EFFECTS OF THYROID HORMONE ON LEYDIG CELL DEVELOPMENT IN THE MT-hMIS TRANSGENIC MICE

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Introduction

In the male, Leydig cells are the primary source of androgens required for sexual differentiation during foetal development and maintenance of sexual health in the mature animal. Two generations of Leydig cells differentiate from their mesenchymal precursors in most mammals and these are named as foetal Leydig cells and adult Leydig cells.

Development of the adult Leydig cell population occurs during the prepubertal period, and their precursor is shown to be the peritubular mesenchymal cells (Ariyaratne *et al.*, 2000a). The differentiation of mesenchymal cells into adult Leydig cells appears to be influenced by a number of factors including thyroid hormone and anti-Mullerian hormone (AMH). Thyroid hormone stimulates differentiation of mesenchymal cells into Leydig cells while AMH is inhibitory to this process. Considering the important effects of thyroid hormone and AMH on testicular development and function, the objective of the present study was to analyze the inhibitory effect of AMH and stimulatory effect of thyroid hormone on postnatal Leydig cell development and function using an AMH over expressing human methaloptrotein promoter MT-hAMH mouse model.

Materials and Mmethods

Animals

Transgenic male mice over expressing AMH gene (MT-hAMH) were kindly donated by Dr. R.R. Behringer. Normal mature female P57BL6 mice were purchased from Harlan Industries. They were housed one male and one female per cage.

Experimental design

Each cage was observed twice a day for litters and the day of birth of pups was considered as the first day of their age. From the resulting litters, some were randomly selected and were given daily subcutaneous injections of triiodothyronine (Sigma, St. Louis, MO) at a dosage of 50 µg/Kg body weight (Ariyaratne *et al.*, 2000a and 2000b) in 50 µl saline from birth to 21 days of age. The other litters were given 50 µl of saline for the same period. Animals in both treatments were sacrificed at days 7, 14, 21, 40 and 90 of their age. At sacrifice, tail clips were collected from each animal and genotyping was performed using specific primers to detect hMIS gene. This protocol resulted in 4 groups of mice at each time point, i.e. saline treated control group (Group C),

thyroid hormone treated control group (Group C+T), saline treated AMH over expressing group (Group AMH) and thyroid hormone treated AMH over expressing group (Group AMH+T). Two hours before sacrifice, each mouse was given an intra-pleural injection of deoxybromouridine (BrdU, Sigma, St. Louis, MO) at a dose of 150 mg/Kg body weight, dissolved in saline. Testes were collected from these mice and fixed in Bouin's, embedded in paraplast and used for immuocytochemical detection of proliferating cells and steroidogenic cells in the same histological section using a double antibody staining technique by employing anti-BrdU and anti-3β-HSD antibodies. Cells undergoing apoptosis were detected by using terminal deoxynucleotide transferase dUTP nick end labeling (TUNEL) assay.

Results

BrdU and 3β-HSD histochemistry

The foetal and adult Leydig cells were intensely stained with 3β-HSD antibody. Many immunostained cells for BrdU were observed in the basal region of the seminiferous tubules (germ cells) and were considered as the positive control for the staining procedure. In the testicular interstium the positively stained cells showed a much intense color development than those cells in semiferous tubules. At the age of 7 days, the testis contained only foetal Leydig cells and Leydig cells immunolabled for BrdU were not seen in the AMH group (Fig.1A). In contrast, there was a significant increase in BrdU positive cells in C+T and AMH+T groups compared to that of the control group (C). At day 14, the Leydig cells are largely foetal type in clusters but a few adult type were also seen. There were more dividing Leydig cells in group C+T compared to group C and in group AMH+T when compared to group AMH. A similar pattern was also observed at day 21, though the most of the Leydig cells were of the adult type. Only a few dividing Leydig cells were seen at day 40 whereas none was encountered at day 90.

The highest percentage of dividing mesenchymal cells (BrdU positive) was seen in group AMH at day 7 and 14 (Fig. 1B). Treatment of animals with T3 resulted in a decrease in the percentage of BrdU stained mesenchymal cells compared to the control animals at these two age points. However, these differences were not apparent in animals at 21 days of age. Only a few dividing mesenchymal cells were seen in animals aged 40 or 90 days.

Apoptosis of interstitial cells

Immunostaining of testicular tissue using TUNEL technique resulted in many positively stained cells in the seminiferous tubules and observation of these cells was considered the positive control for the procedure. However, positively immunostained cells for TUNEL were not seen in the testis interstitium in any of the experimental groups at any age point.

Discussion

Previous studies have shown that while thyroid hormone is stimulatory to prepubertal Leydig cell development (Ariyaratne *et al.*, 2000a and b), the AMH was inhibitory to this process (Behringer *et al.*, 1994). Leydig cell differentiation was arrested

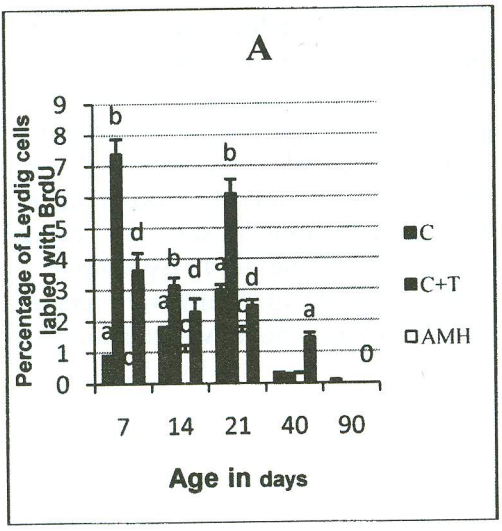

Figure 1. Percentage of BrdU positive cells. Columns with different labels are different ($p<0.05$).

in hypothyroid animals (Mendis-Handagama *et al.*, 1998) while over expression of AMH in MT-hMIS mice resulted in infertility due to impairment of Leydig cell development (Bheringer *et al.*, 1994). The present study revealed that thyroid hormone promotes Leydig cell differentiation by expanding the precursor cell population and enhancing the mitotic division of adult Leydig cells. On the other hand AMH had the opposite effect on precursor cells and adult Laydig cells leading to reduced number of Leydig cells in the mature testis. Furthermore, it appears that increased Leydig cell apoptosis is not the cause for reduced number of Leydig cells in MT-hMIS mice which lead to infertility. Further, the present study showed that short-term treatment of thyroid hormone resulted in significant increase of mitotic activity of Leydig cells in MT-hMIS mice indicating that thyroid hormone may possibly overcome the inhibitory action of AMH. It is interesting to study the long-term effects of thyroid hormone in this respect.

Conclusion

Based on our observations we conclude that AMH negatively regulates Leydig cell development by affecting both precursor and differentiated cell while thyroid hormone positively regulates them affecting the same cell populations. Further, we also conclude that negative effect of AMH on Leydig cells could be overcome, at least in part by the positive effect of thyroid hormone.

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