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Local thyroid renin–angiotensin system in experimental breast cancer

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ABSTRACT

An association between breast cancer and thyroid dysfunction exists although the underlying mechanisms remain to be elucidated. Numerous studies have characterized the role of thyroid hormones in controlling the synthesis and secretion of renin–angiotensin system (RAS) components, but little information is available on the putative role of the local RAS on thyroid function.

Aims: Here we analyze several soluble and membrane-bound RAS-regulating aminopeptidase activities in thyroid gland from rats with mammary tumors and the relationship with the circulating levels of thyroid stimulating hormone (TSH) and free thyroxin (fT_4).

Main methods: We analyze soluble and membrane-bound RAS-regulating aminopeptidase activities fluorometrically using their corresponding aminoacyl-β-naphthylamide as the substrate.

Key findings: We have found in rats with mammary tumors a concomitant change of thyroid RAS-regulating enzymes and thyroid hormone production.

Significance: We suggest that existence of alterations in the regulatory mechanisms mediated by the angiotensins of the local tissue RAS as a consequence of the carcinogenic process which could act alone or in combination with alterations at a higher level of regulation such as the hypothalamus–pituitary axis.

Introduction

Many studies have shown an association between breast cancer and thyroid dysfunction, being hypothyroidism the most frequently observed finding. In fact, many reports even considered hyperthyroidism to be protective against breast cancer because the cancer progressed when the hyperthyroidism was treated (Michalaki et al., 2009; Smyth, 1997, 2003; Smyth et al., 1998). Thus, the hypothesis gained ground that higher levels of thyroid hormones were beneficial because of restricted breast cancer growth, while lower levels were detrimental because of facilitated such growth (Smyth, 1997).

We have recently described, using the N-methyl-nitrosourea (NMU)-induced rat model of mammary tumors, a significant decrease in both thyroid hormone thyroid-stimulating hormone (TSH) and free thyroxin (fT_4) circulating levels in rats with breast cancer, supporting a decreased thyroid function under these experimental conditions (Carrera-González et al., 2011) in the same way that has been majorly described for women with the illness (Gago-Dominguez and Castelao, 2008).

Although it is known that a relationship between thyroid function and the renin–angiotensin system (RAS) exists (Catanzaro and Marzi, 1974; Jimenez et al., 1984; Marchant et al., 1993; Montiel and Jimenez, 1998; Ruiz et al., 1987) it is actually unknown if the components of the local thyroid RAS exert any regulating control of thyroid function. In fact, the RAS is more complex than originally thought, because it operates not only as a circulating endocrine system but also as a local tissue system (Dzau and Herrmann, 1982) playing an important role in the function of the organ. The local RAS appears to be regulated independently from the circulating system, although it can interact with it. Hence, the RAS is a paracrine and intracrine system as well as an endocrine system (Fyhrquist and Saijonmaa, 2008).

In RAS system, several biologically active peptides and regulatory proteolytic enzymes are involved. Angiotensinogen (AGT) is converted into the inactive decapeptide angiotensin I (AngI) by the action of renin. Angiotensin-converting enzyme (ACE), the main effector of RAS, further converts AngI to AngII that acts on angiotensin II type 1 and 2 receptors (AT_1R and AT_2R). Angiotensin II degradation begins with the action of aspartyl aminopeptidase (AspAP) and aminopeptidase A (APA), which remove the N-terminal Asp to produce angiotensin III (AngIII), a less potent vasoconstrictor peptide than AngII (Marc and Llorens-Cortes, 2011).

AngIII is also produced from angiotensin I (AngI) through the production of des-Asp1-AngI, which is further converted to AngIII by the action of ACE. AngIII is further converted to angiotensin IV (AngIV) by aminopeptidase B or aminopeptidase N (Ardaillou and Chansel,

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1997). Whereas AngI is considered inactive, AngII and AngIII are full agonists at the AT_1 and AT_2 receptors subtypes (AT_1R and AT_2R). Also, AngIV binds with low affinity at the AT_1R and AT_2R, but with high affinity and specificity at the AT_4 receptor subtype (AT_4R). Furthermore, there is evidence that the AT_4R may be the insulin-regulated aminopeptidase (IRAP) (Albiston et al., 2001; Chai et al., 2008).

Numerous studies have been performed to characterize the physiological role of thyroid hormones in controlling the synthesis and secretion of RAS components (Marsigliante et al., 2003; Carneiro-Ramos et al., 2006; Diniz et al., 2009). However, to our knowledge, little information is available on the putative role of the local RAS on thyroid function. Thus, only the expression of AT_1R in rat thyroid was confirmed by Marsigliante et al. (2003).

In the present report, we analyze several soluble and membrane-bound RAS-regulating specific proteolytic regulatory enzyme activities in thyroid gland from control rats, from rats with mammary tumors induced by NMU and from rats treated with NMU which did not develop breast cancer. Their relationship with the circulating levels of TSH and fT_4 was also evaluated. They may reflect the functional status of their target peptides on both physiological conditions and under the specific conditions brought about the tumor process.

Materials and methods

Animals and treatment

Forty female virgin Wistar rats (164.7 ± 4.7 g body weight) were used in this work. The animals were provided from the animal house-care of the University of Jaén, and maintained in an environment controlled under constant temperature (25 °C) with a 12 h light/dark cycle. All animals were allowed access to water and food ad libitum. The experimental procedures for animals use and care were in accordance with the 86/609/EEC European Community Council directive. The rats were randomly divided into two groups. One group was injected intraperitoneally with three doses of 50 mg/kg body weight of NMU dissolved in distilled water (10 mg/ml) at 50, 80 and 110 days after birth, as described previously (Carrera-González et al., 2011). All rats were in estrous at the first NMU injection as verified by daily vaginal smears. Control group received the vehicle only. For tumor detection and growth control, rats were examined by palpation 2 days each week after the second NMU injection. NMU-treated rats that did not develop mammary tumors were also included in the study (NMU non-BC group) to analyze the influence of NMU per se in thyroid changes, independently of the presence or not of mammary tumors.

Sample preparation

After 122 days of first NMU injection, animals were sacrificed under equithesin anesthesia (2 ml/kg body weight). Thyroid gland was quickly removed, homogenized in 10 volumes of 10 mM HCl–Tris buffer (pH 7.4) and ultracentrifuged at 100,000 ×*g* for 30 min at 4 °C. The resulting supernatant was used to measure soluble enzymatic activity and protein content, assayed in triplicate. To solubilize membrane-bound proteins, the pellets were rehomogenized in HCl–Tris buffer (pH 7.4) plus 1% Triton X-100. After centrifugation (100,000 ×*g*, 30 min, 4 °C), the supernatants were used to measure solubilized membrane-bound activity and proteins, also in triplicate. To ensure complete recovery of activity, the detergent was removed from the medium by adding to the samples adsorbent polymeric Biobeads SM-2 (100 mg/ml); Bio-Rad (Richmond, CA) and shaking for 2 h at 4 °C. Proteins were quantified by the method of Bradford, using bovine serum albumin (BSA) as standard.

Renin–angiotensin system-regulating aminopeptidase assays

Aspartyl aminopeptidase activity assay

Specific AspAP activity was determined fluorometrically using aspartyl-β-naphthylamide (AspNNap) as the substrate, according to the method previously described by us (Ramírez-Expósito et al., 2001). Briefly, 10 μL of each sample was incubated in triplicate for 30 min at 37 °C with 100 μL of the substrate solution: 100 μM AspNNap, 1.3 μM ethylenediaminetetraacetic acid (EDTA) and 2 mM $MnCl_2$ in 50 mM of phosphate buffer, pH 7.4.

Aminopeptidase A activity assay

Specific APA activity was measured fluorometrically using glutamyl-β-naphthylamide (GluNNap) as the substrate, as previously described (Ramírez-Expósito et al., 2001). Ten microliters of each supernatant was incubated in triplicate for 30 min at 37 °C with 100 μL of the substrate solution: 100 μM GluNNap, 0.65 mM dithiothreitol (DTT) and 50 mM $CaCl_2$ in 50 mM of phosphate buffer at pH 7.4.

Aminopeptidase N and aminopeptidase B assays

Specific APN and APB activities were measured fluorimetrically using alanyl-β-naphthylamide (AlaNNap) or arginyl-β-naphthylamide (ArgNNap) as the substrate, according to the method previously described by us (García et al., 2003). Briefly, 10 μL of each sample was incubated in triplicate for 30 min at 37 °C with 100 μL of the substrate solution: 100 mM AlaNNap, or 100 mM ArgNNap and 0.65 mM dithiothreitol (DTT) in 50 mM of phosphate buffer at pH 7.4.

Insulin-regulated aminopeptidase activity assay

Specific IRAP activity was measured fluorometrically using leucyl-β-naphthylamide (LeuNNap) as substrate. Ten microliters of each sample was incubated in triplicate for 30 min at 37 °C with 100 μL of the substrate solution containing 100 μM of LeuNNap and 0.65 mM dithiothreitol (DTT) in 50 mM phosphate buffer, pH 7.4.

All the reactions were stopped by adding 100 μL of 0.1 M acetate buffer at pH 4.2. The amount of β-naphthylamine released as the result of the enzymatic activities was measured fluorimetrically at 412 nm emission wavelength with and excitation wavelength of 345 nm. Specific soluble and membrane-bound AspAP, APA, APN, APB and IRAP activities were expressed as picomoles of AspNNap, GluNNap, AlaNNap, ArgNNap and LeuNNap, hydrolyzed per min per mg of protein, by using a standard curve prepared with the latter compound under corresponding assay conditions.

Thyroid stimulating hormone (TSH) assay

TSH levels were measured by paramagnetic-beads based chimioluminiscent immunoassay from Beckman-Coulter, according to manufacturer's instructions. The lower limit of assay detection is 0.003 μU/mL. Intra-assay coefficient of variation is between 6.18 and 15.72%. Inter-assay coefficient of variation is between 4.88 and 12.70%.

Free thyroxin (fT_4) assay

fT_4 levels were also measured by paramagnetic-beads based chimioluminiscent immunoassay from Beckman-Coulter, according to manufacturer's instructions. The lower limit of assay detection is 0.15 ng/dL. Intra-assay coefficient of variation is between 1.66 and 4.13%. Inter-assay coefficient of variation is between 4.56 and 7.42%.

Statistical analysis

Data were analyzed by one way ANOVA plus Newman–Keul's test, using IBM Pass V.19. All comparisons with *p*-values below 0.05 were considered significant.

Results

Specific AspAP and APA activities in thyroid gland of control and NMU-treated rats with and without mammary tumors are shown in Fig. 1. Soluble specific AspAP activity significantly increased ($p < 0.05$) in thyroid tissue from NMU-treated animals with mammary tumors when compared with control animals and NMU-treated animals which did not develop mammary tumors. Also, a significant increase ($p < 0.01$) appeared in the membrane-bound fraction of this group of animals (Fig. 1A). On the contrary, APA activity did not show significant differences between groups either in soluble or membrane-bound fractions (Fig. 1B). Specific APN and APB activities are shown in Fig. 2. Soluble APN activity significantly decreased ($p < 0.001$) in thyroid tissue from NMU-treated rats with mammary tumors when compared with control animals and NMU-treated animals which did not develop mammary tumors, whereas membrane-bound APN activity did not show significant differences between groups (Fig. 2A). In the same way, soluble APB activity significantly decreased ($p < 0.001$) in thyroid tissue from NMU-treated rats with mammary tumors when compared with control animals and NMU-treated animals which did not develop mammary tumors, whereas membrane-bound APB activity did not show significant differences between groups (Fig. 2B). Finally, IRAP activity did not show significant differences between groups either in soluble or membrane-bound fractions (Fig. 3).

Table 1 shows circulating levels of both TSH and fT4 in control rats and rats treated with NMU that developed or not mammary tumors. Significant decreases were observed in both hormones ($p < 0.05$ for TSH and $p < 0.01$ for fT4) in NMU-treated rats with mammary tumors, when compared with control animals and NMU-treated animals which did not develop mammary tumors.

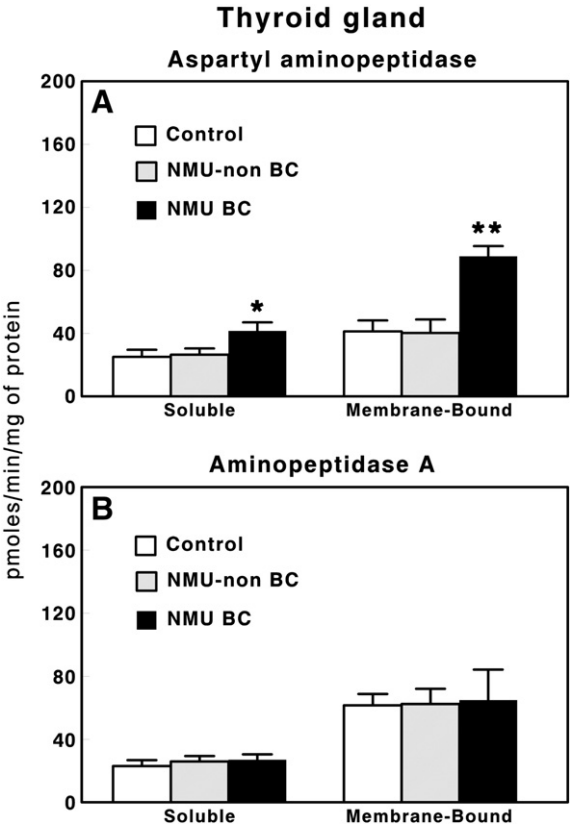

Fig. 1. Specific soluble and membrane-bound AspAP (A) and APA (B) activities in thyroid of control rats, rats treated with NMU that did not develop mammary tumors (NMU non-BC) and rats treated with NMU that developed mammary tumors (NMU BC). Results are expressed in picomoles of their corresponding aminoacyl-β-naphthylamide (see Materials and methods section) hydrolyzed per min and per mg of protein (mean ± SEM; $n = 4$–10; $^{*}p < 0.05$).

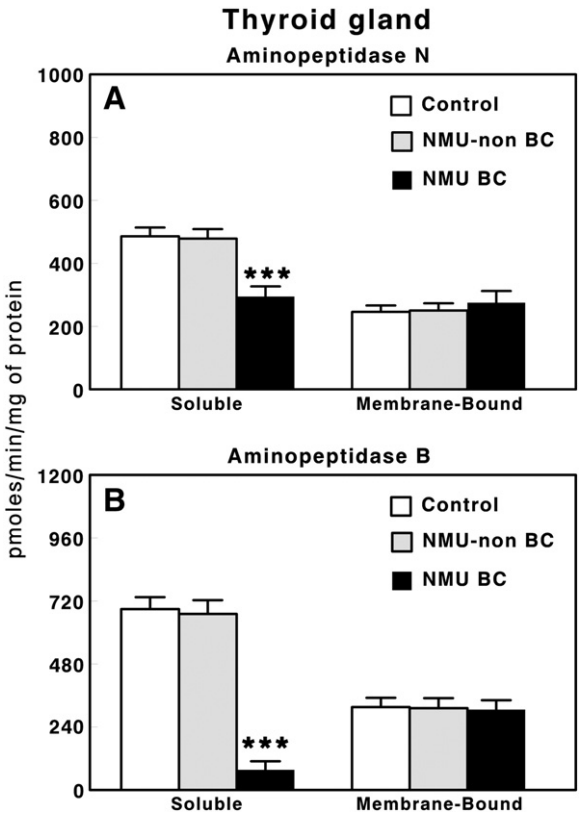

Fig. 2. Specific soluble and membrane-bound specific APN (A) and APB (B) activities in thyroid of control rats, rats treated with NMU that did not develop mammary tumors (NMU non-BC) and rats treated with NMU that developed mammary tumors (NMU BC). Results are expressed in pmoles of their corresponding aminoacyl-β-naphthylamide (see Materials and methods section) hydrolyzed per min and per mg of protein (mean ± SEM; $n = 4$–10; $^{***}p < 0.001$).

Discussion

Several authors have extensively described the influence of thyroid hormones on various components of the RAS in both pathological and physiological conditions. Thus, it has been reported that thyroid hormones may regulate, both in vitro and in vivo, the production of RAS components such as AGT (Ruiz et al., 1987). In fact, the multilocalization of mRNA AGT in many tissues, in addition to the liver that is the major

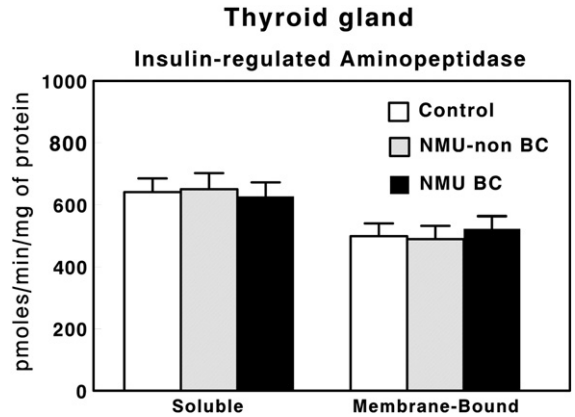

Fig. 3. Specific soluble and membrane-bound specific IRAP activity in thyroid of control rats, rats treated with NMU that did not develop mammary tumors (NMU non-BC) and rats treated with NMU that developed mammary tumors (NMU BC). Results are expressed in pmoles of leucyl-β-naphthylamide (see Materials and methods section) hydrolyzed per min and per mg of protein (mean ± SEM; $n = 4$–10).

site of synthesis AGT, is one of the arguments for the presence and the function of local renin–angiotensin systems (Clauser et al., 1989).

Also, thyroid hormones influence renin gene expression; studies in transgenic mice carrying extra copies of the *Ren2* gene indicate that thyroid hormone can directly stimulate transcription and/or stabilize precursor renin mRNA (Karen and Morris, 1986; Tronik and Rougeon, 1988). The identification in the early 90s of the majority of the RAS components in various tissues and cells not previously considered part the classical RAS suggested the existence of local RAS (Lavoie and Sigmund, 2003), with organ-specific functions that may act independently from the plasma RAS (Paul et al., 2006). These findings have led to the hypothesis of paracrine–autocrine functions for the RAS, which implies that locally generated AngII mediates effects within a tissue or within cell (Kumar et al., 2007). In this context, Montiel and Jimenez (1998) raised that the thyroid gland could be under direct control of local thyroid RAS, since the presence of specific receptors for AngII was described. Indeed, this study demonstrates that the AT1 receptor subtype is only present in the rat thyroid gland, but not the other subtype AT2.

In RAS system, several regulatory proteolytic enzymes, also named angiotensinases, are involved in the synthesis/catabolism of angiotensins (Fig. 4). Besides the traditional role as angiotensinases, the regulatory enzyme activities are directly involved in the tumoral process. In fact, APN has previously been considered a proteolytic enzyme with ability to facilitate the tumor cell invasion through the extracellular matrix (Fukasawa et al., 2006). The APN/CD13 expression in tumor cells significantly correlated with tumor type and neoangiogenesis (Ranogajec et al., 2012). Moreover, a recent report indicated that APA is up-regulated and enzymatically active in the blood vessels of human tumors, but it is not detected in normal blood vessels (Marchiò et al., 2004). On the other hand, APA and APN are considered cell-surface peptidases in the form of integral membrane proteins, which are involved in the control of cell proliferation and differentiation by modulating the access of peptides to their membrane receptors (Kenny et al., 1989).

Unfortunately the available data on angiotensinases expression and function in thyroid gland are minimal. In 2003, Kehlen and collaborators demonstrated, using thyroid carcinoma cells and thyroid tissues from patients with thyroid carcinomas, differences in the absolute levels of the ectopeptidases APN/CD13. This study showed that APN/CD13 is involved in cell motility of thyroid carcinoma cells, and it seems to be a marker for dedifferentiation in thyroid cancer.

Another angiotensinase implicated in the tumoral process is the AspAP. This enzymatic activity is modified in serum (Martínez-Martos et al., 2011) and in breast tissues from women with breast cancer (Martínez et al., 1999) and is significantly increased in head and neck squamous cell carcinoma tissues (Pérez et al., 2009).

In the present report, we have found in rats with mammary tumors a concomitant change of thyroid RAS-regulating enzymes and thyroid hormones production, suggesting that under the conditions brought about by the tumor, a preferential role for AngIII actions occurs due to the increased catabolic activity of AspAP on AngII, and the inhibition of AngIII degradation by APN and APB. The increased bioavailability of AngIII could be responsible of the decrease in the circulating levels of fT4. Therefore, under physiological conditions, the RAS may also be involved in the regulation of thyroid hormones release by the thyroid cells.

Table 1
Circulating levels of TSH and fT_4 in control rats, rats treated with NMU that did not develop mammary tumors (NMU non-BC) and rats treated with NMU that developed mammary tumors (NMU BC).

Group	Control	NMU non-BC	NMU BC	Significance level
TSH levels (ng/dL)	1.78 ± 0.15	1.76 ± 0.14	1.39 ± 0.13*	*$P < 0.05$
fT4 levels (ng/dL)	3.81 ± 0.60	3.68 ± 0.33	2.50 ± 0.17**	**$P < 0.01$

Significant decreases were observed in both hormones (*$p < 0.05$ for TSH and **$p < 0.01$ for fT4) in NMU-treated rats with mammary tumors, when compared with control animals and NMU-treated animals which did not develop mammary tumors.

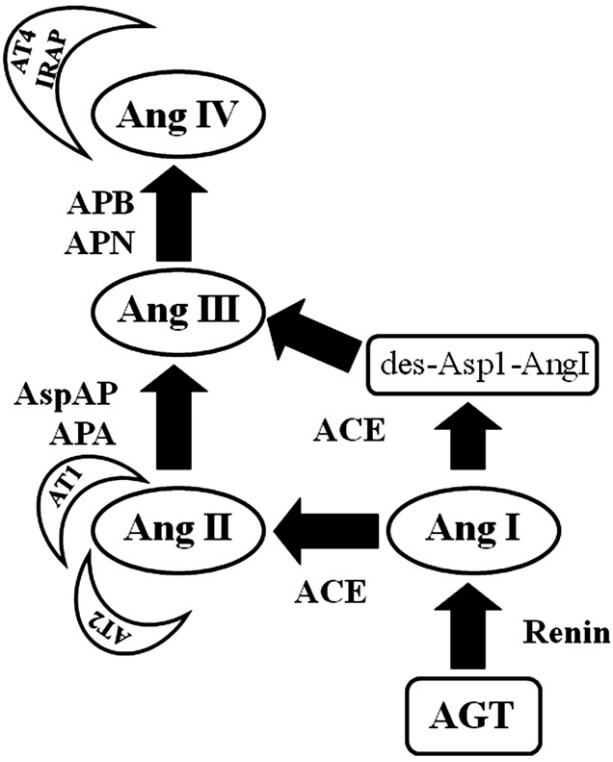

Fig. 4. Scheme for the conversion of angiotensins.

In regulating thyroid cell function, cAMP is perhaps the most important intracellular signaling molecule. However, research carried out by Weiss and collaborators (1984a) has shown that changes in intracellular-free calcium $[Ca^{2+}]_i$ also regulate a multitude of central processes, including regulation of iodide efflux. Even the effects evoked by the TSH and the regulation of TSH receptor (TSHR) expression may also be modified by changes in $[Ca^{2+}]_i$ (Weiss et al., 1984b; Saji et al., 1991). On the other hand, Löf et al. (2012) propose a new mechanism involving extracellular signal-regulated kinases 1 and 2 (ERK1/2), as a positive regulator of TSHR expression. The phosphorylation of ERK1/2 is increased through cAMP/Rap1. Reducing intracellular cAMP reduced the TSHR expression, which further shows that the cAMP pathway is responsible for the up-regulation of the TSHR in the TRPC2 knockdown cells (shTRPC2). In these cells, the ATP-evoked entry of calcium was significantly decreased.

Furthermore, in thyrocytes, AngII stimulated the translocation from the cytosol to the plasma membrane of atypical protein kinase C-zeta (PKC-ζ) with subsequent phosphorylation of the ERK1/2. This effect happens in PC-Cl3 thyroid cells under AT1 activation, angiotensin receptor expressed in thyroid gland in rat (Marsigliante et al., 2003). ERKs are involved in the activation of nuclear transcription factors, such as the c-fos protooncogenes (Wagstaff et al., 2000). c-fos is induced by TSH (cAMP) in Wistar rat thyroid (WRT) cells (Tominaga et al., 1994) and human thyrocytes (Heinrich and Kraiem, 1997), and its expression has been claimed to be required for TSH-dependent proliferation of Fisher rat thyroid low serum (FRTL)-5 cells (Foti et al., 1990). In this context, the activation of AT1 receptors has been shown to induce, in other cell systems, the expression of c-fos (Muscella et al., 2002). However, recent studies showed that AngII activated ERK signal transduction pathway and induced c-fos expression without acting as a mitogen in PC Cl3 cells. Thus, although both ERK and PI3K/Akt pathways are activated, the effects AngII in PC Cl3 proliferation are not evident (Romano et al., 2006).

Therefore, this could be a point of connection between the TSHR and AT1 receptor, although to our view, there remains a deep

research about the connection between them and their regulation and interconnection.

All the same, Marsigliante et al. (2003) have described that PC Cl3 cells, a rat thyroid cell line, express a functional AT1 receptor that regulates the Na^+/K^+ ATPase activity and activates the ERK1/2 pathway. Accordingly, it has been well described in the follicular lumen of thyroid gland that thyroglobulin, the precursor of the thyroid hormones, is concentrated and stored via a concentration process ensured by the extrusion of electrolytes and water out of the thyroid follicle (Smeds, 1972a, b). Indeed, an apical–basal directed transport of fluid has been shown in pig thyrocytes (Pearson et al., 1988). Since the transepithelial flux of Na^+ determines transepithelial secondary flux of water, certain transport systems, including Na^+/K^+ ATPase and Na^+/H^+ antiporter, are involved in these processes. Moreover, the Na^+/K^+ ATPase is strictly implicated in I^- uptake by the Na^+/I^- symport, which is fundamental for the synthesis of thyroid hormones. These authors found that in response to AngII administration the Na^+/K^+ ATPase activity was significantly stimulated.

Although the role of angiotensins in the thyroid is not defined, the proteolytic regulatory enzyme activities modifications showed in this study suggest a possible thyroid RAS involved in thyroid hormones synthesis/release in physiological conditions. This thyroid RAS could be altered under pathological states such as breast cancer. However, it must be taken into account that we have also found decreased levels of TSH in rats with mammary tumors. In this way, we have previously proposed that the development of breast cancer increases the pituitary release of oxytocin (OXT) by decreasing its hypothalamic catabolism and probably due to the alteration of the estrogenic endocrine status. Thus, high circulating levels of OXT decrease the TSH release from the pituitary that also decreases the further production of fT_4 (Carrera-González et al., 2011).

We can conclude that the changes in RAS-regulating enzyme activities in thyroid gland from rats with mammary tumors induced by NMU may lead to alterations in the regulatory mechanisms mediated by the angiotensins. These alterations in angiotensinases activities may be a consequence of the carcinogenic process, which could act alone or in combination with alterations at a higher level of regulation such as the hypothalamus–pituitary axis.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

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References

Albiston AL, McDowall SG, Matsacos D, Sim P, Clune E, Mustafa T, et al. Evidence that the angiotensin IV (AT(4)) receptor is the enzyme insulin-regulated aminopeptidase. J Biol Chem 2001;276:48623–6.
Ardaillou R, Chansel D. Synthesis and effects of active fragments of angiotensin II. Kidney Int 1997;52:1458–68.
Carneiro-Ramos MS, Silva VB, Santos RA, Barreto-Chaves ML. Tissue-specific modulation of angiotensin-converting enzyme (ACE) in hyperthyroidism. Peptides 2006;27:2942–9.
Carrera-González MP, Ramírez-Expósito MJ, de Saavedra JM, Sanchez-Agesta R, Mayas MD, Martínez-Martos JM. Hypothalamus–pituitary–thyroid axis disruption in rats with breast cancer is related to an altered endogenous oxytocin/insulin-regulated aminopeptidase (IRAP) system. Tumour Biol 2011;32:543–9.
Catanzaro OL, Marzi A. Cell proliferaton in mouse kidney by isoproterenol and the relationship with the thyroid function. Experientia 1974;30:1334–5.
Chai SY, Yeatman HR, Parker MW, Ascher DB, Thompson PE, Mulvey HT, et al. Development of cognitive enhancers based on inhibition of insulin-regulated aminopeptidase. BMC Neurosci 2008;9(Suppl. 2):S14.
Clauser E, Gaillard I, Wei L, Corvol P. Regulation of angiotensinogen gene. Am J Hypertens 1989;2(5 Pt 1):403–10.
Diniz GP, Carneiro-Ramos MS, Barreto-Chaves ML. Angiotensin type 1 receptor mediates thyroid hormone-induced cardiomyocyte hypertrophy through the Akt/GSK-3beta/mTOR signaling pathway. Basic Res Cardiol 2009;104:653–67.
Dzau VJ, Herrmann HC. Hormonal control of angiotensinogen production. Life Sci 1982;30:577–84.
Foti D, Damante G, Rapoport B. Studies on the role of c-fos in TSH-stimulated thyroid cell proliferation. Cell Mol Biol 1990;36(4):363–73.
Fukasawa K, Fujii H, Saitoh Y, Koizumi K, Aozuka Y, Sekine K, et al. Aminopeptidase N (APN/CD13) is selectively expressed in vascular endothelial cells and plays multiple roles in angiogenesis. Cancer Lett 2006;243(1):135–43.
Fyhrquist F, Saijonmaa O. Renin–angiotensin system revisited. J Intern Med 2008;264:224–36.
Gago-Dominguez M, Castelao JE. Role of lipid peroxidation and oxidative stress in the association between thyroid diseases and breast cancer. Crit Rev Oncol Hematol 2008;68:107–14.
García MJ, Martínez-Martos JM, Mayas MD, Carrera MP, Ramírez-Expósito MJ. Hormonal status modifies renin–angiotensin system-regulating aminopeptidases and vasopressin-degrading activity in the hypothalamus-pituitary-adrenal axis of male mice. Life Sci 2003;73(5):525–38.
Heinrich R, Kraiem Z. The protein kinase A pathway inhibits c-jun and c-fos protooncogene expression induced by the protein kinase C and tyrosine kinase pathways in cultured human thyroid follicles. J Clin Endocrinol Metab 1997;82(6):1839–44.
Jimenez E, Montiel M, Narvaez JA, Morell M. Renin–angiotensin system in hypothyroid rats: effects of potassium iodide and triiodo-L-thyronine. Acta Endocrinol (Copenh) 1984;105:505–10.
Karen P, Morris BJ. Stimulation by thyroid hormone of renin mRNA in mouse submandibular gland. Am J Physiol 1986;251:E290–3.
Kehlen A, Lendeckel U, Dralle H, Langner J, Hoang-Vu C. Biological significance of aminopeptidase N/CD13 in thyroid carcinomas. Cancer Res 2003;63(23):8500–6.
Kenny AJ, O'Hare MJ, Gusterson BA. Cell-surface peptidases as modulators of growth and differentiation. Lancet 1989;2(8666):785–7.
Kumar R, Singh VP, Baker KM. The intracellular renin–angiotensin system: a new paradigm. Trends Endocrinol Metab 2007;18(5):208–14.
Lavoie JL, Sigmund CD. Minireview: overview of the renin–angiotensin system—an endocrine and paracrine system. Endocrinology 2003;144(6):2179–83.
Löf C, Sukumaran P, Viitanen T, Vainio M, Kemppainen K, Pulli I, et al. Communication between the calcium and cAMP pathways regulate the expression of the TSH receptor: TRPC2 in the center of action. Mol Endocrinol 2012;26(12):2046–57.
Marc Y, Llorens-Cortes C. The role of the brain renin–angiotensin system in hypertension: implications for new treatment. Prog Neurobiol 2011;95:89–103.
Marchant C, Brown L, Sernia C. Renin–angiotensin system in thyroid dysfunction in rats. J Cardiovasc Pharmacol 1993;22:449–55.
Marchiò S, Lahdenranta J, Schlingemann RO, Valdembri D, Wesseling P, Arap MA, et al. Aminopeptidase A is a functional target in angiogenic blood vessels. Cancer Cell 2004;5(2):151–62.
Marsigliante S, Muscella A, Elia MG, Greco S, Storelli C. Angiotensin II AT1 receptor stimulates Na+-K+ATPase activity through a pathway involving PKC-zeta in rat thyroid cells. J Physiol 2003;546:461–70.
Martínez JM, Prieto I, Ramírez MJ, Cueva C, Alba F, Ramírez M. Aminopeptidase activities in breast cancer tissue. Clin Chem 1999;45(10):1797–802.
Martínez-Martos JM, del Pilar Carrera-González M, Dueñas B, Mayas MD, García MJ, Ramírez-Expósito MJ. Renin angiotensin system-regulating aminopeptidase activities in serum of pre- and postmenopausal women with breast cancer. Breast 2011;20(5):444–7.
Michalaki V, Kondi-Pafiti A, Gennatas S, Antoniou A, Primetis H, Gennatas C. Breast cancer in association with thyroid disorders. J Buon 2009;14:425–8.
Montiel M, Jimenez E. Characterization of angiotensin II receptors (binding and mRNA) in the rat thyroid gland. J Mol Endocrinol 1998;20:299–304.
Muscella A, Greco S, Elia MG, Storelli C, Marsigliante S. Angiotensin II stimulation of Na+/K+ATPase activity and cell growth by calcium-independent pathway in MCF-7 breast cancer cells. J Endocrinol 2002;173(2):315–23.
Paul M, Poyan Mehr A, Kreutz R. Physiology of local renin–angiotensin systems. Physiol Rev 2006;86(3):747–803.
Pearson J, Bourke JR, Manley SW, Huxham GJ, Matainaho T, Gerard C, et al. Electrophysiological correlates of fluid transport in cultured porcine thyroid cells. J Endocrinol 1988;119:309–14.
Pérez I, Varona A, Blanco L, Gil J, Santaolalla F, Zabala A, et al. Increased APN/CD13 and acid aminopeptidase activities in head and neck squamous cell carcinoma. Head Neck 2009;31(10):1335–40.
Ramírez-Expósito MJ, Martínez-Martos JM, Prieto I, Alba F, Ramírez M. Angiotensinase activity in mice fed an olive oil-supplemented diet. Peptides 2001;6:945–52.
Ranogajec I, Jakić-Razumović J, Puzović V, Gabrilovac J. Prognostic value of matrix metalloproteinase-2 (MMP-2), matrix metalloproteinase-9 (MMP-9) and aminopeptidase N/CD13 in breast cancer patients. Med Oncol 2012;29(2):561–9.
Romano S, Muscella A, Storelli C, Marsigliante S. Angiotensin II does not stimulate proliferation of rat thyroid PC Cl3 cell line. J Endocrinol 2006;191(3):727–35.
Ruiz M, Montiel M, Jimenez E, Morell M. Effect of thyroid hormones on angiotensinogen production in the rat in vivo and in vitro. J Endocrinol 1987;115:311–5.
Saji M, Ikuyama S, Akamizu T, Kohn LD. Increases in cytosolic Ca++ down regulate thyrotropin receptor gene expression by a mechanism different from the cAMP signal. Biochem Biophys Res Commun 1991;176(1):94–101.
Smeds S. A microgel electrophoretic analysis of the colloid proteins in single rat thyroid follicles. I. The qualitative protein composition of the colloid in normal thyroids. Endocrinology 1972a;91:1288–99.

Smeds S. A microgel electrophoretic analysis of the colloid proteins in single rat thyroid follicles. II. The protein concentration of the colloid single rat thyroid follicles. Endocrinology 1972b;91:1300–6.
Smyth PP. The thyroid and breast cancer: a significant association? Ann Med 1997;29:189–91.
Smyth PP. The thyroid, iodine and breast cancer. Breast Cancer Res 2003;5:235–8.
Smyth PP, Shering SG, Kilbane MT, Murray MJ, McDermott EW, Smith DF, et al. Serum thyroid peroxidase autoantibodies, thyroid volume, and outcome in breast carcinoma. J Clin Endocrinol Metab 1998;83:2711–6.
Tominaga T, Dela Cruz J, Burrow GN, Meinkoth JL. Divergent patterns of immediate early gene expression in response to thyroid-stimulating hormone and insulin-like growth factor I in Wistar rat thyrocytes. Endocrinology 1994;135(3):1212–9.
Tronik D, Rougeon F. Thyroxine and testosterone transcriptionally regulate renin gene expression in the submaxillary gland of normal and transgenic mice carrying extra copies of the Ren2 gene. FEBS Lett 1988;234:336–40.
Wagstaff SC, Bowler WB, Gallagher JA, Hipskind RA. Extracellular ATP activates multiple signalling pathways and potentiates growth factor-induced c-fos gene expression in MCF-7 breast cancer cells. Carcinogenesis 2000;21(12):2175–81.
Weiss SJ, Philp NJ, Grollman EF. Iodide transport in a continuous line of cultured cells from rat thyroid. Endocrinology 1984a;114(4):1090–8.
Weiss SJ, Philp NJ, Grollman EF. Effect of thyrotropin on iodide efflux in FRTL-5 cells mediated by Ca2+. Endocrinology 1984b;114(4):1108–13.