Inhibition of human thyroid peroxidase gene expression by interleukin 1

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Abstract. We have already demonstrated the inhibitory effect of interleukin 1 on thyroglobulin gene expression. Recent availability of thyroid peroxidase cDNA has allowed us to investigate the regulation of thyroid peroxidase gene. Therefore, the regulation of thyroid peroxidase mRNA by interleukin 1 in cultured human thyrocytes was investigated. Thyrocytes dispersed from thyroid tissues from patients with Graves’ disease were incubated with TSH with or without recombinant human interleukin 1. Unstimulated human thyrocytes did not contain any detectable thyroid peroxidase mRNA, however, TSH-stimulated thyrocytes expressed four thyroid peroxidase mRNA transcripts (4.0, 3.2, 2.1 and 1.7 kb, respectively). Both interleukin 1α and β inhibited TSH-induced thyroid peroxidase mRNA in a dose responsive manner; 10³ U/l interleukin 1 caused maximal suppression of TSH-induced thyroid peroxidase mRNA level to nearly basal levels. Interleukin 1 also inhibited cAMP analogue 8-bromo-cyclic AMP induced thyroid peroxidase mRNA level. In contrast the γ-actin mRNA hybridization signal was not altered in control or treated cells. These results demonstrate that interleukin 1 directly inhibits TSH-induced thyroid peroxidase gene expression and provide further evidence for a paracrine role of interleukin 1 as a local inhibitor of thyroid hormone synthesis.

Recently attention has been focused on the role of cytokines for cellular communication not only in immune responses but also in non-immune systems. Nagayama et al. (1) have previously demonstrated that interferon-γ (IFN-γ) induced HLA-DR antigen and also inhibited thyrotropin-induced T₃ and thyroglobulin (Tg) secretion from cultured human thyrocytes. Furthermore, Yamashita et al. (2) have recently presented that interleukin 1 (IL-1) inhibited TSH-stimulated Tg gene expression in a dose-responsive manner. These results suggest the existence of the microenvironmental interaction between IL-1 and thyroidal epithelial cells on thyroid hormone secretion.

IL-1α and β are generally produced by antigensensitized macrophages, and involved in the lymphokine cascade reaction (3). Furthermore, IL-1 has different action on various endocrine systems (4—6). IL-1 has been shown to release ACTH and corticosterone in vivo (7) and in vitro (8). Krogsh-Rasmussen et al. (9) have already demonstrated the influence of IL-1 on the function of in vitro cultured normal human thyroid cells in monolayers. Recent in vivo studies also demonstrated the inhibitory effect of IL-1 on circulating thyroid hormone level (10, 11). In contrast, thyroid peroxidase (TPO) plays a major role for thyroid hormone synthesis (12) and the recent cloning of human TPO cDNA fragments (13) have provided the probes allowing analysis of TPO gene expression in cultured human thyrocytes. Therefore, we have examined the TPO gene expression in order further to elucidate the molecular mechanism of the inhibitory effect of IL-1 on thyroid hormone secretion.

Materials and Methods

Cell cultures
Thyroid tissues were obtained at subtotal thyroidectomy of patients with Graves’ disease. Isolation of thyrocytes
was performed as previously described (14). Thyrocytes dispersed by collagenase (100 mg/l) and dispase (3.3 mg/l) in Hank’s Balanced Salt Solution (HBSS) were distributed to 10 cm-diameter culture plates with Ham’s F-12 medium supplemented with 5% heat-inactivated fetal bovine serum (FBS), penicillin (10⁵ U/l) and streptomycin (100 mg/l). Medium was changed every other day and cells were incubated for 7 days. The thyrocyte preparations were more than 95% reactive with antithyroglobulin antibody and/or antimicrosomal antibody. Reactivity was identified by an indirect immunofluorescence method using a fluorescence-activated cell sorter (14). Medium was changed to serum-free Ham’s F-12 medium with 0.3% bovine serum albumin (BSA) for 24 h. Then cells were treated with bovine 5 U/l TSH or 1 mmol/l 8-bromo-cAMP (8-Br-cAMP) with or without IL-1.

**RNA extraction**

After the indicated treatments, total RNA was extracted by acid-guanidinium (15). Briefly, the thyrocytes were lysed directly on the culture plates by addition of denaturing solution (4 mol/l guanidinium thiocyanate, 25 mmol/l sodium citrate pH 7, 0.5% sarcosyl, and 0.1 mol/l 2-β mercaptoethanol). The solution mixture was extracted by sodium acetate (2 mol/l, pH 4) and phenol-chloroform. The isopropanol precipitation was rinsed with ethanol and dissolved. The concentration of mRNA was determined by absorbance of 260 nm. The ratio of OD 260/280 was usually 2.0.

**Northern gel analysis**

Total RNA (30 µg each lane) was subjected to gel electrophoresis through a 1% agarose gel with 2.2 mol/l formaldehyde as described previously (16). The RNA was transferred to Hybond nylon membrane paper (Amersham, Japan) after electrophoresis. After prehybridization, hybridization was performed with [³²P]labelled human TPO cDNA probe (13). Autoradiographic results were obtained after the exposure to X-ray filter as described previously (16). Rehybridization with γ-actin cDNA probe was performed after washing in 0.1 × sodium citrate and sodium chloride (SSC) and 0.1% sodium dodecyl sulphate (SDS) at 95°C for 30 min.

Human TPO cDNA probe HTPO-31 which contain 565 bps of cDNA was kindly provided by Dr B. Rapoport (13) and γ-actin cDNA by Dr L. Kedes (17). The probes

**Fig 1.**

Northern gel analysis of thyroid peroxidase (TPO) mRNA in thyrocytes. Thyrocytes (5 x 10⁶ cells) were treated with TSH (5 U/l) with or without varying concentrations of interleukin 1 (IL-1) for 48 h and total RNA was extracted. Northern gel analysis of RNA extracts (30 µg RNA, each lane) was performed as described in methods. Sizing was accomplished by running Hind III-digested λDNA fragments in adjacent lanes. Top: The blotted paper was hybridized with [³²P]TPO cDNA. Lane, left to right, showed: 1: control; 2: TSH (5 U/l); 3: TSH + IL-1α (10² U/l); 4: TSH + IL-1α (10⁶ U/l); 5: TSH + IL-1α (10⁴ U/l); 6: TSH + IL-1β (10² U/l); 7: TSH + IL-1β (10³ U/l); and 8: TSH + IL-1β (10⁴ U/l). Bottom: The same paper was re-probed with [³²P]γ-actin cDNA after washing with 0.1% SDS and 0.1 × SSC at 95°C for 30 min. The same results were obtained from 11 independent experiments.
used in this study were labelled with $[^{32}P]\alpha$CTP by random prime labelling to a specific activity of $5\times 10^{7}$ cpm/μg DNA.

Reagents
All molecular biology reagents were purchased from Sigma Chemical Co, Ltd (St. Louis, MO), Amersham Japan Co, Ltd (Tokyo, Japan) and Takara Shuzo Co, Ltd (Kyoto, Japan).

Recombinant human IL-1α and β were kindly provided by Dr Y. Hirai, Otsuka Pharmaceutical Co, Ltd (Kushikima, Japan).

Results

Effect of IL-1α and β on thyroid peroxidase mRNA
To investigate the expression of TPO mRNA, thyrocytes were treated with TSH with or without IL-1 for 48 h and total RNA was extracted for Northern gel analysis. Basal control thyrocytes did not express any detectable TPO mRNA, however, 5 U/l TSH stimulated TPO mRNA expression. Both IL-1α and β significantly inhibited TSH-induced TPO mRNA in a dose-responsive manner (Fig. 1). The suppressive activity of IL-1α and β was similar. The sizes of TPO mRNA in cultured human thyrocytes were about 4.0, 3.2, 2.0, and 1.7 kb, respectively, which were not different from those reported in a previous report (18). In contrast, γ-actin mRNA hybridization signal was not altered in control or treated cells. Furthermore, the cyclic AMP analogue 8-Br-cAMP stimulated TPO mRNA. Both IL-1α and β also inhibited 8-Br-cAMP induced TPO mRNA levels (Fig. 2).

Discussion
The results of this study indicate that IL-1 was capable of inhibiting TSH and cAMP-induced TPO mRNA in cultured human thyrocytes in a dose-dependent manner. TPO mediates thyroid hormone synthesis and is also a major product of cloned thyrocyte gene expression (12). Cultured human thyrocytes did not express IL-1 mRNA, indicating that thyrocytes themselves could not produce IL-1 (19). It is, therefore, conceivable that IL-1 released from the microenvironmetal cells (i.e. monocytes, endothelial cells) plays an important role as a paracrine mediator of inhibition of thyroid hormone synthesis. Although the biochemical mechanism through which IL-1 exerts the inhibitory effect on TPO gene expression is not clear, IL-1 itself directly inhibits TSH-induced TPO mRNA level in
cultured human thyrocytes. Furthermore, IL-1 was not cytotoxic to thyrocytes, because IL-1 stimulated DNA synthesis in cultured human thyrocytes (2, 19).

A stimulatory effect of proliferation of rat thyrocytes by IL-1 has also been demonstrated (20), suggesting the growth promoting effect of IL-1 on thyrocytes. Although both TSH and cAMP stimulate the induction of TPO mRNA (18), the inhibition of TPO gene in cultured human thyrocytes has not yet been demonstrated. Our results are the first evidence of the inhibitory effect of IL-1 on human TPO gene expression in vitro. Since thyroid peroxidase proteins were reported to be generated from the 3.2 kb mRNA (21), the nature of other RNA species is unclear at present. Other transcripts may be reflected from the TPO precursor and alternative splicing products because Northern gel analysis of poly(A)RNA yielded the same results. Although we used Graves' thyrocytes, the same inhibitory effect of IL-1 on T3 release was already demonstrated using normal thyrocytes (unpublished data). Taken together, IL-1 is considered to be a potent inhibitor of TPO gene expression in cultured human thyrocytes.

We have further examined the effect of other cytokines on TPO gene expression. Tumour necrosis factor α (TNF-α) and IFN-γ also inhibited the TSH-induced TPO mRNA level (22), suggesting the inhibitory role of some cytokines on thyroid hormone synthesis. On the other hand, IFN-α and β and IL-2 did not affect the TPO mRNA level (unpublished data). It is, therefore, important to consider a role of IL-1 on the inhibitory effect of thyroid hormone synthesis. IL-1 may contribute to the development of autoimmune thyroid disease, especially chronic thyroiditis. Furthermore, IL-1 is generated in response to an inflammatory reaction. Overproduction of circulating IL-1 may induce the inhibitory effect of thyroid hormone synthesis. The decreased thyroid hormone level, especially observed in the low T3 and T4 syndrome, may be in part due to the inhibition of thyroid hormone synthesis by cytokines such as IL-1, TNF-α and IFN-γ. Recent in vivo studies also demonstrated the inhibitory effect of IL-1 on circulating thyroid hormone level (10, 11). As the thyroid microenvironmental cell-cell interaction has been well characterized (14, 23), paracrine regulation within the thyroid gland should be considered in thyroid diseas.

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