SAGE-Hindawi Access to Research
Journal of Thyroid Research
Volume 2011, Article ID 875125, 7 pages
doi:10.4061/2011/875125

Review Article

Role of Estrogen in Thyroid Function and Growth Regulation

Ana Paula Santin[1] and Tania Weber Furlanetto[1,2]

[1] *Postgraduation Program in Medicine and Medical Sciences, Federal University of Rio Grande do Sul, 90035-003 Porto Alegre, RS, Brazil*
[2] *Divisão de Medicina Interna, Hospital de Clínicas de Porto Alegre, Universidade Federal do Rio Grande do Sul, Rua Ramiro Barcellos 2350/700, 90035-903 Porto Alegre, RS, Brazil*

Correspondence should be addressed to Tania Weber Furlanetto, taniafurlanetto@gmail.com

Received 14 January 2011; Accepted 24 February 2011

Academic Editor: Guillermo Juvenal

Thyroid diseases are more prevalent in women, particularly between puberty and menopause. It is wellknown that estrogen (E) has indirect effects on the thyroid economy. Direct effects of this steroid hormone on thyroid cells have been described more recently; so, the aim of the present paper was to review the evidences of these effects on thyroid function and growth regulation, and its mechanisms. The expression and ratios of the two E receptors, α and β, that mediate the genomic effects of E on normal and abnormal thyroid tissue were also reviewed, as well as nongenomic, distinct molecular pathways. Several evidences support the hypothesis that E has a direct role in thyroid follicular cells; understanding its influence on the growth and function of the thyroid in normal and abnormal conditions can potentially provide new targets for the treatment of thyroid diseases.

1. Introduction

Thyroid diseases are more prevalent in women particularly between puberty and menopause [1], and women are more susceptible to the goitrogenic effect of iodine deficiency [2]. Carcinomas of the thyroid are three-times more frequent in women than in men, and the peak rates occur earlier in women [3]. These epidemiological data suggest a role of estrogen in the pathogenesis of thyroid diseases.

Estrogen has a well-known indirect effect on thyroid economy, increasing the thyroxine binding globulin [4], and the need for thyroid hormone in hypothyroid women [5]. Direct effects of estrogen on thyroid cells have been described more recently [6], so the aim of the present paper was to review the evidences of these effects on thyroid function and growth regulation, and its mechanisms.

2. Estrogen and Its Receptors

17-β-estradiol (E2) is a lipophilic hormone with low-molecular weight that occurs naturally. Cellular signaling of estrogen is mediated classically upon the binding on two soluble intracellular nuclear receptors, estrogen receptor (ER) alpha, and ER beta [7]. The isoform β is smaller than the isoform α, and the DNA-binding domains of both subtypes are highly conserved. After binding of E2, ER forms a stable dimer that interacts with specific sequences called estrogen response elements (EREs) to initiate the transcription of target genes. Ligand-bound ERs can also interact with other transcription factors complexes and influence transcription of genes that do not harbor EREs. Third and fourth mechanisms of ERs regulatory actions are, respectively, non-genomic and the ligand independent pathway. A variety of rapid signaling events such as activation of kinases and phosphatases and increases in ion fluxes across membranes has been described. These and other aspects of signaling and targets of ERs have been reviewed recently [7].

Recently, a transmembrane intracellular nonclassical ER mediating rapid cell signaling was described, a G protein-coupled receptor (GPCR), named GPR30 [8].

2.1. Expression of ERs in Human Thyroid Tissue. Classically, the presence of ER is fundamental for a direct action of estrogen in a given cell. ER has been described in both neoplastic and nonneoplastic human thyroid tissues, but the results are discordant. Immunohistochemical assays,

Table 1: Estrogen receptor (ER) in human normal thyroid, and benign and malignant thyroid diseases.

Study	Method	Normal	All benign lesions	All neoplastic lesions	All carcinoma	Benign lesions		Carcinoma			
						Adenoma	Goiter	Papillary	Follicular	Medullary	Anaplastic
Tavangar et al. [10]; 2007	IHC					8/37	31/130	37/119	2/18	0/35	0/12
Arain et al. [11]; 2003	IHC	0/25				0/9	0/8	0/19	0/10	0/4	
Lewy-Trenda et al. [12]; 1998	IHC					2/19	0/20	4/8	3/5		0/4
Valle et al. [13]; 1998	RT-PCR	28/33				12/12	6/7	26/26	1/1	1/1	1/1
Bonacci et al. [14]; 1996	DCC	26/38	11/28		7/20						
Jaklic et al. [15]; 1995	IHC					0/1	0/5	0/4		0/1	
Colomer et al. [16]; 1996	IHC									1/7	
Inoue et al. [17]; 1993	IHC							24/74			
Inoue et al. [18]; 1993	IHC							18/70			
Yane et al. [19]; 1994	RT-PCR			5/27							
Yane et al. [20]; 1993	IHC	0/10			2/19	2/12	0/7				
Hiasa et al. [21]; 1993	IHC					44/130	23/39	19/115	7/23		0/6
Diaz et al. [22]; 1991	IHC					20/30		23/30	11/20		
Mizukami et al. [23]; 1991	IHC		8/18			4/8		47/62			
Takeichi et al. [24]; 1991	IHC							11/12			1/6
Hong et al. [25]; 1991	IHC							1/27	1/20		
Miki et al. [26]; 1990	DCC	0/14	12/46		7/23	5/11	2/12	6/20	0/1		1/1
Haruta et al. [27]; 1990	IHC							30/52			0/12
Chaudhuri et al. [28]; 1989	SDG	3/8				7/9	5/23	8/8		0/6	
Money et al. [29]; 1989	IHC		20/22								
Clark et al. [30]; 1985	SDG			14/15							
Hampl [15]; 1985	RBA	0/8			0/5						
Molteni et al. [37]; 1981	SDG	0/2						2/4			

Data are shown as number of ER-positive samples/total number of samples. IHC: immunohistochemical assay; DCC: dextran-coated charcoal assay; RT-PCR: reverse transcriptase-polymerase chain reaction technique; SDG: sucrose density gradient assay; RBA: radioligand binding assay.

Table 2: Estrogen receptors (ER) α and β in human normal thyroid, and benign and malignant thyroid diseases, by immunohistochemistry (IHC).

Study	Isoform	All benign	All carcinoma	Benign lesions		Carcinoma			
				Adenoma	Goiter	Papillary	Follicular	Medullary	Anaplastic
Vaiman et al. [31]; 2010	ERα			0/34	0/150	0/90	0/6	0/4	0/5
	ERβ			30/34	126/150	60/90	4/6	3/4	3/5
Winters et al. [32]; 2010	ERα					1/1			
Vannucchi et al. [33]; 2010	ERα		12/38						
Cho et al. [34]; 2007	ERα							10/11	
	ERβ							8/11	
Bléchet et al. [35]; 2007	ERα							0/28	
	ERβ							26/28	
Ceresini et al. [36]; 2006	ERα				0/17	0/17			
	ERβ				17/17	14/17			

Data are shown as number of ER-positive samples/total number of samples.

with monoclonal antibodies, are the most commonly used methods for establishing receptor status. As may be seen in Table 1, some studies have found ER-positivity in normal and abnormal thyroid tissue while others have not detected ER protein in any tissue studied. This discrepancy could be due to methodological issues; the development of monoclonal antibodies against ER with high sensitivity and specificity, and others factors such as tissue fixation, tissue processing, interpretation of immunohistochemistry, and *cutoffs* for positive results, could have contributed to the sensitivity of the techniques employed [9].

2.2. Expression of ERα and ERβ in Human Thyroid Tissue. ER expression in human thyroid was first reported in 1981 [37]. ERα was first described in 1973 [38], and ERβ was identified in 1996 [39], so only from this moment on it was possible to evaluate the relationship between isoforms of ERs in thyroid tissue. An important role of different patterns of distribution and expression of subtypes ERs in thyroid carcinoma has been proposed: estrogen binding to ERα would promote cell proliferation and growth, and, in contrast, ERβ would promote apoptotic actions and other suppressive functions in thyroid tumors, as reviewed by Chen et al. [40]. Then, ERα : ERβ ratio could have a role in the pathophysiology of thyroid cancer [40], similar to that postulated for breast cancer [41].

In differentiated thyroid follicular tumors, the expression of ERα has been associated with well-differentiated tumors and reduced incidence of disease recurrence [54]. ERα protein [55] and ERα mRNA [19, 56] are expressed in normal and neoplastic follicular cells of the thyroid. Also, the expression of ERα and ERβ was detected in human medullary thyroid cancer [34] with an increased ratio of ERα/ERβ, suggesting a possible role in tumor growth and progression. A few studies evaluated ERα and ERβ expression in normal and abnormal thyroid tissue, as shown in Table 2.

The effects of the agonists of ERα and ERβ, respectively, propyl-pyrazole-triol (PPT) and diarylpropionitrile (DPN), in the proliferation of thyroid cancer cell lines has been studied: PPT had a stimulatory effect, while inhibition of proliferation and DNA fragmentation were observed after DPN [45]. In the same study, small interference ribonucleic acid (siRNA) blocking ERα or ERβ demonstrated that knockdown of the ERα attenuated E2-mediated B-cell lymphoma 2 (Bcl-2) expression, an important antiapoptotic protein, while knockdown of the ERβ enhanced E2-induced Bcl-2 expression [45].

2.3. Expression of GPR30 in Thyroid Cells Lines. Growing evidence suggests that estrogens are also able to exert non-genomic events mediated by GPR30 [8]. Vivacqua and colleagues analyzed the effects of E2 and the phytoestrogen genistein in human follicular thyroid carcinoma cell lines, WRO and FRO, and ARO, a human anaplastic thyroid carcinoma cell line [46]. Both hormones stimulated *in vitro* proliferation of these cell lines through the GPR30 and mitogen-activated protein kinase signaling cascade [46]. In other human benign and malignant thyroid tissue, the expression of GPR30 has not been studied.

3. Response to E2 Stimulation *In Vitro*

3.1. Proliferation. Several studies described proliferation of thyroid cells induced by E2, as shown in Table 3. Some of the most commonly used assays are incorporation of bromodeoxyuridine (BrdU) [6], 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) [45, 47, 50, 57], [(3)H]-thymidine incorporation [48, 52, 53], and trypan blue solution [43]. Cotreatment with ICI182780, fulvestrant, an antagonist of E2 by inhibition and degradation of ER [58], significantly attenuated these proliferative effects.

Based in these studies, E2 increases proliferation of thyroid cells.

3.2. ER-Dependent Effects on Thyroid Differentiation Proteins. Few studies evaluated E2 effect on gene transcription of

Table 3: E2 effects on thyroid protein expression, function, and proliferation *in vitro*.

Study	Thyroid cells	Presence of ERα/ERβ	Erα expression	Erβ expression	Proliferation	Nis expression	Iodide uptake	TG mRNA
Kumar et al. [42]; 2010	NPA87	ERα+/ERβ+			↑			
	KAT5	ERα+/ERβ+			↑			
	WRO	ERα+/ERβ+			↑			
Rajoria et al. [43]; 2010	BCPAP	ERα+/ERβ+			↑			
	Nthy-3-1	ERα+/ERβ+			↑			
Zeng et al. [44]; 2008	KAT5	ERα+/ERβ+	↑	0	↑			
	FRO	ERα+/ERβ+	0	↑	↓			
Zeng et al. [45]; 2007	KAT5	ERα+/ERβ+	↑	0	↑			
	FRO	ERα+/ERβ+	↑	↑	↑			
	ARO	ERα+/Erβ+	↑	0	↑			
Vivacqua et al. [46]; 2006	WRO	ERα+/ERβ−			↑			
	FRO	ERα+/ERβ−			↑			
	ARO	ERα−/ERβ−			↑			
Lee et al. [47]; 2005	KAT5				↑			
Banu et al. [48]; 2001	NPA87	ER+		↑	↑			
	WRO	ER+			↓			
Manole et al. [6]; 2001	HTC-TSHr	ERα+/ERβ+	↑	↑	↑			
	Goiter	ERα+/ERβ+	↑	↑	↑			
	XTC 133				↑			
Furlanetto et al. [49]; 2001	FRTL-5				↑		↓	
Furlanetto et al. [50]; 1999	FRTL-5	ERα+			↑	↓		
Nagy et al. [51]; 1999*	Mng				↑			
	Ca				↑			
	Ade				↓			
Del Senno et al. [52]; 1989**	N				↑			↑
	Ade				↑			↑
	Ca				0			0
Yang et al. [53]; 1988	TT				↑			

Estrogen receptor (ER) +: presence of expression, –: absence of expression; NPA87, KAT5, and BCPAP: human papillary thyroid carcinoma cell lines; WRO and FRO: human follicular thyroid carcinoma cell lines; Nthy-3-1: human normal transformed thyroid cell line; ARO: human anaplastic thyroid carcinoma cell line; HTC-TSHr: human thyroid carcinoma cell line lacking endogenous TSH receptor; XTC-133: thyroid cancer cell line of Hurthle cell origin; FRTL-5: Fischer rat thyroid cell line. Mng: multinodular goiter; Ca: carcinoma; Ade: adenoma; N: normal thyroid; TT: human medullary thyroid carcinoma cell line; ↑: increase, ↓: decrease, and 0: no effect, after E2 exposure. *: thyroid tissue obtained in surgical resection, under organotypic culture conditions for 48 hours; **: suspension cultures of thyroid follicles.

differentiation proteins in thyroid cells. In Fischer rat derived thyroid cell line, FRTL-5, E2 treatment decreased the sodium-iodide symporter (NIS) gene expression [50], and the iodide uptake [49]. E2 increased the thyroglobulin gene expression in suspension cultures of human thyroid follicles of adenoma and carcinoma [52]. These data are shown in Table 3. The opposite effects of E2 on the NIS gene expression and iodide uptake, in FRTL-5 cells, and the thyroglobulin gene expression, in suspension culture of thyroid cells, could be due to the different systems studied; it cannot be excluded that estradiol affects these genes by different intracellular pathways. These results, together with the increase in cell growth caused by estrogen, could implicate this hormone in the pathogenesis of goiter and thyroid carcinoma; nevertheless, as just one study evaluated the effect of estrogen on thyroid differentiated proteins in human thyroid tissue, more studies should be done to better understand the role of estrogen in thyroid differentiated protein expression.

3.3. Non-Genomic Effects of E2. Some of the actions of E2 in the proliferation of thyroid cells are mediated by the activation of signal transducing pathways, as shown in

Table 4: Non-genomic estrogen effects on thyroid cells.

Study	Cells	GPR30	MAPK	PI3k	Cyclin D1	*c-fos*	BcL-2	Bax
Kumar et al. [42]; 2010	NPA87	−	↑	↑				
	KAT5	−	↑	↑	↑			
	WRO	+	↑	↑	↑			
Zeng et al. [45]; 2007	KAT5						↑	↓
	FRO						↑	↓
	WRO						↑	↓
Vivacqua et al. [46]; 2006	WRO	+	↑		↑	↑		
	FRO	+	↑		↑	↑		
	ARO	+	↑		↑	↑		
Manole et al. [6]; 2001	HTC-TSHr		↑		↑			
	Goiter		↑		↑			
	XTC 133		↑		↑			

NPA87 and KAT5: human papillary thyroid carcinoma cell lines; WRO and FRO: human follicular thyroid carcinoma cell lines; HTC-TSHr: human thyroid carcinoma cell line lacking endogenous TSH receptor; XTC-133: thyroid cancer cell line of Hurthle cell origin; Goiter: primary culture of human thyroid cells isolated from goiter nodules. (+): presence of expression; (−) absence of expression; (↑): increase, (↓): decrease, and (0): no effects, after E2 exposure.

Table 4. E2 can induce activation of phosphatidylinositol 3-kinase (PI3K) [42] and phosphorylation of extracellular signal-regulated kinase 1/2 (ERK1/2) in follicular thyroid carcinoma cells, mainly due to interaction via membrane-associated ER [42, 45, 46]. PI3K and Erk1/2 signaling may play a critical role in preventing apoptosis and inducing cell cycle progression by induction of key genes expression [59].

Expression of early response genes and regulatory genes of the cell cycle are necessary for proliferation of cells. As E2 has been demonstrated to stimulate the growth of thyroid cells, it is important to study the expression of key cell-cycle genes such as cyclin D1 after stimulation with E2. Cyclin D1 regulates the cell progression cycle facilitating G_1 to S phase transition and also has an estrogen-responsive regulatory region [60], that is likely different from the canonical EREs. Overexpression of cyclin D1 in thyroid malignancies has been reported [61–65], moreover, its expression has been associated with an aggressive behavior in papillary thyroid microcarcinomas, because over 90% of the metastasizing microcarcinomas expressed cyclin D1 [66].

E2 significantly increased the expression of cyclin D1 in a human thyroid carcinoma cell line lacking endogenous TSH receptor (HTC-TSHr cells), and in a thyroid cancer cell line of Hurthle cell origin (XTC-133), which was abolished by PD.098059 that blocked G0/G1 to S phases [6]. E2 upregulated cyclins A and D1, as well as the proto-oncogene *c-fos*, in WRO, FRO, and ARO cells [46]. Cyclin D1 was also shown to be upregulated by E2 in KAT5, a papillary thyroid cancer cell line, and WRO cells [42].

Together, these results are very compelling, pointing to an ability of E2 to regulate genes mediating cell cycle progression in thyroid cells, and potentially contributing to the pathogenesis of thyroid cancer or thyroid hyperplasia.

4. Conclusions

There are evidences that estrogen may have direct actions in human thyroid cells by ER-dependent mechanisms or not, modulating proliferation, and function. Different patterns of distribution, expression, and ratios of ERα and ERβ may have a role in thyroid cancer cells proliferation, as well as in the outcome of thyroid cancer. Studying estrogen effects on thyroid cells is a potential tool to better understand the pathogenesis of thyroid diseases, and to develop targets to its treatment. Further studies on the influence of E2 on the growth and function of the thyroid are needed, preferably in primary culture of normal and abnormal human thyroid cells.

Conflict of Interests

The authors declare that there is no conflict of interests.

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