A study on the possible pro-inflammatory effects promoted by thyroid hormone signaling on the rat ovaries

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By

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ABSTRACT

The present study aimed at exploring the possible pro-inflammatory effects of thyroid hormones on the rat ovary. The experimental animals were assigned into eight groups, four normal groups (group 1, 2, 3, and 4) and four hyperthyroid groups (group 5, 6, 7, and 8). Hyperthyroidism was induced by daily IP injection of L-thyroxine, in a dose of 25µg/100 gram body weight, for two weeks. Group (1) was considered the normal control group whereas the rats in group (5) were assigned as the control hyperthyroid group. While the animals in groups (2, and 6) received tamoxifen, those in groups (3, and 7) were given progesterone. In addition, the animals in groups (4, and 8) were provided with combined tamoxifen and progesterone treatment.

The results of the control hyperthyroid group revealed significant upregulation of ovarian COX2, ER, and TR expression, while the PR expression was significantly down-regulated. Both ER and TR are significantly correlated with COX2, whereas PR is negatively correlated with it. Both tamoxifen and progesterone in the hyperthyroid groups resulted in down-regulation of ER, TR, and COX2, as well as up-regulation of PR, in the treated groups as compared to the control hyperthyroid group. However, the effect of progesterone appeared to be more prominent.

In conclusion, our results provided evidence that induced hyperthyroidism is associated with an inflammatory state in the rat ovary. The administration of tamoxifen and/or progesterone appeared to exert protective effects against this inflammation.

Key words: thyroid hormones, thyroid receptors, estrogen, estrogen receptors, progesterone, progesterone receptors, cyclooxygenase-2, tamoxifen, ovary

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LIST OF ABBREVIATIONS

AA: Arachidonic Acid

COX: Cyclooxygenase

COX1: Cyclooxygenase-1

COX2: Cyclooxygenase-2

COX3: Cyclooxygenase-3

E: Estrogen

eNOS: Endothelial Nitric Oxide Synthase

ER: Estrogen Receptor

GAPDH: Glyceraldehyde 3-Phosphate Dehydrogenase

IL: Interleukin

MPR: Membrane Progestin Receptors

NSAIDs: Non-steroidal Anti-inflammatory Drugs

OSE: Ovarian Surface Epithelium

P: Progesterone

PG: Prostaglandin

PIs: Pregnanolone Isomers

PR: Progesterone Receptor

SERM: Selective Estrogen Receptor Modulator

SHBG: Sex Hormone Binding Globulin

TH: Thyroid Hormone

TNF-α: Tumor Necrosis Factor-α

TR: Thyroid Receptor

TxA_2: Thromboxane A_2

VEGFR-2: Vascular Endothelial Growth Factor-2

INTRODUCTION

Thyroid hormones (THs) play critical roles in growth, differentiation, and metabolism. In fact, TH is required for normal function of nearly all tissues, with major effects on oxygen consumption and metabolic rate (**Oppenheimer** et al., **1987**).

It has become increasingly clear that adequate levels of circulating THs are of primary importance for normal female reproductive functions. In both humans and animals, disturbed TH levels resulted in menstrual disturbances, impaired fertility, and altered pituitary gonadotropin secretion (**Dellovade** et al., **1996**; and **Longcope, 1991**).

Ovulation is a natural recurrent inflammatory reaction causing regular and frequent local injury to the ovarian surface during follicular rupture (**Rae** and **Hillier, 2005**).

Failure of ovulation is linked with severe hypothyroidism, and menstrual irregularities occur in both hypo- and hyperthyroidism (**Krassas**, **2000**). However, the mechanisms by which THs impact ovarian function remain poorly understood since they have broad ranging effects throughout the body (**Boelaert** and **Franklyn**, **2005**).

Accumulating evidence has shown that a majority of ovarian cancers are thought to arise from the ovarian surface epithelial (OSE) cell layer (**Auersperg** et al., **2001**), possibly due to gene mutation caused by repeated episodes of inflammation-associated DNA damage (**Beachy** et al., **2004**).

The inflammation-associated gene, cyclooxygenase-2 (COX2), encodes prostaglandin synthase-2, which is responsible for pro-inflammatory prostaglandin biosynthesis (**Sirois** et al., **2004**). Some evidence suggests that COX-2 could contribute to the early stages of ovarian cancer formation, as the enzyme was found to be over-expressed in the pre-neoplastic changes of the OSE (**Roland** et al., **2003**).

The significance of estrogen in the etiology of ovarian carcinoma has been emphasized by the fact that anti-estrogenic intervention may inhibit the growth of ovarian carcinoma both in vitro and in vivo (**Langdon** et al., **1994**).

Further studies have reported that estrogen replacement therapy may even induce ovarian cancer (**Lacey** et al., **2002**). On the other hand, opposing studies reported that this estrogen role in inducing tumor progression may not be precise (**Chadwick** et al., **2005**). Thus, the impact of estrogen in ovarian tumors is a matter of debate.

Inter-relationships between THs and estrogen actions have been documented with regard to a variety of physiological functions involving both hormones, such as growth of the uterine epithelium, change in bone density and determination of sexual behavior (**Vasudevan** et al., **2002**). Indeed, up-regulation of estrogen receptors by the THs in the rat pituitary cell lines was reported by **Fujimoto** et al. (**2004**).

It was recently suggested that chronic hyperthyroidism might contribute to a state in which OSE cells are more susceptible to neoplastic transformation (**Balkwill** et al., **2005**).

Furthermore, the OSE cells are established sites of progesterone action (**Lau** et al., **1999**). It was reported that progesterone may exhibit immuno-modulatory properties mediated via progesterone receptors (**Van der Burg B** and **van der Saag, 1997**). In this issue, progesterone has been shown to inhibit IL-1α activity (**De Oliveira** et al., **2007**). However, the underlying receptor-mediated mechanisms are not fully understood (**Peluso** et al., **2008**).

Based on the above mentioned findings, the present work aimed to study the effect of hyperthyroidism on the ovarian function in female rats. Measurement of COX2 gene expression in the ovary was planned to further investigate the possible pro-inflammatory role of thyroxine administration on the ovarian tissues. Furthermore, this work aimed to clarify whether this thyroid hormone action on the ovary is mediated through modulation of the secretion of sex steroids (estrogen and progesterone) and/or their receptors expression.

An additional objective in this study was to examine the exact role exerted by ERs on the ovarian events in hyperthyroidism by administrating tamoxifen, one of the selective ER modulators. Furthermore, we extended our study to investigate whether progesterone can impact a possible anti-inflammatory protective role in these events.

REVIEW

THYROID HORMONES

The thyroid gland is an important endocrine organ which is located in the neck region in mammals. It regulates almost all body functions, including growth, development, reproduction, digestion, neural function, as well as the cardiovascular system (**Ganong**, **2005**). The thyroid gland which is composed of two lateral lobes joined by an isthmus is the first endocrine gland to start developing in the embryo (**Sankar** et al., **2009**).

The gland produces thyroid hormones (THs) and calcitonin in two distinct cell types; the thyroid follicular cells and the parafollicular or C cells, respectively. The thyroid follicular cells form the thyroid follicles, whereas the C cells are scattered in the inter-follicular space (**Mauchamp** et al., **1998**). The two diverse cell types, responsible for the dual endocrine function of the gland, originate from different embryological origin (**Fontaine**, **1979**).

SYNTHESIS AND RELEASE OF THYROID HORMONES

The thyroid gland releases a combination of T_4 (thyroxine; 3, 3', 5,5' -tetra-iodothyronine), and T_3 (3,3',5-tri-iodothyronine), in a ratio of approximately 17:1 (**Pilo** et al., **1990**). Iodide is actively transported and concentrated into the thyroid gland by the Na^+/I^- symporter (**Dai** et al., **1996**). The trapped iodide is oxidized and incorporated into the tyrosine residues of the glycoprotein; thyroglobulin. Eventually, this yields monoiodinated and diiodinated residues (MIT, monoiodo-tyrosines; DIT, diiodo-tyrosines) that are enzymatically coupled to form T_4 and T_3. The iodinated thyroglobulin containing MIT, DIT, T_4, and T_3, is then stored as

an extracellular storage polypeptide in the colloid within the lumen of thyroid follicular cells (**De Vijlder** et al., **1997**).

The secretion of THs requires endocytosis of the stored iodinated thyroglobulin from the apical surface of the thyroid follicular cell. The internalized thyroglobulin is then incorporated in phagolysosomes where it undergoes proteolytic digestion, releasing THs into the circulation via the basal surface (**Taurog**, **1996**).

The majority of the released THs are in the form of T_4, as total serum T_4 is 40-fold higher than serum T_3. Only 0.03% of the total serum T_4 is free (unbound), with the rest bound to carrier proteins such as thyroxine binding globulin (TBG), albumin, and thyroid binding pre-albumin. Approximately 0.3% of the total serum T_3 is free, with the rest bound to TBG and albumin. It is the free TH that enters target cells and generates a biological response (**Yen**, **2001**).

EXTRA-THYROIDAL PRODUCTION OF THYROID HORMONES

Several reports in the literature have suggested an extra-thyroidal source of thyroid hormone. **Meischl** et al. (**2008**) reported that cardiomyocytes may produce thyroid hormone under specific experimental conditions. Furthermore, the expression of the transporters that act through thyroid hormone secretion (**Cosmo** et al. **2010**) and I^- intake (**de Carvalho** and **Quick**, **2011**) have been reported in extrathyroidal organs.

An interesting observation was that although serum thyroid hormones (T_4, T_3 and rT_3) were decreased as a result of surgical thyroidectomy, yet, they were not completely abolished. Therefore, it was

concluded that in thyroidectomized rats, serum T_4 may be maintained at a constant low level by T_4 supply from extra-thyroidal tissues (**Nagao** et al., **2011**).

REGULATION OF THYROID HORMONE SECRETION

Thyroid hormone synthesis and secretion is regulated through a negative-feedback system that involves the hypothalamus, pituitary, and thyroid gland (hypothalamic/pituitary/thyroid axis) (**Shupnik** et al., **1989**). Thyrotropin releasing hormone (TRH) is a tripeptide synthesized in the paraventricular nucleus of the hypothalamus. It is transported via axons to the median eminence and then to the anterior pituitary via the portal capillary plexus. TRH binds to TRH receptors in pituitary thyrotropes, a subpopulation of pituitary cells that secrete thyroid stimulating hormone (TSH) (**Yen**, **2001**).

The glycoprotein hormone, TSH, is composed of α- and β-subunits designated as glycoprotein hormone α- and TSH β-subunits. Both TRH and TSH secretion are negatively regulated by TH (**Yen**, **2001**). Besides being the primary regulator of TH secretion, TSH also plays a critical role in the thyroid gland growth and development. It's noteworthy that, a number of thyroid genes, including Na^+/I^- symporter, thyroglobulin, and thyroid peroxidase, are stimulated by TSH, and promote the synthesis of TH (**Paschke** and **Ludgate**, **1997**).

GENDER DIFFERENCES IN THE THYROID HORMONE

Females experience more thyroid problems throughout life, such as goiter, nodules, and autoimmune disorders (**Castanet** et al., **2001**). Excluding cancer, the lifetime prevalence of thyroid disease for women is about 4 to 10 times the estimates for men (**Whybrow**, **1995**).

Major depression is more common in women; with approximately 5-10% having some metabolic evidence of an impaired thyroid function. Interestingly, adjunctive T_3 was shown to speed recovery from depression in women but not in men (**Whybrow**, **1995**).

THYROID HORMONE CELLULAR UPTAKE

Trans-membrane passage of THs has been thought to be passive, given their lipophilic nature. However, it is now evident that TH uptake is transporter and energy dependent (**Hennemann**, **2005**), and that the intracellular TH levels are highly variable in different tissues (**Quignodon** et al., **2004**).

This TH passage is achieved via thyroid hormone transporters, which have different tissue distributions and ligand affinities (**Hennemann**, **2005**). The TH transporters, unlike many other transporting molecules, have a high affinity and consequently a high specificity for THs. The activity of these transporters in any given tissue is likely to be a key factor in determining the effect of serum T_3 concentration on intracellular T_3 levels (**Dayan** and **Panicker**, **2009**).

LOCAL REGULATION OF THYROID HORMONE ACTIONS

The active cellular form of TH is T_3, which is largely produced through the deiodination of T_4 in extrathyroidal organs (**Greenspan** and **Greenspan**, **1999**). The conversion of T_4 to T_3 is catalyzed by iodothyronine deiodinases, which influence the relative balance of these hormones in the circulation (**Bianco** and **Kim**, **2006**). Therefore, a change in serum TH concentrations, especially T_3, is very often related to altered deiodinase activity (**Panda** and **Kar**, **2007**).