Metabolism
Clinical and Experimental

VOL 44, NO 10 OCTOBER 1995

PRELIMINARY REPORT

Increased Plasma Levels of Endothelin-1 in Patients With Hyperthyroidism

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Plasma concentrations of endothelin-1 (ET-1) were measured in 25 hyperthyroid subjects, 15 hypothyroid subjects, and 21 age-matched normal controls. In hyperthyroid patients, plasma concentrations of ET-1 were significantly higher than in the control group ($P < .0001$) and in hypothyroid patients ($P < .0001$). In contrast, no differences were found between hypothyroid patients and controls. Plasma levels of ET-1 were similarly elevated as in patients with Graves' disease and those with toxic adenoma. No correlations were found between plasma ET-1 levels, thyroid hormones, and thyrotropin (TSH) in hyperthyroid, hypothyroid, and euthyroid groups. The results of our study clearly indicate that in hyperthyroidism, circulating levels of ET-1 are strongly increased, although the pathogenesis of the increase is unclear.

THERE IS INCREASING evidence that in addition to vasoactive substances such as norepinephrine, acetylcholine, dopamine, and 5-hydroxytryptamine, several peptides are involved in the regulation of thyroid function.[1-3] Moreover, many of the interactions between numerous peptides and thyroid hormones, although complex and still incompletely understood, have been partially clarified in recent years.

We have previously shown that plasma activity of angiotensin-converting enzyme increased in hyperthyroidism[4] and decreased in hypothyroidism.[5]

Recently, endothelin (ET), a peptide of vascular endothelial origin, has been isolated from culture media of porcine aortic endothelial cells.[6] ET circulates as a 21–amino acid peptide after proteolytic processing from a 203-residue preproendothelin via a 183-residue proendothelin to a 39-residue intermediate "big-endothelin."

In human tissues, ET binding sites have been identified not only in the cardiovascular system, but also in the lung, kidney, adrenal gland, brain, spinal cord, gastrointestinal tract, liver, and spleen.[7]

ET exerts several biological actions, including stimulation and release of aldosterone,[8] atrial natriuretic peptide,[9] and catecholamines,[10] and inhibition of renin release.[11]

Thus, ET has a broad range of binding sites and may function as a regulator in many organ systems.

ET/thyroid relationships have been recently investigated by Jackson et al,[12] who have reported the presence of specific high-affinity receptors for ET on human thyrocytes. Also, Eguchi et al[13] have demonstrated that ET stimulates proliferation of epithelial cells derived from thyroid tissues of normal subjects and patients with Graves' disease.

Human ET has been found in three isoforms: ET-1, ET-2, and ET-3,[14] and increasing evidence indicates that in addition to its known role as an autacoid, ET-1 is present in the plasma of human beings, and animals.[15,16]

Moreover, Lam et al[17] have demonstrated that in the rat lung the major immunoreactive ET is ET-1, and its concentration is reduced in both the hypothyroid and hyperthyroid states. However, no data are available on the effect of thyroid function on ET-1 levels in humans. Therefore, the aim of the present study was to determine plasma levels of ET-1 in subjects with different thyroid homeostasis.

SUBJECTS AND METHODS

Subjects

Plasma levels of ET-1 were measured in 61 subjects divided into the following groups: (1) 21 healthy subjects (15 men and six

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Submitted December 12, 1994; accepted April 7, 1995.
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0026-0495/95/4410-0001$03.00/0

women; mean age, 42.6 ± 12.2 years) used as a control; (2) 25 patients with definite hyperthyroidism (eight men and 17 women; mean age, 44.3 ± 15.0 years), 16 of whom were affected by Graves' disease and nine by toxic adenoma; and (3) 15 patients with hypothyroidism (five men and 10 women; mean age, 45.6 ± 13.4 years). Of 15 patients, 10 showed definite hypothyroidism and five had subclinical hypothyroidism.

Diagnosis was made on the basis of clinical and laboratory findings. No patient enrolled in this study was undergoing medical treatment. Also, no subjects exhibited either clinical or laboratory signs of diabetic, hepatic, and/or cardiovascular disease.

A fasted venous blood sample was obtained from the forearm; plasma and serum were frozen and stored in a polypropylene tube at −70°C until assayed for ET-1, free triiodothyronine (FT_3), free thyroxine (FT_4), T_3, T_4, basal thyrotropin (TSH) or, where appropriate, TSH-releasing hormone–stimulated TSH, antimicrosomal antibodies, antithyreoglobulin antibodies, and antithyroid peroxidase antibodies.

Systolic (S) and diastolic (D) blood pressure (BP) and heart rate (HR) were measured; arterial pressure was taken from the left arm of each subject using a mercury-column sphygmomanometer.

Methods

Extracted plasma ET-1 was determined by specific radioimmunoassay (RIA) using rabbit anti–ET-1 (RIK-6901; Peninsula Laboratories, Belmont, CA) serum. Recovery of the extraction procedure was 90%. According to the manufacturer's instructions, the antibody cross-reacts 100% with human, rat, porcine, canine, bovine, and mouse ET-1 and 7% with ET-2 and ET-3 (respectively), but not with atrial natriuretic factor, angiotensin I or II, corticotropin, or vasopressin. To perform the RIA, samples were dissolved in 0.2 mL assay buffer consisting of 0.01 mol/L sodium hydrogenphosphate buffer (pH 7.4), 0.05 mol/L NaCl, 0.1% bovine serum albumin, and 0.01% NAN. After incubation of samples and standards with antiserum for 24 hours at 4°C, approximately 12,000 cpm ^{125}I-ET was added to the reaction mixture, which was then reincubated for 24 hours at 4°C. Subsequently, 100 μL diluted goat antirabbit IgG serum was added and the samples were centrifuged at 1,700 × *g* for 2 minutes. The residual pellet was counted using an automatic gamma counter. All assays were performed in duplicate. Concentrations of ET-1 were expressed as picograms per milliliter.

T_3 and T_4 were assayed by RIA using standard T_3-Bridge and T_4-Bridge kits with coated tubes (Biodata-Serono; Guidonia, Rome, Italy); FT_3 and FT_4 by a RIA method using isophase kits (Sclavo, Siena, Italy); and TSH by a immunoradiometric assay Maia-Clone method (Biodata-Serono; Guidonia).

Antimicrosomal antibodies and antithyreoglobulin antibodies were evaluated using a Bridge-Irma method (Biodata-Serono; Guidonia). Antithyroid peroxidase antibodies were assayed by an immunoradiometric method (Radim, Pomezia, Italy). The intraassay coefficient of variation for all thyroid determinations was less than 5%. Normal values in our laboratory are as follows: T_3, 0.6 to 1.77 ng/mL; T_4, 52 to 102 ng/mL; TSH, 0.4 to 3.5 μUI/mL; FT_3, 2.2 to 5.5 pg/mL; and FT_4, 7.8 to 19.4 pg/mL.

Statistical Analysis

Results are expressed as the mean ± SD. Statistical analysis was performed with an IBM personal computer (IBM, Atlanta, GA) using Primer software (Biostatistics; Glantz, McGraw-Hill, San Francisco, CA, 1987). Individual values were inserted by group and evaluated using ANOVA, followed by Bonferroni's test and whenever appropriate by Student's *t* test.

Correlations between hormones and other variables were examined with linear regression analysis (*r*). Statistically significant differences were assumed at *P* less than .05.

Table 1. Clinical Data (mean ± SD) for Each Group

	Normals	Hypothyroid Patients	Hyperthyroid Patients
Age (yr)	42.6 ± 12.2	45.6 ± 13.4	44.3 ± 15.1
SBP (mm Hg)	122.1 ± 5.4	122.0 ± 7.9	153.0 ± 7.1
DBP (mm Hg)	75.2 ± 6.9	75.7 ± 5.6	65.8 ± 4.9
HR (beats per min)	75.0 ± 6.2	72.1 ± 2.8	87.0 ± 5.0
T_3 (ng/mL)	1.5 ± 0.4	1.2 ± 0.3	3.5 ± 1.2
T_4 (ng/mL)	81.5 ± 18.0	50.4 ± 16.8	160.6 ± 33.1
FT_3 (pg/mL)	3.1 ± 0.9	2.1 ± 1.3	11.8 ± 6.5
FT_4 (pg/mL)	10.3 ± 1.7	6.1 ± 1.2	19.0 ± 10.1
TSH (μUI/mL)	2.4 ± 0.7	25.1 ± 29.0	0.1 ± 0.1

RESULTS

Thyroid function parameters of the different groups are summarized in Table 1.

Circulating levels of ET-1 are shown in Table 2. In the control group, plasma levels of ET-1 were 11.1 ± 2.1 pg/mL, which are similar to the normal values reported in other studies.[18] Mean plasma levels in hyperthyroid subjects were significantly higher than in the control group (showing an increase of 44%, $P < .0001$) and in hypothyroid subjects (+40%, $P < .0001$; Table 2). Overall, 22 of 25 hyperthyroid patients (88%) showed ET-1 levels higher than those of the control group. In contrast, no differences were found between hypothyroid subjects and controls, with only one of 15 (6.6%) hypothyroid subjects having serum values above the normal range (Table 2).

The same table shows that no statistically significant differences were found between hyperthyroid patients with toxic adenoma and those with Graves' disease or between overt and subclinical hypothyroidism subjects.

Analysis of the data also showed that no correlations exist between ET-1 plasma levels and TSH, thyroid hormones, BP, and HR in all the groups studied.

DISCUSSION

The relationship between thyroid gland and ET-1 has been recently established by Jackson et al[12] and Colin et al,[19] who demonstrated the presence of specific high-affinity

Table 2. Plasma ET-1 Levels (mean ± SD) in Each Group

Subjects	ET-1 (pg/mL)
Hyperthyroid patients (n = 25)	19.7 ± 5.4*
Graves' disease (n = 17)	19.9 ± 5.6*
Toxic adenoma (n = 8)	19.4 ± 6.2*
Hypothyroid patients (n = 15)	11.8 ± 1.3
Definite hypothyroidism (n = 10)	11.5 ± 1.4
Subclinical hypothyroidism (n = 5)	12.5 ± 0.8
Controls (n = 21)	11.1 ± 2.1

*$P < .0001$ *v* hypothyroid patients and *v* controls.

receptors for ET in human thyroid cells and ET-1 immunoreactivity in cat thyroid cells. Moreover, the study reported by Eguchi et al[13] demonstrated that ET-1 stimulates proliferation of thyroid epithelial cells in tissues of patients with Graves' disease.

In the present study, we evaluated ET-1 levels in patients with clinical hyperthyroidism and patients with untreated hypothyroidism to reveal any possible correlation between ET-1, thyroid hormones, and TSH.

The results indicated that at the time of diagnosis, ET-1 plasma levels were elevated in hyperthyroid patients as compared with euthyroid controls. In contrast, ET-1 levels were normal in overt and subclinical hypothyroidism patients. There were no differences in plasma ET-1 levels between hypothyroid patients and the control group. ET-1 plasma levels were similarly elevated both in patients with Graves' disease and in those with toxic adenoma. No correlations were found between plasma ET-1 levels, thyroid hormones, TSH, and hemodynamic parameters (SBP, DBP, and HR) in hyperthyroid, hypothyroid, and euthyroid groups.

Such behavior of plasma ET-1 in altered human thyroid homeostasis is almost identical to that found by Rebello et al[20] in rats. In fact, these investigators induced a state of hyperthyroidism and hypothyroidism in rats by administering T_4 and methimazole, respectively, for 8 weeks. They reported an increase in ET-1 plasma levels in rats treated with T_4. In rats treated with methimazole, plasma ET-1 levels were identical to those of the control group. According to these data, it appears that the hyperthyroid state per se promotes the increase of ET-1 plasma levels independently from the etiology of hyperthyroidism. The exact mechanism by which hyperthyroidism increases circulating levels of ET-1 is not known. Up to now, no data are available about a direct effect of thyroid hormone on ET-1 synthesis and/or secretion by endothelial and nonendothelial cells. However, interleukin-1B, which appears to be involved in the pathogenesis of Graves' disease,[21,22] also represents one of the substances (vasopressin, angiotensin II, thrombin, and catecholamines)[12,13] that increase ET-1 secretion. Thus, one possibility is that ET-1 secretion would be indirectly stimulated by immunomodulating factors involved in hyperthyroidism. However, this hypothesis would not explain the increase of ET-1 in subjects affected by toxic adenoma, in which the immune system plays no role.

A more likely explanation would be that the increase of ET-1 is due to vessel endothelial cell damage. ET-1 is preformed and stored in vessel endothelium[23]; an altered metabolism of endothelial cells due to hyperthyroidism could disrupt the continuity of the endothelium, thus releasing the preformed ET-1. Whatever the pathogenesis of this event, elevated levels of ET-1 should be a primary factor in the pathogenesis of hypertension in hyperthyroid subjects. In fact, ET-1 has been shown to increase peripheral resistance and arterial BP[24] and has been found to be elevated in patients with essential hypertension.[25,26] However, the hypertension present during chronic hyperthyroidism may not be explained by the effect of ET-1 alone, since DBP is typically reduced in this condition.

In conclusion, the results of this study clearly indicate that in hyperthyroidism, but not hypothyroidism, circulating levels of ET-1 are significantly increased. Future studies on the effect of therapy may contribute to understanding the present findings.

ACKNOWLEDGMENT

We are grateful to Giovanni Clemente for excellent technical assistance.

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