of reversing these changes in the aged Leydig cells by exogenous supplementation of thyroid hormone and/or LH to increase their testosterone producing capacity similar to those of young. Rats have been suggested as suitable models for human aging studies (Hazzard 1991) and the Brown Norway rats were chosen for this study as this strain of rats has been recommended as a suitable model for aging studies (Wang *et al*. 1993, Zirkin *et al*. 1993).

MATERIALS AND METHODS

1. Animals and treatments

Male Brown Norway rats of 3, 6, 12 (n = 8 per group) and 18 months (32 rats) were purchased from

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Hanil animal laboratory (Chonju, Chonbuk, South Korea). Eighteen month old rats were divided into four groups (n = 8 per group). Under deep inhalation anesthesia (Metofane, Malincroft Veterinary Inc, Mundelein, IL, USA) these 18 month old rats were implanted subdermally with Alzet mini osmotic pumps (Alza Corporation, Palo Alto, CA) containing saline (control), LH (24 μg/day, National Hormone and Pituitary Program), thyroxine (Thy, 5 μg/day, Sigma, St Louis, MO, USA) and LH and Thy (LH+Thy, 2 pumps, 24 μg/day of LH and 5 μg/day of Thy), respectively for four weeks. These doses of LH (Mendis-Handagama *et al*. 1998a) and thyroid hormone (Ariyaratne *et al*. 2000) were used based on previously published studies. Rats were maintained under conditions of controlled temperature (25°C) and lighting (14 hours light: 10 hours darkness), and were housed 1 per cage. The animals were fed with rat chow and water ad libitum until sacrifice. At the end of 4 weeks, rats were sacrificed to be used for studies described below. In addition, implanted pumps were removed and examined to verify that they have delivered the contents as expected.

2. RIA for serum LH, T_4, tri-iodothyronine, and testosterne

Serum LH hormone was quantified using a commercially available rLH (rat luteinizing hormone) kit (Amersham Pharmacia Biotech, Piscataway, NJ, USA). Serum T_4, tri-iodothyronine (T_3), and testosterone were assayed using commercially available kits (Coat-A-Count; DPC, Los Angeles, CA, USA).

3. LH-stimulated testicular steroidogenesis in vitro

Under Metofane anesthesia (Malincroft Veterinary Inc, Mundelein, IL, USA) one testis of each rat (including 3, 6 and 12 months of ages) was removed, cleaned of fat, and weighed on a Mettler H54 balance to obtain the fresh testis weight. Using the flotation technique (Mori & Christensen 1980, Mendis-Handagama & Ewing 1990), the fresh testis volume was determined by specific gravity (in metric units specific gravity = density) and the fresh testis weight. Specific gravity of testes were determined by the flotation technique as described previously (Mori & Christensen 1980, Mendis-Handagama & Ewing 1990). Testis was then decapsulated and incubated in 2 mL of Krebs-Ringer-bicarbonate buffer saturated with air and containing 2% glucose (KRBG) and 100 ng/mL of LH, at 34°C in a shaking water bath (90 oscillations/minute) for 3 hours as described previously (Mendis-Handagama *et al*. 1998b, Ariyaratne & Mendis-Handagama 2000, Ariyaratne *et al*. 2000a). The incubation chamber was aerated continuously throughout the process. At the end of 3 hours, the incubation medium was separated from the tissue by centrifugation (80 g) for 10 minutes, and the supernatant was stored at -80°C. Testosterone levels in these media were measured by radioimmunoassay using commercially available kits (COAT-A-COUNT, Diagnostic Products Corporation, Los Angeles, CA). Testosterone secretory capacity per Leydig cell was calculated by dividing the testosterone secretory capacity per testis by the number of Leydig cells per testis.

4. Fixation and processing of testis tissue

The other testis of each rat (n = 8 per group) was fixed by whole body perfusion. This step was followed by sending a fixative containing 2.5% glutaraldehyde buffered with 0.1M cacodylate buffer (pH 7.4) through the same cannula to achieve fixation of tissue. The fixed testis was weighed, the specific gravity was measured, and the fixed testis volume was calculated. The fixed testis was cut into approximately 2 mm cubes and processed for microscopy and stereology as described previously (Ariyaratne & Mendis-Handagama 2000, Ariyaratne *et al*. 2000b). Shrinkage of testis tissue from fresh to processed state was determi-

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ned as described previously (Mendis-Handagama & Ewing 1990) to use in the stereological studies.

5. Microscopy and stereology

Two tissue sections, 1 µm thick and 4 sections apart were cut from the testis tissue blocks prepared for stereology using a LKB IV ultramicrotome and glass knives. These sections were stained with methylene blue. The volume density of testicular components which is defined as the volume of the component per unit volume of testis tissue) was obtained via point counting as described previously (Mendis-Handagama *et al*. 1988, Mendis-Handagama *et al*. 1998) using a 400-point lattice grid fitted to one eyepiece of the light microscope. Each line intersection was used as a test point. With a ×40 objective lens, four corners of every tissue section were analyzed with a Leica DMRBE light microscope. The tested components include, seminiferous tubules, testis interstitium, and Leydig cells. The formula used to obtain the volume density of each testicular component is given below.

Volume density of a component = number of points on the component ×100/total number of points on the testis tissue.

The absolute volume (mm^3) occupied by each testicular component was calculated by multiplying the volume density of each component by the volume (mm^3) of the fresh testis. The numerical density of Leydig cells (number of cells per unit volume of testis) was obtained via the disector method (Sterio 1984) as described in detail previously (Ariyaratne & Mendis-Handagama 2000). The average volume of a Leydig cell was obtained by dividing the volume density of Leydig cells by the numerical density as published previously (Mendis-Handagama *et al*. 1998). The number of Leydig cells per testis was calculated by multiplying the numerical density by the fresh testis volume (Sterio 1984, Ariyaratne & Mendis-Handagama 2000). Leica DMRBE light microscope was used for photography.

7. Statistical analysis

The results are expressed in the tables as mean, and standard error of the mean in parenthesis. Significant differences ($p<0.05$) between the means were determined by the Duncan's multiple-range test after analysis of variance (Duncan 1975).

RESULTS

Significant differences were not observed with the values for testis volumes among all experimental groups. At 3 months of age the absolute volume of seminiferous tubules and interstitium per testis was 1465.65 mm^3 and 180.77 mm^3, respectively. The absolute volume of seminiferous tubules and interstitium per testis was not significantly different in all groups. The absolute volume of Leydig cells per testis did not change with age advancement from 3 to 6 months, but significantly reduced first at 12 months and then at 19 months (saline-treated). By contrast 19 month-LH, 19 month-Thy and 19 month-LH+Thy had significantly greater values than that of 19 month-saline rats. Moreover, this value of 19 month-LH rats was not significantly different from those at 3 and 6 months of age. In addition, 19 month-Thy and 19 month-LH+Thy values were not significantly different from those at 12 months of age (Table 1).

Fig. 1 shows the number of Leydig cells per testis in 3-, 6-, 12-month-old rats, and rats treated with saline, LH, Thyroxine, and LH+thyroxine pumps. The number of Leydig cells per testis showed a significant increase ($P<0.05$) in thyroxine-treated rats compared to the value in 3 and 12 months. No change was observed in the number of Leydig cells per between the saline-treated rats and LH-, thyroxine or LH+thyroxine treated rats (Fig. 1).

The average volume of a Leydig cell was unchanged with the age advancement from 3 to 6 months,

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Table. 1. Mean testis volume (mm^3) and absolute volume (mm^3) of seminiferous tubule, interstitium and Leydig cells

Parameter	3M	6M	12M	19M			
				Control	LH	T_4	LH+T_4
Testis volume	1674	1736	1683	1716	1804	1838	1783
	(75)[a]	(82)[a]	(68)[a]	(56)[a]	(72)[a]	(66)[a]	(71)[a]
Seminiferous tubule (mm^3)	1465.65	1552.85	1497.77	1510.16	1595.38	1608.77	1556.50
	(46.69)[a]	(23.93)[a]	(83.01)[a]	(57.35)[a]	(25.77)[a]	(24.63)[a]	(85.82)[a]
Interstitim (mm^3)	180.77	183.15	184.73	205.84	208.62	226.74	203.17
	(14.45)[a]	(10.02)[a]	(7.25)[a]	(33.85)[a]	(11.29)[a]	(8.57)[a]	(35.54)[a]
Leydig cells (mm^3)	59.99	58.14	47.38	38.22	50.26	43.57	45.02
	(3.19)[a]	(1.95)[a]	(2.17)[bc]	(3.69)[c]	(4.72)[ab]	(0.37)[bc]	(2.52)[bc]

[a-d] Numbers in parentheses are SEM. In each row, values with different superscripts are significantly different ($P<0.05$)

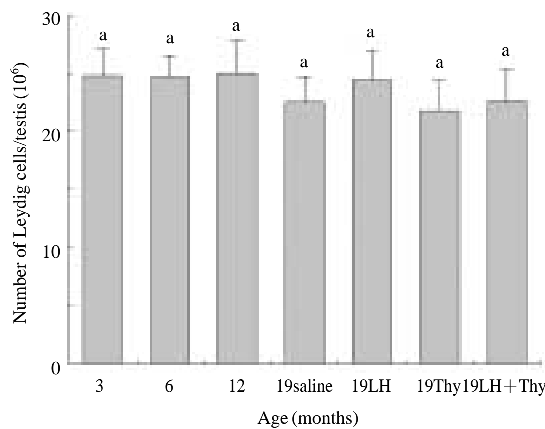

Fig. 1. The number of Leydig cells per testis in 3-, 6-, 12-month-old rats, and rats treated with saline, LH, Thyroxine, and LH+thyroxine pumps (mean ± SEM).

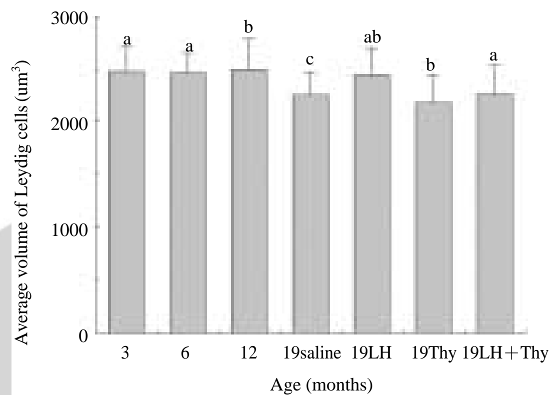

Fig. 2. Average volume (μm³) of a Leydig cell in 3-, 6-, 12-month-old rats, and rats treated with saline, LH, Thyroxine, and LH+thyroxine pumps (mean±SEM). Different letters indicate statistically significant differences ($P<0.05$).

however, a significant reduction was observed first at 12 months and then at 19 months (saline-treated). The average volume of a Leydig cell in 19 month-LH and 19 month-LH+Thy rats was not significantly different from those at 3 and 6 months of age. The value for this parameter in 19 month-Thy rats was significantly lower than those of 3 and 6 months of age, but was not significantly different from those of 19 month-LH and 19 month- LH+Thy rats as well as 12 month old rats (Fig. 2).

LH-stimulated testosterone secretory capacity per testis (Fig. 3) and per Leydig cell (Fig. 4) in vitro. There was no difference between 3 and 6 month old rats for both of these parameters, but significant reductions were observed at 12 and 19 months (saline-treated) of age. Both of these parameters in 19 month-LH and 19 month-Thy rats were comparable in values to those of 12 months of age. Both of these parameters in 19 month-LH+Thy rats were similar to those at 3 and 6 months of age.

Serum LH levels were unchanged from 3 M to 12 M rats but were reduced significantly in 19 M control and T_4-treated rats compared with 3 M through 12 M

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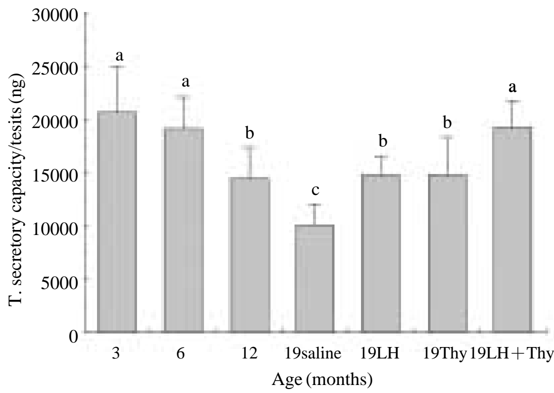

Fig. 3. Testosterone secretory capacity per testis (ng) in 3-, 6-, 12-month-old rats, and rats treated with saline, LH, Thyroxine, and LH+thyroxine pumps (mean±SEM). Different letters indicate statistically significant differences ($P<0.05$).

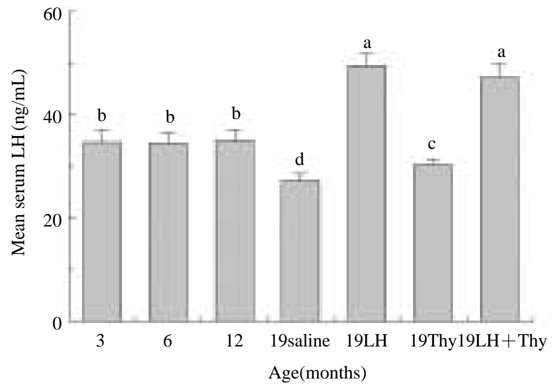

Fig. 5. Mean serum LH (ng/mL) in 3-, 6-, 12-month-old rats, and rats treated with saline, LH, Thyroxine, and LH+thyroxine pumps (mean±SEM). Different letters indicate statistically significant differences ($P<0.05$).

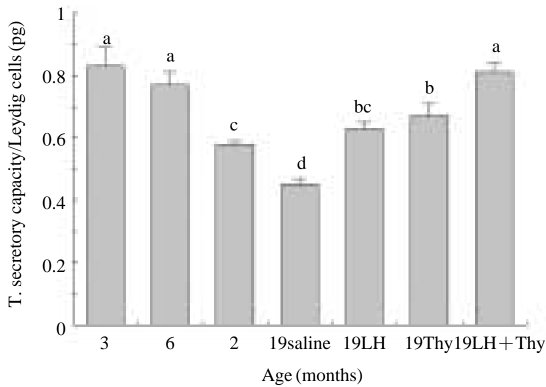

Fig. 4. Testosterone secretory capacity per Leydig cell (pg) in 3-, 6-, 12-month-old rats, and rats treated with saline, LH, Thyroxine, and LH+thyroxine pumps (mean±SEM). Different letters indicate statistically significant differences ($P<0.05$).

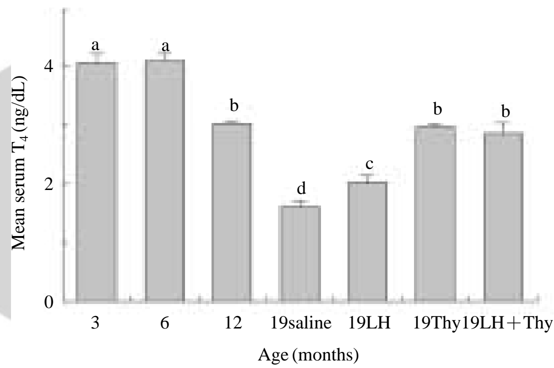

Fig. 6. Mean serum T_4 (ng/dL) in 3-, 6-, 12-month-old rats, and rats treated with saline, LH, Thyroxine, and LH+thyroxine pumps (mean±SEM). Different letters indicate statistically significant differences ($P<0.05$).

rats. In 19 M LH- and LH+T_4-treated rats, LH levels were greater than those of 3 M to 12 M rats (Fig. 5). Both T_4 (Fig. 6) and T_3 (Fig. 7) levels in serum were highest in 3 M and 6 M rats and lowest in 19 M control rats. These hormone levels in 19 M T_4-and LH+T_4-treated rats were similar to those in 12 M rats and were higher than those in 19 M LH-treated rats. Serum testosterone levels did not change significantly from 3 M to 12 M rats, although the mean value in 12 M rats was approximately 20% lower than those in 3 M and 6 M rats. The lowest value was observed in 19M control rats; the values in 19 M LH-, T_4-, and LH+T_4-treated rats were significantly greater than 19 M controls. Serum testosterone levels in 19 M LH- and T_4- treated rats were significantly lower than

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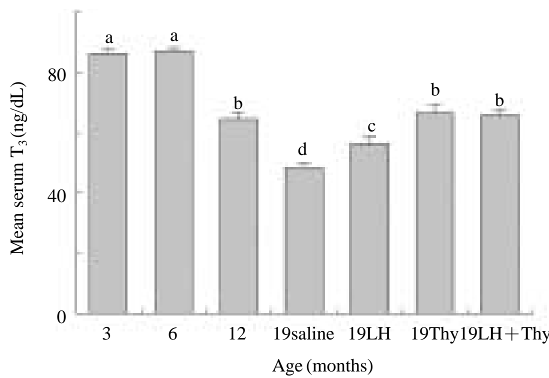

Fig. 7. Mean serum T_3 (ng/dL) in 3-, 6-, 12-month-old rats, and rats treated with saline, LH, Thyroxine, and LH+thyroxine pumps (mean±SEM). Different letters indicate statistically significant differences ($P < 0.05$).

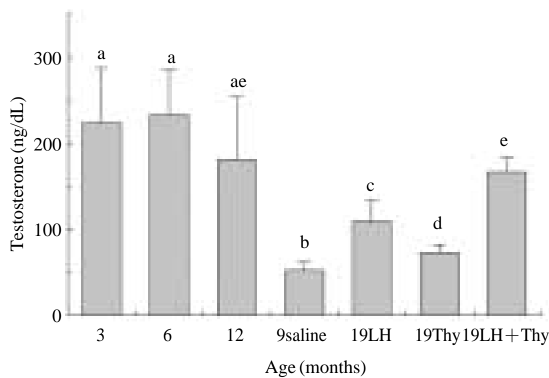

Fig. 8. Mean serum testosterone (ng/dL) in 3-, 6-, 12-month-old rats, and rats treated with saline, LH, Thyroxine, and LH+thyroxine pumps (mean±SEM). Different letters indicate statistically significant differences ($P < 0.05$).

those in 3 M through 12 M rats. Although the serum testosterone levels in 19 M LH+T_4-treated rats were not significantly different from those in 12 M rats, they were 27% lower ($P < 0.05$) than those in 3 M and 6M rats (Fig. 8).

DISCUSSION

The present investigation showed that there is no change in the mean testis weights in brown Norway rats with aging which compares favorably with previously published studies on this strain of rats (Zirkin *et al*. 1993, Chen *et al*. 1994). This is in contrast to the observations in Sprague Dawley (Mendis-Handagama & Gelber 1995) and Wistar strains (Ichihara *et al*. 1993) which show increase in testes weights with aging. In a previous study occurrence of small and large testes in aged brown Norway rats has been reported (Chen *et al*. 1994), however, we did not see a size difference in aged brown Norway rats used in this investigation which is difficult to explain at this juncture.

The effects of aging on Leydig cell steroidogenic function has been studied in great detail in many strains of rats such as Long-Evans (Leathem & Albrecht 1974), Wistar (Ichihara *et al*. 1993), Fischer (Bethea & Walker 1979), Sprague Dawley (Lin *et al*. 1980, Liao & Azhar 1993) and brown Norway (Wang *et al*. 1993, Zirkin *et al*. 1993). These studies have shown clearly that the steroidogenic capacity is impaired in the aged rat testes compared to those at younger ages. The present study on brown Norway rats is in agreement with the above studies on the testicular steroidogenesis with aging. Moreover, our quantitative information on unchange in Leydig cell number per testis with aging and the reduction in the average volume of a Leydig cell in the aged rats is in agreement with previously published studies in brown Norway rats (Wang *et al*. 1993, Chen *et al*. 1994). These findings further confirm that there is no change in Leydig cell number per testis with aging in brown Norway rats although there is Leydig cell hypotrophy. Latter feature (i.e. Leydig cell hypotrophy) is also observed with aging in Sprague Dawley (Mendis-Handagama & Gelber 1995) and Wistar rats (Ichihara *et al*. 1993), however, the number of Leydig cells per testis is increased with aging in Sprague Dawley (Mendis-Handagama & Gelber 1995) and Wistar rats (Ichihara *et al*. 1993) demonstrating differences among these strains for this

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parameter. Occurrence of Leydig cell hyperplasia in the aged testes has also been reported in stallions (Johnson & Neaves 1981) and human (Kothari & Gupta 1974) and suggest the existence of differences among the species for this parameter with aging. Increase in numbers of Leydig cells have also been observed following chronic human chorionic gonadotropin (hCG; Christensen & Peacock 1980) and LH (Mendis-Handagama *et al.* 1998) treatments in Sprague Dawley rats although it was absent in brown Norway rats of the present study. Previous studies have shown that triiodothyronine treatment of neonatal rats (Teerds *et al.* 1998, Ariyaratne *et al.* 2000a) and adult rats treated with ethane dimethane sulphonate (Ariyaratne *et al.* 2000) causes a stimulation of Leydig cell differentiation from the precursor cells. However, to our knowledge effects of thyroid hormones on the testis intertstitium and/or the Leydig cells in the sexually mature testis is poorly understood.

The observation of Leydig cell hypertrophy in LH-treated rats compares favorably with the results of previous studies on the effects of chronic hCG (Christensen & Peacock 1980) and LH (Mendis-Handagama *et al.* 1998) treatments in sexually mature young rats. However, together with cell hypertrophy, hyperplasia of Leydig cells (Christensen & Peacock 1980, Mendis-Handagama *et al.* 1998) was also observed in those rats, in contrast to the aged Brown Norway rats of the present study. Whether these differences in response to LH are due to age effects or to strain effects is not clear at present. Whereas LH treatment alone was able to fully restore the average volume of a Leydig cell to those of 3 M and 6 M rats, it was sufficient only to upgrade the steroidogenic potential of Leydig cells in vitro to that of 12 M rats (i.e., above 19 M control rat values). A1though serum testosterone levels in the aged Brown Norway rats were raised significantly above the 19 M control rat value with LH treatment, this increased level was still much less than the serum testosterone levels of 3 M and 6 M rats. Therefore, it is apparent that exogenous LH substitution alone is not sufficient to rejuvenate the functional aspect of Leydig cells in aged Brown Norway rats toward that of 3 M and 6 M rats, although a complete recovery in cell size was achieved with LH treatment alone.

LH is produced by the gonadotrophs of the anterior pituitary gland. It has a positive correlation with Leydig cell steroidogenic function; deprivation of endogenous LH causes Leydig cell hypotrophy and reduced steroidogenic capacity (Ewing *et al.* 1983, Russell 1992) and chronic stimulation of Leydig cells with LH produces Leydig cell hypertrophy and increased steroidogenic capacity (Mendis-Handagama *et al.* 1988). Substantial evidence from studies by Liao and Azhar (1993) supports the idea that aging directly affects LH-mediated cholesterol transfer into mitochondria and within the inner mitochondrial membrane. This view is further supported by the observations that significant reductions occur in steroidogenic acute regulatory protein (StAR) (Luo *et al.* 2001) and peroxisomes (Ichihara *et al.* 1993) in Leydig cells, which are considered to be crucia1 factors in cholesterol transport into mitochondria in steroidogenic cells (Stocco 1996, Mendis-Handagama 2000). In addition, enzymes responsible for converting cholesterol to testosterone in Leydig cells are reduced with aging (Luo *et al.* 1996); these include P450 cholesterol side chain cleavage enzyme, 3beta-hydroxysteroid dehydrogenase, 17 alpha-hydroxy1ase/C_{17-20} lyase, and 17 beta-hydroxysteroid (Luo *et al.* 1996). Because substitution of LH to the aged Brown Norway rats has aided tremendously in upgrading the steroidogenic potential of their Leydig cells, testicular aging clearly is close1y associated with aging of the pituitary gland.

Thyroid hormones (T_4 and T_3) are produced by the follicular cells of the thyroid gland and are regulated by TSH made by the thyrotrophs of the anterior pituitary gland. With aging of the pituitary gland, circulating thyroid hormone levels are reduced (Cizza *et*

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al. 1992, 1995). Hypothyroidism arrests differentiation of Leydig cells in the neonatal (Mendis-Handagama *et al*. 1998, Teerds *et al*. 1998) and adult (Ariyaratne *et al*. 2000b) testis and, more important1y, causes hypotrophy and hypoplasia of Leydig cells in the sexually mature testis (Amin & El-Sheikh 1977). Although direct effects of T_4 on Leydig cells in vivo or in vitro have not been extensively studied, acute treatment of Leydig cells with its metabolite, T_3, directly enhances Leydig cell steroidogenesis in vitro (Maran *et al*. 2000, Manna *et al*. 2001). Additionally, treatment of mouse Leydig cells with T_3 coordinately augments the levels of StAR protein, StAR mRNA, and steroid production (Manna *et al*. 2001). Because the effects of T_4 in vivo (restricted to the vascular pool) are mediated via T_3 (T_4 is converted to T_3 in target tissues; T_3 is 3-to 5- fold more active than T_4) (Norman & Litwack 1997), it is possible to suggest that the stimulatory effects of T_3 in vitro on Leydig cell steroidogenesis (Maran *et al*. 2000, Manna *et al*. 2001) reflect the acute effects of thyroid hormone on Leydig cells in vivo as well. Taken together, it is apparent that thyroid hormone is an important factor for Leydig cell steroidogenesis, and that thyroid hormone deficiency during aging could be corrected with exogenous substitution. Manifestation of thyroid hormone deficiency with aging and its effects on testicular steroidogenic function are also in agreement with the concept that aging of the testis is closely associated with pituitary aging.

With LH+T_4 treatment, the aged Leydig cells of 19 M Brown Norway rats were rejuvenated remarkably; their average cell volume and the steroidogenic potentia1 in vitro were completely reversed, to values similar to those of 3 M and 6M rats. Although the serum testosterone levels of these rats were raised only to the level of 12 M rats (i.e., 73% recovery compared to 3 M rats), this was a 300% increase compared to 19 M control rats. Therefore, it is possible to be optimistic regarding the potential of this line of research toachieve the desired serum testosterone levels by adjusting the present LH and T_4 doses (to be lower and higher, respectively). However, even with a 100% success compared to 3 M Brown Norway rats, it would still be too early to predict whether this line of treatment could be effective in other strains of rats, other rodents, or other mammalian species in rejuvenation of aged Leydig cells and, thereby, treatment of the androgen deficiency caused by the process of aging.

Currently, androgen deficiencies in aging humans are treated with androgen therapy (Anderson *et al*. 1996, Googn 1996). The risks of administering androgens to aging men mainly concern the cardiovascular system and the prostate (Googn 1996). Cardiovascular effects of androgens are ascribed to the atherogenic effects of androgens on blood-lipid profiles (Googn 1996). Apart from these effects, androgens could have other possible deleterious metabolic effects on the cardiovascular system. They induce insulin resistance (Polderman *et al*. 1994) and increased plasma levels of endothelin, a substance with vasoconstrictor properties produced by the vascular wall (Polderman *et al*. 1993). Regarding the effects of androgens on the prostate, benign prostatic hyperplasia and prostate cancer are the main concerns (Googn 1996). Therefore, if the present findings are, in fact, applicable to humans, a potential exists to develop an alternative to androgen therapy for minimizing the effects of androgen deficiency during age advancement. Clear1y, additional research is required to evaluate the feasibility of this approach.

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Abstract

Brown Norway rat에서 노화 간질세포에 미치는 LH와 thyroxine의 영향

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간추림 : 노화된 Brown Norway 흰쥐에서 luteinizing hormone (LH)과 thyroxine (Thy)을 주사하여 고환내 스테로이드 형성능이 복구될 수 있는 가능성을 알아보기 위하여 본 연구를 수행하였다. 3, 6, 12, 18개월령 쥐를 구입하고 18개월령 쥐는 4군으로 나누어 대조군(saline), LH (24 μg/day), Thy (5 μg/day), LH+Thy (24 μg/day+5 μg/day)를 함유하는 Alzet mini osmotic pump를 피부 밑에 이식하고 30일 후에 각각 희생하여 실험에 이용하였다. 고환용적은 모든 실험군에서 차이를 보이지 않았다. 고환조직 당 간질세포 숫자는 변화가 없었고 간질세포 평균용적은 12개월령부터 19개월령 대조군까지 유의성 있게 감소하였으며, LH, LH+Thy를 처리한 군에서는 3개월령, 6개월령과 차이가 없었고 Thy를 처리한 실험군에서는 12개월령과 차이를 보이지 않았다. 간질세포 테스토스테론 분비능은 6개월령부터 대조군인 19개월령까지 유의성 있게 감소하였고 LH와 Thy를 처리한 실험군은 12개월령과 유사하였으며, LH+Thy를 처리한 군에서는 3개월령, 6개월령과 차이가 없었다. 혈청내 테스토스테론 농도는 3개월령부터 12개월령까지 변화가 없었고 19개월령 대조군에서 감소하였으며, LH와 Thy를 처리한 실험군에서는 대조군보다 높게 관찰되었으나 3~12개월령 보다는 낮았고 LH+Thy를 처리한 실험군에서는 12개월령과 유사하였으나 3~6개월령 보다는 낮게 관찰되었다. 이상의 결과를 요약하면 노화된 간질세포에 LH 또는 thyroxine을 처리하면 스테로이드 형성능을 복구함에 있어 효과적임을 입증하였고, 가장 효과적인 처리는 노화된 고환이 3개월령, 6개월령과 비슷한 결과를 나타내는 LH+Thy를 처리하는 것임을 알 수 있었다.

찾아보기 낱말 : Luteinizing hormone, Thyroxine, 노화, 간질세포

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